ADVANCES IN GENETICS

VOLUME I

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Edited by

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Editorial Board

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PREFACE

As material for their research geneticists use higher and lower plants, higher and lower animals, and recently also viruses and bacteriophages. They study heredity in man. In their experiments they may use biophysical methods, they may investigate the chemical synthesis of organic compounds, they may study the components of living cells. A considerable part of genetic research deals with practical problems related to the breeding of plants and animals. As a consequence of these several aspects of research in genetics, the results of such research are published in a wide variety of journals, and summary reviews are scattered among a considerable number of review periodicals.

This series of review articles, ADVANCES IN GENETICS, has been started in order that critical summaries of outstanding genetic problems, written by competent geneticists, may appear in a single publication. The articles are expected to deal with both theoretical and practical problems, and to cover plant breeding, animal breeding, and human heredity, as well as the related fields of biophysics, biochemistry, physiology, and immunology. The aim is to have the articles written in such form that they will be useful as reference material for geneticists and also as a source of information to nongeneticists.

The editors of ADVANCES IN GENETICS appreciate the fine response of their colleagues who have been asked to prepare reviews. They take it as an indication that this undertaking will fulfill a real need.

M. DEMEREC.

Cold Spring Harbor, New York

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Cytogenetics and Breeding of Forage Crops[1]

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[1] Paper No. 245, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

I. Introduction

The value of forage plants is receiving increasing recognition in diverse types of agriculture. This is attributable in part to economic factors which tend to limit cash-crop production, but more generally it seems due to a growing realization of the multiple values of grass (66). This tendency is particularly noticeable in certain land areas, where the soil and climate seem especially well adapted to a fairly permanent grassland type of farming.

Coincident with this greater appreciation of forage plants has been the greater emphasis on the breeding of improved types as well as on the related problems of fundamental cytogenetics. An excellent review of the literature on these subjects was presented in the several articles relating to forage crops in the U.S.D.A. Yearbook of Agriculture published in 1937. In addition, the Fourth International Grassland Congress was held in Great Britain in 1937, at which time several phases of forage breeding and cytogenetic research were summarized. Since that time, work along the same lines has been accelerated in many respects, so an attempt will be made in this review to include the most pertinent litera-

ture of the past 8 to 10 years. The species to be included will be the principal grasses and legumes used for pasture and hay, but work on certain problems and with certain species has received more effort than others, and so will be emphasized accordingly. The species on which the greatest amount of work has been done appear to be those which are important in northern Europe and the northern humid sections of the United States. This can be explained by both the importance of forage crops in these areas and the regional concentration of research workers interested in forage investigations. A few other species that are used at least in part for forage have been consciously omitted from this review due to lack of space, for example lupins, soybeans, sorghum and root crops used for fodder.

II. Breeding Methods with Forage Plants

Most perennial forage species are naturally cross-pollinated to a large extent (118), but there is great variation in this regard both between species and between plants within certain species. This suggests that the breeding methods may have to be varied depending on the fertility relationships of the material involved and the nature of the objective to be attained (306).

1. *Range in Self-Fertility in Relation to Breeding*

The entire range from complete self-sterility to complete self-fertility has been found in many of the forage crops that have been studied thoroughly, but the proportion of plants in the different fertility classes varies markedly between species. This was emphasized in the reviews of the literature and the results, relating to fertility in grasses, which were included in the articles by Beddows (37), Nilsson (233), Smith (292), and others. A more detailed account of this work will be included in the sections dealing with certain species.

Investigations on self-fertility are particularly useful when they can be related critically to methods of breeding. For example, in brome grass, meadow fescue and orchard grass, Hayes and Schmid (119) obtained a considerable amount of selfed seed by bag isolation during four out of five years. A sufficient number of fertile plants for selection purposes were found if about three times as many plants were bagged as could be used as parents for the production of selfed lines. In this case it was concluded that "selection in selfed lines practiced for only a few generations seems a logical and efficient method of isolating relatively vigorous inbred lines with the extent of homozygosity in important characters that is desirable." With crested wheatgrass, on the other hand, Murphy (203) stated that unless more self-fertile lines are found, selection within

selfed lines would not seem to be a very promising method of improvement because of the difficulty of obtaining selfed seed and because of the lack of vigor and uniformity found in many one-year selfed progenies.

When a species consists of only naturally self-pollinating plants, the most practical breeding procedure appears to be hybridization and selection, as practiced with wheat and other cereals. On the other hand, if most plants in the species are self-sterile but cross-fertile, inbreeding can be done only by sib matings, so that procedures other than inbreeding may be more fruitful. The greatest number of opportunities are presented within a species when self-sterile and self-fertile plants as well as intermediates are found. In this case inbreeding is easy with the self-fertile plants, and it is practical with many of the plants that have intermediate degrees of self-fertility. The latter may have the advantage of giving rise to inbred lines which, because of their relatively low self-fertility, will tend to outcross rather than self when exposed to open-pollination, thus making the combining of inbreds relatively simple. With highly self-fertile plants, it may be possible to include the factors for self-fertility during the inbreeding process but eliminate them before outcrossing is attempted, according to some such scheme as outlined by Rinke and Johnson (266) for red clover. The possibility of isolating self-fertile single plant lines which may be increased without loss of vigor should not be overlooked, but when inbred lines are obtained in most species, there still remains the important problem of the best method of utilizing the lines.

2. *Inbred Lines and Their Utilization*

Since inbred lines can be obtained easily in many forage species, much has been written to suggest that a procedure involving selection within selfed lines and subsequent hybridization, such as is done with modern corn breeding practices, should be useful also with forage crops. At present, however, there are no examples among the forage plants of commercial utilization of inbred lines in hybrid combinations and, from current evidence, it appears that it may be several years before results comparable to those with corn will be practically feasible on a large scale with most grasses and legumes. But the facts remain that inbred lines can be developed in many species and that hybrid vigor is commonly observed when the lines are outcrossed. These facts suggest that the possibilities of this method of approach have not been explored completely. It hardly seems fair to condemn the method until a large number of lines have been tested on a scale of approximately the magnitude of most corn breeding programs. The results to date are encouraging to this method of approach, but it seems too early to decide definitely on its practicability.

Part of the reason why more inbreds have not been developed may be that difficulties involving loss of vigor and fertility seem to be inherent with most forage species. The difficulties encountered in loss of fertility are so severe that they have influenced several workers to change their viewpoints. For example, when Kirk (163) first found certain selfed lines in red clover to be more self-fertile than his random sample of plants, he concluded that self-fertilization, together with selection within the selfed lines, provided the best attack in developing varieties improved in general agronomic characters. After several more years, during which more difficulties incident to self-sterility and loss of vigor were encountered, he (167) reversed his previous conclusion, stating that some method of composite crossing was, perhaps, better adapted to red clover improvement than selfed line breeding. Similarly Williams and Silow (345) found in red clover a self-fertility gene which can be transferred to any desirable plant, but in later writings Williams (340) favored strain-building, concluding that self-fertility, together with the decrease of vigor accompanying inbreeding, "must be regarded as nothing less than an unmitigated ill." In the same way Stapledon's (299) earlier writings suggested the possibilities of using some degree of inbreeding with orchard grass, but his later work (300) advocated strain-building.

Part of these difficulties may be avoided if inbreeding is continued only through a limited number of generations or if inbreds are combined as a synthetic variety. A few strains of grasses and legumes have been and are being developed with these procedures. In many instances they show marked improvement over parental stocks, but in very few cases have the trials been carried out over a number of years under several environmental conditions, so that here again it seems too early to pass judgment on the merits of this method of improvement.

3. *Mass Selection*

When inbreeding proves too difficult or impossible, mass selection provides a practical alternative. This procedure has given rise to most of the improved varieties in the past, and it is still being used with considerable success. For example the work of Law and Anderson (178) with big bluestem, *Andropogon furcatus*, showed that by five generations of selection in open-pollinated lines of desirable plants as maternal parents, it was possible to increase leaf area more than 12 times for plants in their first season of growth, and likewise to effect marked improvement in number of culms, plant height and basal diameter. Inbreeding in this same material was usually accompanied by a marked and progressive loss of vigor. Similar results were obtained by Popravko (260) in Russia, using several forage species. He concluded that mass selection and family

selection gave the best results, especially when using a wide range of pollen types. The progeny of different plants differed sharply and variability was regarded as necessary for the maintenance of high yield.

4. *Strain Building*

A method which compromises some of the features of both inbreeding and mass selection is known as strain building. It consists of selecting individuals, which conform to certain standards, and subsequently combining the chosen plants in a composite strain. The method has been employed for many years in Great Britain, particularly at Aberystwyth, where some of the most extensive breeding work with forage crops has been done, but it has found wide acceptance elsewhere, especially in Canada.

The term strain building was defined by Jenkin (139, 140) to include a range of procedures from mass selection through relatively complicated progeny testing, but Kirk (167, 169) limited its usage to any system of mating by which a strain is built up by the crossing of carefully selected plants. The use of the term was limited even more by Stevenson (306), and in this form it has received its widest acceptance. It is considered as a modified method of mass selection, which, in addition to selection on the basis of type or of a certain physiological response to a particular environment, provides for thorough exploration of the genetic constitution of the provisionally selected plants and a final selection of parents on the basis of their breeding behavior. The aim is to maintain vigor by incorporating into the strain a reasonably large number of genotypes, while at the same time approaching homozygosity with respect to the genetic factors governing the particular characters which formed the basis of selecting the parent plants. Strain building allows for progressive improvement through the uncovering of superior parent plants from time to time, and the use of these new exceptional plants, possibly in place of others, in reconstituting the strain. An essential feature is the preservation, for all time, of the parent plants, which are used as stock in each additional increase. In many respects, the process of selecting rigidly the type on the basis of progeny tests is the same as the approach used successfully by many livestock breeders.

The most extensive application of the strain-building method undoubtedly has been at the Welsh Plant Breeding Station. Recently, Jenkin (142) listed the strains which were produced there as follows: perennial ryegrass, S.23, S.24 and S.101; cocksfoot, S.26, S.37 and S.143; timothy, S.48, S.50 and S.51; meadow fescue, S.53; creeping red fescue, S.59; red clover, S.123 and S.151; and white clover, S.100 and S.184.

5. *Hybrid Alfalfa*

A modification of the hybrid corn technique has been proposed by Tysdal, Kiesselbach and Westover (325) and by Tysdal and Kiesselbach (324) for producing hybrid alfalfa. Since its principle seems applicable to other forage crops, it will be mentioned here. A more detailed description is in the section on alfalfa.

It is stated (324) that the chief reason for inbreeding corn is to be able to duplicate a given genotype. In perennial crops, such as alfalfa, which can be propagated vegetatively with ease, the need for self-fertilization is eliminated. Instead all the emphasis can be placed on selecting desirable clones with high combining ability, planting cuttings of them in isolated crossing-blocks for the production of F_1 seed and sowing two resultant single-crosses in alternating rows in another field to produce the double-cross commercial seed. It is estimated conservatively that two 25 acre fields for the production of single-cross seed would furnish stock for one million acres of double-cross alfalfa. If this procedure proves profitable, it may well change the basis for much of the breeding work with forage crops.

6. *Cytogenetic and Breeding Technics*

The technics which are used in plant breeding often become limiting factors in the progress of the work. As a consequence, most breeders are attempting continually to find new and improved methods which will facilitate the rate of work. Generally these improvements are developed when a specific need is recognized in working with certain species. It is apparent, however, that these new ideas can often be adapted to a wide range of material. Some of the more pertinent ones of these technics, which have been described recently and which appear to have potentialities for greater usefulness, will be mentioned briefly.

The process of hot water emasculation has been applied to a number of grasses by several investigators; some of the factors influencing the success of the process have been studied particularly by Domingo (90), Keller (156), Clark (69), and Tsiang (319). Treatments for five minutes at 47° C. have proved satisfactory under several conditions, but this was conditioned in part by the time of day and the accompanying air temperature. Following bulk emasculation, higher seed set resulted from exposure to natural pollination than from any of the methods of controlled pollination. Seed was obtained by Keller (155) on grass culms detached prior to pollination. A number of species ripened seed in this way, but in every case the seed weighed less and it often had lower germination than normal seed. The literature on methods of isolation was summarized by Keller (157); his results with brome grass showed that 35-pound parch-

ment bags were superior to both heavier weight parchment and kraft paper bags in number of selfed seeds produced. The seeds from the 35-pound parchment bags tended to be heavier and to germinate better than those from the other treatments. Burton (55) described a blower for cleaning grass seed, and James (138) and Higbee (124) each described devices for rapid hulling of small samples of clover heads.

A technic for growing grass and legume seedlings in newsprint paper bands in flats was described by McAlister (189). A space of only 4×12 feet in the greenhouse was required to grow 9,000 seedlings and the cost per seedling was relatively low. Transplanting to the field was rapid and a 94% survival was obtained in a dry-land planting. The problem of inoculating legumes with nodule bacteria while at the same time disinfecting the seed can be handled, according to Albrecht (10), by mixing the bacterial culture with well-composted, finely-ground stable manure, which is applied to open rows or mixed with the seed and broadcast. A system for numbering and note taking in the field was outlined by Newell and Tysdal (225) for use with perennial forage crops. By prefixing to the accession number both a number for the year of selection and a number representing the treatment, ready recognition of plant and seed material is possible throughout the process of improvement. In testing large numbers of plants in the field, Burton (62) found that very satisfactory estimates of yield could be made by inexperienced individuals when the plants were scored on the basis of 1 to 5. Significant differences between individuals were noted not only in their scoring ability but also in their ability to improve their scoring with training.

Many modifications of the picric acid test for HCN have been proposed, but those which allow for testing large numbers of plants rapidly, such as those of Nowosad and MacVicar (245) and Hogg and Ahlgren (130), appear to have the greatest applicability to breeding or inheritance studies. Similar technics were used with white clover, except that here purified preparations of both the glucoside and enzyme were added separately (80, 33). Likewise, in tests for coumarin, the rapid fluorometric methods, suggested by Ufer (327) and Slatensek and Washburn (289), appear to be adaptable to studies of large populations. The correlation between dark green leaf color and protein content has been suggested by many investigators as a valuable aid to breeding for higher protein. The correlation is not high for all materials, but Leitzke (180) concluded from a study of several species that it could be a valuable step in the selection process.

In testing seedlings for drought resistance, McAlister (190) found that results from a control chamber agreed closely with the known behavior under natural conditions. The per cent of plants renewing growth fol-

lowing the drought treatment was used as the index of relative drought resistance. Likewise, using control chambers, Atwood and MacDonald (32) found highly significant differences between selected brome grass plants in their ability to produce aftermath yield at continuous high temperature.

A technic for separating ergot-infected seeds from normals by means of acid treatment was suggested by Burton (64). This method was much less tiresome, gave more accurate results, and was only half as time consuming as the old method of dissecting florets. Many different cytological procedures have been found especially adapted to certain materials, and most of these are described in the papers dealing with the particular plants. A schedule for treating root tips of certain grasses at low temperature was found by Hill and Myers (126) to contract the chromosomes, thus facilitating determinations of chromosome number.

The assembling and testing of all promising strains is a necessary part of every complete breeding program. This type of collection is now being sponsored by the U. S. Department of Agriculture through the Uniform Nurseries and it has proved very helpful to plant breeders in many respects.

III. Bluegrasses

The species of bluegrass are widely distributed in the cooler humid portions of both North America and northern Europe, where they are cultivated for forage and turf purposes. The most important single species agronomically is Kentucky bluegrass, *Poa pratensis*, but several other species are cultivated extensively. A turning point in the cytogenetic and breeding investigations with bluegrass was the report by Müntzing (192) that an apomictic form of reproduction occurred in Poa.

1. *Apomixis and Embryo Development*

The evidence for apomixis has been deduced both from progeny tests and from direct observations of the embryo sac. The latter line of work has provided explanations for most of the phenomena observed in the progeny tests.

In apomictic plants of *Poa pratensis*, Åkerberg (3, 6) observed the nucellar cells taking over the functions of the megaspores. In sexual parents, no evidence of aposporic embryo sacs was found. It was concluded that reproduction in *Poa pratensis* may take place with or without apospory and with or without fertilization. Nilsson (235) pointed out that pollination was necessary for seed development.

At about the same time, Tinney (316) described the development of embryo sacs in biotypes collected from old pastures in Wisconsin. He

stated that the single macrospore mother cell undergoes meiosis in the usual manner, forming generally three macrospores, all of which subsequently disintegrate. The embryo sac then develops, without meiosis, from a cell of the nucellus, which is located near the chalazal end of the macrospore mother cell. The typical mature embryo sac consists of three antipodals, a primary endosperm cell with two nuclei, an egg and two synergids. The diploid egg develops by parthenogenesis into a proembryo, frequently before pollination. Since endosperm development was not observed until after pollination, it was concluded that pollination or the growth of pollen tubes in the style may be necessary for endosperm development and consequently for seed development. It was pointed out also that diploid parthenogenesis and occasional fertilization of an egg by a sperm possessing a varied chromosome number would account for the occurrence of (a) the extreme polymorphism and varied chromosome number between different biotypes, as well as (b) the constant type and chromosome number within biotypes, which previously had been described by Müntzing (192). Twin embryo sacs were observed occasionally.

Engelbert (92) found that pollination was necessary for seed formation in strains of *P. arctica*, *P. alpina*, *P. alpigena* and *P. pratensis*, but in every case the progeny of crosses resembled the mother parent. In these species, pollen apparently acted only as a stimulus to ovule development. Åkerberg (8) considered both autonomous embryo development and induced endosperm formation to be general phenomena in apomictic types of *P. pratensis*. Similar situations were described by Kiellander (159, 160) for *P. serotina* and *P. palustris*. Most of these findings were confirmed by Kiellander (161), in a somewhat fuller account of the cytological conditions relating to seed formation in *P. pratensis*.

A somewhat different development was described by Håkansson (111) for two apomictic forms of *P. alpina*. Here the division of the macrospore mother cell was invariably mitotic, and the resulting diploid egg cell gave rise directly to the embryo. The diploid chromosome number was observed in most of the embryos, although one was apparently triploid, and in a few cases polyembryony was noted.

2. *The Progeny Test as Evidence of Apomixis*

All the cytological work has been helpful in explaining the process of apomixis. It is apparent, however, that even a greater amount of evidence has been derived from measuring apomixis indirectly through the progeny test. This procedure has been utilized with a number of species, but again the most extensive studies have been with *Poa pratensis*. In these tests it is generally assumed that the predominating type of progeny is associated with asexual seed formation, whereas the variant progeny are con-

sidered to arise as a result of sexual reproduction. The classification is usually done in the field during the second growth year, and it is based on a number of morphological characters such as growth habit, aggressiveness, leaf width, color, culm height, vigor and disease reaction. Ordinarily the distinction between normal and abnormal types is clear cut.

The progeny test was used first by Müntzing (192) and has since been developed into almost a standard technique through the work of many others. Müntzing found that three Swedish biotypes of *P. alpina* produced morphologically uniform progenies with constant chromosome numbers. In contrast, the Swiss forms of *alpina* were sexual and characterized by variable chromosome numbers and variable morphology. Similarly, in four of the eight biotypes studied in *P. pratensis*, morphological constancy and aneuploid chromosome number suggested apomixis as a general phenomenon. Müntzing (198) reported that different frequencies of aberrants were found in four apomictic strains of *P. alpina*. The apomicts were characterized by various aneuploid numbers, and biotypes from the same geographical region tended to have the same number. In a sexual strain with oscillatory chromosome number, there was a tendency for the individual progeny to decrease in number in comparison with the mother plant. In addition, haploid parthenogenesis resulted in lower chromosome numbers, but functioning of unreduced gametes and possibly selection of high chromosome gametes operated in the opposite direction. In one strain, this selection for low chromosome number resulted in a stable sexual line with 11 bivalents at IM. No correlation was observed between chromosome number, vigor and fertility. Müntzing also described the cross between a sexual strain with 24 chromosomes and an apomictic strain with 38. In the F_1 generation numbers ranged from 25 to 43, suggesting that both reduced and unreduced female gametes functioned. All F_1 plants were fertile and sexual, but variation in both morphology and chromosome number was noted in the 17 F_2 progenies. In the offspring of some F_1 plants with high chromosome number, a regular (15%) formation of haploids was observed; this was considered due to a combination of the parental tendencies to chromosome reduction and parthenogenetic development. All F_2 plants were sexual; consequently, it was concluded that apomixis does not result from the action of a single gene but "rather to special constellations of genes and chromosomes brought about by natural selection."

Of 44 families from panicles collected in nature, Åkerberg (6) found that 37 gave uniform progeny; a total progeny of 185 plants were examined, and only 5.9% were aberrants. Likewise, in a population of 703 from over 25 biotypes in the breeding nursery, only 9.2% were aberrants. Further studies along this line were reported by Åkerberg (7) in 1942.

The progeny test was applied to breeding material of *P. pratensis* by Tinney and Aamodt (317). The seeds were collected from single open-pollinated panicles, and the progenies were grown as individual spaced plants. In 48 of the 102 progenies, no variant plants were found, and the highest per cent of variants in any progeny was 21.9. In the total population, only 1.6% of the progeny plants were variant. Similar results were obtained by Brown (51) using 11 lots of *P. pratensis* grown from seed produced by open pollination; the per cent aberrant types within strains ranged from 0.09 to 18.18. Apomixis was prevalent in the high polyploid forms. In some strains both apomictic and sexual reproduction was observed, and this was used as a partial explanation for the extreme diversity within the species.

A very exhaustive study of the variation in the progeny of 115 plants of *P. pratensis* was presented by Brittingham (49). The per cent variability for all offspring was 14.6, but values for different families ranged from 0 to 65.5. The 16 progenies from introductions and numbered strains averaged 18.6 variability; the 28 progenies from pasture sources 16.2; and 71 progenies from commercial sources 13.3. The differences between these averages were not significant. Brittingham noted 80.3% germination and 86.3 survival in his total population, and a highly significant negative correlation ($r = -0.380$) was found between survival and variability. All of the seeds were germinated in Petri dishes, thus permitting equal chances for survival, and this may account for the higher per cent of variability than was found by Tinney and Aamodt (317). A second generation progeny test of part of this material was made by Myers (211) using three progenies, which in the first generation had been classed as containing 3, 27 and 48% variant-type plants. Although the results agreed in general with the first-generation test, some errors in classification were found; some plants which had been classified as parental types were proved by their progeny to be variant types. In general, variant-type plants produced a higher proportion of sexual seeds than did their parents or parental type sibs. It was concluded that the first-generation test provided a satisfactory preliminary evaluation of method of reproduction.

Succeeding generations were studied also by Smith and Nielsen (293) using the lines started by Tinney and Aamodt (317). A wide range of variation in per cent normals was found when progenies were grown from both normal and aberrant plants. For instance, one normal showed 100% segregation in its progeny whereas five other normals produced completely uniform progeny, but no families were completely uniform for two successive generations. The most constant families studied showed the following per cent of normals in three successive generations: 99, 91,

98; 98, 91, 100; 99, 87, 81; 98, 79, 88; 100, 83, 75; 100, 87, 87; and 99, 96, 59. In contrast to this, 29 progenies from aberrant plants were entirely aberrant, whereas 14 other progenies from aberrants were all normal. Nine families showed complete aberrancy for two successive generations. When the per cent normals was compared in progenies of four families each from normal and abnormal plants, it was found that the apomicts tended to give rise to more uniform progenies than did the aberrants, but this relationship was not marked enough to make selection for normal types very effective in increasing the constancy of the average progeny. The average per cent of aberrants was high in the progenies of both normal and abnormal plants.

3. *Cytological Behavior as Related to Apomixis*

One feature of the genus Poa is its extensive range of chromosome numbers. This is particularly true of *P. pratensis*. When the reports up to 1939 were summarized by Brown (50), the lowest somatic number reported was 28 and the highest 95. Since then Åkerberg (7) reported a high of 124, but he pointed out that values below 49 and above 91 were exceptional. All values between these limits seemed to be equally frequent. Brown (50) concluded that there was a strong mode at $2n=56$, indicating that most plants were octaploid.

Observations on meiosis have been reported by Armstrong (14), Müntzing (192, 198), Tinney (316), and Håkansson (111). In general, the division of the macrospore mother cell is regular, but many irregularities have been reported in microsporogenesis. An exceptional case in *P. pratensis* was reported in detail by Kiellander (162). A biotype with $2n=\pm 72$ was morphologically constant throughout three generations, but among the twin plants were some aberrant individuals, two of which, a twin with $2n=40$ and a triplet with $2n=18$, were described in detail. The open-pollinated progeny of the 18 chromosome plant ranged in chromosome number from 15 to 19 with an average of 16. Like the mother plants these progenies resembled *P. trivialis* ($2n=14$), suggesting that the latter may be one of the species that has contributed to the formation of *P. pratensis*. Nielsen (228) reported another cytological abnormality, consisting of globular inclusions in the developing microsporocytes, particularly during anaphase and telophase stages. In addition, highly significant differences in frequency of laggards were found in most plants.

Chromosome numbers in other species of Poa have been reported by Armstrong (14), Nannfeldt (223), Brown (50), Flovik (98), Litardiere (188) and Åkerberg (7).

4. *Species Hybrids*

A hybrid between *Poa compressa* and *P. pratensis* was reported by Brittingham (48). The hybrid had ±72 somatic chromosomes and resulted from the cross Canada bluegrass female ($2n=42$)×Kentucky bluegrass male ($2n=56$). The hybrid came from a head which had been emasculated with hot water, but it was the only hybrid in the progeny, all 41 other plants being unmistakably matroclinous. It was suggested that the hybrid resulted from the union of an unreduced egg cell of *compressa* and an approximately reduced pollen grain of *pratensis*. The hybrid was fairly fertile, produced seed about 45% heavier than seed of either parent, and was intermediate between the two parents in most other characters.

A second species hybrid, namely between *Poa pratensis* and *P. alpina*, was described by Åkerberg (4, 7), Nannfeldt (222), and Müntzing (198). A complete description of several hybrid plants which were widely different morphologically was presented by Åkerberg (7). These plants arose partly by fertilization of reduced and partly by fertilization of unreduced gametes of the female parent, *pratensis*. The F_1 plants had highly sexual seed production, even after crossing with apomictic races of *pratensis* and *alpina*. In two F_1 plants, observations were made of reduction division, megaspore formation and aposporous embryo sac initials; the behavior was similar to *pratensis*. Müntzing's (198) hybrids, which arose from a haploid *pratensis* type, all had chromosome numbers in the expected range of 31 to 35, except one plant which had 52; the latter arose presumably from an unreduced female gamete. Åkerberg (7) also reported a hybrid between *pratensis* and *glauca* and mentioned that several other supposed hybrids have been found in nature.

Hardison (113) suggested that hybrids between *P. arachnifera* and *P. pratensis* may be useful for breeding because of the possibility of their inheriting disease resistance and fertility from *arachnifera*.

5. *Polyembryony*

The occurrence of twins and other multiple embryos was reported many times for the genus. Recent reports were those of Müntzing (196, 197), Skovsted (288), Engelbert (93), and Brittingham (49). In every case, this type of material resulted in many morphological and cytological aberrants.

Müntzing (196) observed that the frequency of twins with deviating chromosome numbers seemed to be unusually high in comparison with other species. A total of 19 pairs of twins, one set of triplets and 15 single twin plants comprised not less than 10 plants with deviating numbers; often the deviating number was approximately triploid in comparison with the other. When this study was enlarged to include 270 twin plants

(197), 18 were found to be triploids, two were haploids, and five showed other deviations. Seven of these approximately $2n$–$3n$ pairs were studied from a number of aspects by Müntzing (198). In most cases the twin with the increased number gave a greater weight in the total of three years cutting. The higher number plants had thicker and broader leaves, and this proved to be one of the best diagnostic characters of those studied. There were no significant differences between high and low chromosome number twins in any of the chemical properties analyzed. The high chromosome plants had better pollen fertility and appeared to be more sexual than their corresponding $2n$ members. Brittingham (49) found that out of 1441 plants from seed containing more than one embryo, between 16 and 20% of the pairs or triplets contained plants that differed morphologically; this per cent was significantly higher than was the 13.1% found among the plants from single embryo seeds. There was a significant negative correlation ($r = -0.205$) between per cent variability and per cent polyembryony for the 115 progenies studied.

The cytological origin and development of twin embryo sacs was described by Engelbert (93) for *Poa arctica*. One sac developed from the innermost of a row of four macrospores with reduced chromosome numbers; the other came from an aposporous cell that originated in the nucellus near the chalaza and behind the normal archespore. Both types of eggs developed parthenogenetically. Multiple embryos were observed likewise in *P. pratensis*, but Armstrong (14) found no evidence of apospory, whereas Tinney (316) interpreted all of the sacs as arising from the nucellus. Kiellander (161) explained the 13% polyembryony found in the biotype studied as due to the occurrence of aposporous embryo sacs.

6. *Improved Strains*

During the past several years, numerous attempts have been made to breed improved strains of bluegrass. Some of the resultant types have been superior, but thus far no improved strains have been grown commercially to any considerable extent in North America (2). It was reported by Nilsson-Leissner and Nilsson (242) that five varieties of *P. pratensis* were released in Sweden prior to 1940. The acreage of these and other improved varieties in Europe is still limited, and preliminary observations of improved European varieties in parts of the United States (2) indicate that they have no particular superiority in these regions. It is undoubtedly too early to assess the value of all this breeding work, but it seems reasonable to assume that outstanding strains may be infrequent and difficult to isolate. Many different types appear to result from natural selection (158), but it is not known in most cases whether they are the best types to choose in a breeding program.

It is generally assumed that the process of apomixis will be utilized directly for the maintenance of varietal purity (317). The question then arises of how many off-type plants can be tolerated. It is generally observed that the sexually produced plants in apomictic families are usually lower in vigor than the normal types. For this reason, Smith and Nielsen (293) suggested that the weak plants might be submerged and have little effect in the mass productivity; relatively pure seed probably could be harvested from old sods. On the other hand, it has been pointed out on numerous occasions (192, 7, 49) that other breeding methods beside straight selection are available in bluegrass.

In an attempt to evaluate the yielding ability of individual plants of *P. pratensis*, Myers and Garber (217) used clonal plots, established from 81 selected plants. Following two years of clipping to simulate pasture treatment, yields were determined in the third season. Significant differences among strains were found for total annual yield. Differences in seasonal yield and in competitive ability with white clover also were noted. Further studies of 13 of these strains plus two commercial checks, all seeded in plots, were reported by Myers and Sprague (221). Significant differences among strains for yield of total herbage, yield of bluegrass, and per cent of associated white clover were obtained at each clipping date for the three years. The short-leaved, dense sod-forming strains were consistently inferior to the commercial checks in yield, whereas two tall, long-leaved strains exceeded the checks in yield. There was no noticeable tendency for the differences among strains to disappear in the third year as compared with the first.

Similar experiments, conducted by Hayes and Thomas (120), involved clones of bluegrass from 150 pastures and waste places in Minnesota. A total of 281 clones were selected and, together with 5 other types, were measured for yield in clonal plots throughout two years. Highly significant differences were obtained and there was good agreement in yield between the two years. Seeded plots from 48 of these strains, together with eight others from Wisconsin, were clipped for yield seven times in 1944. Five strains were significantly better than the check in total yield and 9 in summer yield. There was little or no relationship, however, between yield of the clones and yield of the corresponding seed progenies.

In contrast, Ahlgren, Smith, and Nielsen (2) state that only one of the 74 selections from a nursery of 11,800 plants was superior to commercial bluegrass in all three years; the strains were seeded in small plots on two soil types. There was a marked tendency for the original differences between most strains to become less pronounced as the plots increased in age. There was little or no relation between yields of the same selections during the first and second years, but there was good relation between the

yields of the same selections during the second, third and fourth harvest years. There was little or no relation between estimated yields of spaced plants and yield of the progeny of these plants seeded in plots; nor was there a relation between yields in plots and disease reaction. The taller growing clones appeared to be the better competitors with white clover, but the correlations between yield and competitive ability were so low as to be of little value for prediction purposes. All this work suggested the need of better technics in the initial stages of a breeding program.

IV. Timothy

Phleum pratense is the most widely cultivated hay grass in the northeastern and north central United States, eastern Canada and parts of northern Europe and Asia. It was one of the first forage grasses to receive extensive attention from the breeding standpoint, and many studies have been made of its cytogenetic behavior. The literature on these subjects up to 1937 was reviewed by Evans (95). Since that time the cytogenetic investigations have emphasized species hybrids, polyploidy and twinning; the breeding studies have advanced along several lines.

1. *Species Hybrids*

The first species hybrids were obtained by Gregor and Sansome (109) and Gregor (108). The cross *P. pratense* ($2n$)×*P. alpinum* ($4n$) gave rise to triploids, which were partially sterile, but which, in turn, gave rise to four hexaploids. One of these $6n$ plants was completely fertile and crossed easily with hexaploid *P. pratense*. The cross *P. pratense* ($6n$)×*P. alpinum* ($4n$) gave rise to one pentaploid.

Spontaneous hybrids between *P. pratense* ($6n$) and *P. nodosum* ($2n$) — called *P. pratense* by Gregor and Sansome (109)—were investigated by Müntzing (194). The hybrids, with 35 and 36 chromosomes, were characterized by IM configurations of $14_{II}+7_{I}$ and $15_{II}+6_{I}$, respectively, although a few quadrivalents were observed. Presumably the 35 and 36 chromosome plants arose from backcrossing the primary hybrid, $2n=28$, with *pratense*. As a result of this work, Müntzing (194) formulated the hypothesis that the *nodosum* genome should be designated N and the *pratense* genome NAB. Thus, the hexaploid timothy was considered an allohexaploid with one of its 7 chromosome complexes similar to that of *nodosum*. The I_1 families from selfing the 35 and 36 chromosome plants showed a striking variation in morphology, vigor, fertility and chromosome number; as the chromosome numbers approached 42, there was a marked increase in vigor and fertility.

Subsequently Nordenskiöld (243) made a thorough study of several species hybrids; in the cross *P. nodosum* ($2n$)×*P. pratense* ($6n$) both 35

and 49 chromosome plants were obtained. This was explained by assuming that the female parent produced $2n$ and $4n$ gametes. The assumption was confirmed in studies of the progeny of the *nodosum* parent, and it explains the origin of Müntzing's (194) hybrids without backcrossing. From the reciprocal cross two 28 chromosome plants were obtained, and these individuals had 14_{II}, and in a few cells one ring-shaped quadrivalent. This indicated that the A and B genomes of *pratense* were homologous *inter se* and at least partially homologous with the N genome. Additional evidence supporting this conclusion was the pairing of chromosomes in hybrids between *P. nodosum* ($2n=14$) and *P. alpinum* ($2n=14$ and 28). Considering all hybrids which were investigated, it was concluded that triploid hybrids tend to be male sterile, forming univalents, bivalents and trivalents, while the hybrids with 35 or 49 chromosomes usually are fertile and form multivalents of higher degrees. Several other species not in the so called *pratense* group were used but all crosses between groups failed.

Among the additional hybrids and their offspring studied by Olah (247), there was one progeny plant with 56 chromosomes. This plant was sterile and feeble, and during meiosis bivalents, trivalents and quadrivalents were formed. Soon after flowering the plant died. It was suggested that octoploidy is the highest polyploid form in Phleum which is viable.

Another hybrid, *P. pratense* ($6n$)$\times$*P. subulatum* ($2n$) was described by Myers (206). At IM, chromosomal association ranged from $7_{II}+14_{I}$ to $12_{II}+4_{I}$. The hybrid was completely male sterile, but was partially female fertile with *P. pratense* pollen. Here also bivalent formation was higher than would be expected if the several genomes were not partially homologous.

A general conclusion (243) from all of the work on species hybrids is that $2n$, $4n$ and $6n$ types of the *pratense* group are probably closely related. They cross readily, and cytological investigations have indicated close homology between their genomes; even so, the species are for the most part distinct.

2. *Cytological and Genetical Investigations*

The results of Müntzing and Prakken (202) using three twin plants, with 63 chromosomes each, provided additional evidence on the origin and relationship of the several species. The sum of the bivalents and the occasional trivalents was rarely lower than 28, and the number of univalents not higher than seven. The frequency of bivalents was higher and the frequency of trivalents much lower than expected. All gametes carried at least 28 chromosomes, and the average was 30.03. In the 186 offspring of the twins, chromosome numbers ranged from 56 to 64, the average being 59.66. Since the "triploid" twins supposedly consisted of 3 genomes each

replicated three times, it was expected that the sum of bivalents plus trivalents would not exceed 21, whereas the number obtained was at least 28. Since this can be explained only by assuming homology between two of the three genomes, Müntzing's original hypothesis (194) of an NAB complex for *P. pratense* was revised (202) to be NA_1A_2.

Additional information on the cytological background of timothy was derived from the study of "haploids," $2n=21$. Meiosis was studied by Levan (183) in two out of seven haploids derived from twin seedlings. Many irregularities were found, some P. M. C.'s having $7_{II}+7_I$, whereas others had 21_I. The most striking irregularity was cell fusion at meiotic prophase. From two to ±30 P.M.C.'s fused to form one large syncytium. At IM these large cells developed one bipolar spindle, on which all the bivalents (at least 150 in one cell) were arranged into one regular equatorial plate. These results were confirmed by Nordenskiöld (244), but in addition she found trivalents in a few cells. This evidence of pairing as bivalents and some trivalents supports the theory (202) that at least two of the three genomes in *P. pratense* are homologous and that there is partial homology between these two genomes and the third.

At IM in hexaploid timothy, Müntzing (194) described 21_{II}. Likewise, Nordenskiöld (243) stated that multivalent associations were not found in the species. Müntzing and Prakken (202) noted that quadrivalent formation was rare. A greater proportion of quadrivalents was observed by Myers (206) in a male sterile clone of hexaploid timothy. Among the 72 sporocytes examined at diakinesis, 63.9% had one, two or three quadrivalents. In a few additional cells not analyzed completely, 4_{IV} were seen. Two exceptional sporocytes with 84 and 126 chromosomes resulted presumably from reduplication in premeiotic divisions; associations of eight chromosomes were seen in both of these cells, but most of the chromosomes paired as bivalents. This study was extended by Myers (213) to include seven plants of diverse origin. Of the 102 sporocytes examined at diakinesis, only two had 21_{II}; all others had quadrivalents, sexivalents, or both, in addition to bivalents. The frequency of quadrivalents per cell ranged from one to seven, and the plant averages ranged from 2.9_{IV} to 4.9_{IV} per sporocyte. The average number of sexivalents per cell ranged from 0 to 0.9; 26 of the 102 cells had one sexivalent, six had two, and one had three. In the cell with three sexivalents, none consisted of nucleolar chromosomes, but in one of the cells with one, nucleolar chromosomes were involved. All these data on multivalents supported the hypothesis (202) of homology between certain of the genomes in *P. pratense*. Quadrivalents were distinguished also at IM, and in addition univalents were found in 1.1–14.3% of the cells in the 10 plants studied. Lagging and dividing chromosomes, dicentric bridges and acentric frag-

ments were seen at anaphase I in varying per cents for the different plants. The frequency of quartets with micronuclei ranged from 0–15.1% for the 10 plants and appeared to be correlated with frequency of laggards at I anaphase.

Most of the recent evidence (243, 202, 183, 244, 206, 213) has suggested that the A_1 and A_2 genomes are homologous and that parts of either or both of them are also partly homologous with the N genome; if this is the case, it is difficult to understand why multivalents are not formed more frequently. Müntzing and Prakken (202) suggested that each of the bivalents might not always be formed from the same two chromosomes, but that sometimes they might be formed by random two by two pairing among four or six homologous chromosomes. They suggested also that there might be "a special genotypically controlled tendency to bivalent formation." Multivalent formation has not only been low but it has varied considerably in different plants; Myers (213) suggested this might be due to inherent differences in the material studied or to different conditions under which the plants were grown.

As pointed out by Myers (213) there is a paucity of critical genetic evidence from hexaploid timothy. Barker and Hayes (35), Proytchoff, according to Horsfall (136), and Myers and Chilton (unpublished work cited by Myers (213)), obtained typical monohybrid ratios for resistance to rust. On the other hand, triplicate factors were assumed by Clarke (71) to explain the segregations he obtained for chlorophyll-deficient seedlings. Each of four types of chlorophyll-deficient seedlings, studied by Wexelsen (334), were shown to be conditioned by three independently inherited homomeric factors, and at least two such factors were operating in the case of a fifth deficiency. Normal green was dominant to all types so that 3:1, 15:1, and 63:1 ratios were obtained. In the case of one family, tetrasomic inheritance was suggested as a more suitable explanation. Myers (213) likewise studied segregations for chlorophyll deficiency in the I_1 and I_2 generations from five plants. In this case dihybrid and trihybrid ratios were not obtained, but the data were insufficient to distinguish between possible tetrasomic and hexasomic segregations.

3. *Twins*

In a study of 45 pairs and 20 single twins, Müntzing (196) found three plants with $2n = \pm 63$ and one with $2n = 21$. Later (197) he listed six triploids and three haploids among 446 twin plants examined; all others had the normal chromosome number. Skovsted (288) also reported twins in *P. pratense*. The cytological studies (202, 183, 244) of the aberrant plants have been very illuminating in regard to the origin and relationship of species in the *pratense* group.

Additional studies were made by Müntzing and Prakken (202) in regard to several morphological characters. The "triploid" members of the twin pairs had good vigor and fertility. It was also observed that the triploids had longer, broader and thicker leaves than the corresponding diploids; similarly, their stems were thicker and longer, their culms thicker, the spikelets bigger and the pollen grains larger. Plant weight was measured in 1371 twin progeny plants, in comparison with the standard variety, "Gloria." In the first summer, the twin progenies gave a yield of 114 in comparison with the yield of the standard variety rated as 100, but in the total yield for two and three years, respectively, the twins averaged 97 and 91. This change in relative value was explained in part by differences in winter-hardiness and in part by differences in seedling establishment; the latter factor may be related to the greater seed size of the triploids. Progeny plants with about 56 chromosomes were slightly more vigorous than those with numbers between 56 and 63, but pollen fertility was good in all plants. Müntzing (199) reported later that certain lines from 63 chromosome twins have a higher weight than Gloria.

4. *Breeding Methods and New Strains*

As pointed out by Evans (95), several breeding methods have been used successfully with timothy. Recent reports from Sweden by Nilsson-Leissner (241) and Julén (152) reëmphasize the value of inbreeding as a method of improving timothy. In general, their inbred lines decreased in yield in each of four successive generations, but 27 of the 199 I_4 lines gave higher yields than their parents. Originally, nine highly self-fertile lines were selected and the elimination was very strict in each generation. The ability of regrowth was not generally influenced by inbreeding but selection for earliness was very effective. It was also possible to select strains which were very resistant to rust throughout. Myers and Chilton (216) observed that stem rust infection was correlated with degree of winter injury in 60 parental clones of timothy.

The twinning method of increasing variability has been suggested by Müntzing (see discussion above) as a means of increasing both yield and fertility. Some of the types developed by this method are being increased. The 84-chromosome type (199) produced by means of colchicine proved to be above the optimum.

In 1937, Evans (95) listed 21 improved varieties of timothy. Several others have been developed since then, but there is little evidence that any have received widespread acceptance, particularly in the United States. Problems of increase and distribution seem to be major factors in limiting the use of these as well as other improved forage crops. Better success in promoting such materials has been obtained in Sweden; recently Julén

(153) described two new superior varieties, Bore II and Sv. 0812, the latter of which may be adapted to a wide area.

V. Orchard Grass

1. *Cytological and Genetical Investigations*

Brief reports of cytological behavior in Dactylis were made first by Müntzing (193) and Rancken (263); a later report of Müntzing's (195) included detailed descriptions of meiosis, vigor, fertility and morphology in normal and aberrant plants of *D. Aschersoniana* ($2n=14$), *D. glomerata* ($2n=28$), and their hybrids. Meiosis was studied in three biotypes of *D. Aschersoniana;* one had regular meiosis with 7_{II}, the second contained a large fragment, and the third was found to be heterozygous for a segmental interchange, causing quadrivalent formation in some cells. There was a positive correlation between total chiasma frequency and the occurrence of quadrivalents. Müntzing (193) first observed that the chromosome number in *D. glomerata* was unstable; these observations were extended (195) to include determinations of 233 plants, and in 177 of them the determinations were considered absolutely accurate. Of the 233 counts, 3 showed 26 chromosomes, 22 showed 27, 188 showed 28, 18 showed 29, and 2 showed 30. Thus, 19% were classed as aberrants with other than 28 chromosomes; 10% of the 177 accurate counts were aberrants. At IM in the Skandia II original clone, Müntzing (195) found an average of about half the chromosomes associated as quadrivalents. All possible combinations of quadrivalents and bivalents were found; in addition, two cells had the maximum association of 7_{IV}. Similar results were observed in wild biotypes. Altogether, 70% of the quadrivalent rings or chains were arranged in a zig-zag pattern. Detailed studies were also made of meiosis in the aberrants and chromosome variation in progenies of the aberrants. The pentasomic plants differed significantly in meiotic behavior but in the progenies of the aberrants there was a general tendency to revert to 28. Natural hybrids between *Aschersoniana* and *glomerata* had 21 chromosomes, which formed from one to seven trivalents at meiosis, with an average of 4.58. Many univalents were eliminated at later stages of meiosis, so that the average chromosome number in the gametes was less than 21/2. All $3n$ hybrids were male sterile but offspring were obtained from backcrossing to each parent. The backcross to *Aschersoniana* resulted in plants with all numbers between 14 and 21. The other backcross, with *glomerata*, gave several $\pm 5n$ plants but a majority with lower numbers. Meiosis in a $5n$ plant was irregular, and its progeny ranged in chromosome numbers from 28 to 41. Chiasma frequency was found to be the same in $4n$ and $2n$ types, but it was lower in $3n$ and $5n$ plants, due to their inca-

pability of forming closed rings. Müntzing (195) also reported that the plant weights of individuals with 14, 21 and 28 chromosomes represented maxima of increasing size, with the 28 chromosome plants being at least twice as vigorous as those with 14. The ranges 15–20, 22–27 and 29–35 represented minima, the poorest plants having 16–18 chromosomes. Almost complete pollen fertility was noted in the plants with 14 and 28 chromosomes; the plants with intermediate numbers showed varying degrees of male sterility, but most plants with more than 28 chromosomes had good pollen. A detailed study of morphological differences in the two species led to the conclusion that the differences were caused mainly by quantitative differences in chromosome number rather than by specific genes.

Further information of this species relationship was obtained by Müntzing (201) in a study of two "haploid" *D. glomerata* plants, found in an examination of 198 twins. The haploids, which had dimensions less than half their normal sisters, resembled *D. Aschersoniana*. One of the haploids had completely degenerate anthers; in the other, meiosis was regular with 7_{II}, but no functional pollen was produced. The latter plant, however, did give rise to a number of $3n$ plants and one $4n$ when pollinated with *D. Aschersoniana* pollen.

Similar studies of *D. glomerata* have been made by Myers and Hill (218, 219, 220) and Myers (210). In their first report (218) the somatic chromosome numbers of 116 plants were listed as 22% with 27, 59% with 28, 12% with 29 and 7% with 30. In three plants, the number of quadrivalents ranged from one to seven, and the plant averages were 3.3, 3.8 and 4.2. The per cent of IM cells with univalents was less than the per cent of IA cells with dividing laggards. In turn the per cents of I interphase cells and quartets showing micronuclei were lower than expected, on the assumption that no univalents were included in the nuclei. Chromatin bridges and fragments, presumably from crossing over in inversions, were noted in all three plants. Additional data (219) from 12 plants showed a range in quadrivalent frequency from 3.42 to 4.39, with the differences between plants significant. Similar significant differences between plants were noted for per cent of univalents at IM, laggards at IA and quartets with micronuclei; the ranges were, respectively, 3.4–13.8, 6.4–34.0, and 4.4–25.6. Quadrivalent frequency was not correlated with any of these three factors, but the three correlations between them were significant. A further study of eight of these clones, together with one other, was described by Myers (210); collections from six clones one year and nine the next were made from each of three replications growing in the field. In general, the results confirmed those obtained previously, and in addition, a significant clones×years interaction was observed for each of the characters. Chiasma frequency, recorded in one year, showed

a significant negative correlation with univalents at IM and a significant positive correlation with quadrivalent frequency. It was found also that all significant differences between plants in IA laggards could be accounted for by variations in IM univalents, although the per cent of the former usually was higher. To test the hypothesis that these differences were due to genes and/or chromosomal differentiation, Myers and Hill (220) studied first generation inbred plants from eight of the parental clones examined previously. With inbreeding, chiasma frequency was significantly decreased in one of the two families studied in this regard, whereas quadrivalent frequency was significantly increased in two out of eight families. The per cents of IM univalents, IA laggards and quartets with micronuclei were increased two to three times in the inbreds as compared with their parents. Within the families, quadrivalent frequency was negatively correlated with IM univalents, IA laggards and quartets with micronuclei, whereas these correlations in the previous work on the parents had not been significant. The seeds set per panicle under bag and with open-pollination were both negatively correlated with IM univalents, IA laggards and quartets with micronuclei. Since the differences between families in these meiotic irregularities were significant in comparison with the variation within families, it was concluded that the differences were heritable; this suggested the possibility of breeding for strains which have greater regularity of meiosis and consequently greater fertility. A general conclusion from all these investigations (218, 219, 220, 210) was that the unpaired chromosomes appear to be the most important factors in the formation of aneuploid gametes in *D. glomerata*, whereas in other autopolyploids, the quadrivalents which disjoin unequally generally have been assumed to be the most important factors.

On the basis of this cytological behavior in *D. glomerata*, tetrasomic ratios would be expected. Myers (207) obtained such ratios in I_1 and I_2 families of duplex and triplex plants segregating for chlorophyll deficient seedlings.

2. *Self-Fertility and Seed Set*

The work on this subject up to 1937 was reviewed by Vinall and Hein (329). In most of this research (300, 233), it was observed that some plants were completely self-sterile, others were completely self-fertile and, in addition, almost every transition type between these extremes was found.

More recent results on seed set in orchard grass include those of Schultz (280), Myers (208, 209), and Hayes and Schmid (119). In general, sufficient self-fertility was found for the production of inbred lines. Schultz (280) expressed self-fertility as a ratio of F/I, which refers to free

over isolated seed set. The entire group of 38 parental clones averaged 8, 4 and 49 for F/I in the three years when the plants were bagged. Results from the best two years gave a correlation of $r = 0.30$, which only approaches significance. One- and 2-year selfed plants also showed a complete range in self-fertility; in one year a nearly significant correlation ($r = 0.32$) was obtained between the values of the parental plants and their 1-year selfed progenies.

Myers (208) presented data on number of seeds per panicle set under bag and with open-pollination on 46 replicated parental clones and their I_1 progenies. The parental clones ranged from 1.8 to 145.6 selfed seeds per head, and the parent-I_1 correlation ($r = 0.618$) was significant. The average set of 14.9 seeds per panicle for the I_1 was significantly lower than the 40.2 average for the parents, but no simple Mendelian basis for inheritance of self-fertility was apparent from these data. Selfed seed was also expressed as per cent of open seed. Here also the differences between parental clones were significant but the reduction from parent to I_1 was not significant. The inter- and intra-annual variations in seed set under bag were also analyzed by Myers (209) for 51 clones in three replications; the differences between years and the clones×years interaction were significant. The mean square for error was high, with the S. E. of a single determination approaching the magnitude of the mean. It was concluded from a sampling study of these data that only by increasing the number of replications could much increase in precision be obtained. Myers (209) found also that protection of the culms with cotton at the base of the bag was without effect. The number of seeds per head from one, two or four panicles per bag were not significantly different, but eight panicles resulted in a decreased seed set. Earlier flowering panicles set more seed than the later panicles on the same plant.

It was reported by Hayes and Schmid (119) that F_1 crosses did not set seed as well, on the average, as third and fourth generation inbred plants.

3. *Breeding*

Significant differences in winter injury were observed by Myers and Chilton (216) in 59 clones of orchard grass. The correlation between parents and inbreds in this regard was $r = 0.905$, but segregation occurred within inbred progenies, suggesting the possibility of selecting both within and between progenies.

Methods of breeding orchard grass were studied in detail by Schultz (280). Wide variations in winter-hardiness were observed, with the hardy plants tending to produce hardy selfed progenies. The results in the field, however, were not correlated with results from the freezing chamber.

Variation was obtained also in resistance to rust and leaf spot, but uniformly resistant lines were found in the selfed progenies. Reductions in yield, plant height and number of culms were observed in the selfed lines, but some inbred clones were equal to the superior open-pollinated individuals. Yield showed a significant positive correlation with winter-hardiness, plant height and number of culms in the first crop, and a significant negative correlation with erect habit, per cent rust and number of culms in the second crop.

Similar studies by Hayes and Schmid (119) showed that several 2- to 4-year selfed lines were as vigorous as the commercial checks, although the average of all lines showed a reduction in vigor. The 49 F_1 crosses which were studied gave about the same yield as the commercial check. The differences between selfed lines and F_1 crosses in both yield and winter injury were significant.

An interesting breeding experience was described by Nilsson-Leissner (240) for two orchard grass strains, Skandia II and Brage. Each of these varieties originated from one single plant, which was at first propagated vegetatively in locally isolated plots. Seed from the clonal plots was sown in other isolated fields, and the seed from these fields was sown in turn in adjacent fields, thus establishing large seed propagations containing plants of two or three successive inbred generations. A comparison was made between the yield of the I_1 and the yield of the composited I_2–I_4. Trials of Skandia II were repeated in 12 years and Brage in 10; most trials were harvested twice during two consecutive harvest years. With Skandia II, the I_1 yielded 31.140 kg. per hectare and the I_2–I_4 32.960; the difference was significant. A similar comparison (36.710 and 38.120) was found with Brage, but here the difference was not significant. The combined mean yield difference, however, was highly significant. These differences were explicable, not on the basis of seed quality or treatment, but by the fact that many weak and abnormal plants appeared to be suppressed in each successive generation; this resulted in a continuous selection for strong and normal types and caused an improvement in the mean vitality of the strains.

Altogether, 11 strains of orchard grass were listed (121, 122) in the Uniform Grass Nurseries. The three Aberystwyth strains and the Brage variety from Sweden are probably the best known strains in this country, although no strain has received widespread increase here. A need has been felt for more winter-hardy types as well as types that would mature later and produce good aftermath growth.

VI. Brome Grasses

1. *Cytological and Genetical Investigations*

Up to 1937 chromosome numbers had been reported for 32 species of brome grass (329), and since then numbers for several other species have been described (36, 234, 229, 226, 87, 301, 172). In the case of *B. inermis* Stählin (298) reported 42 chromosomes and Avdulov (34) reported 56. These numbers were confirmed by several investigators and, in addition, Nielsen (226) reported a race with 70 chromosomes. Meiosis was found by Knobloch (170) to be irregular in 42 and 56 chromosome races of *B. inermis;* lagging chromosomes were frequent.

Johnson and Miller (147) studied per cent of total carotenoid pigments, β-carotene and total chlorophyll in 76 clones of Parkland brome grass. The clones differed significantly in regard to these characters as well as in green weight yield. There was no significant relationship between yield and either chlorophyll or pigment concentration.

Tsiang (319) found highly significant differences between clones in reaction to leaf spot, *Selenophoma bromigena.* Many of the first generation inbreds were more resistant to the leaf spot than commercial checks, and the reaction of the inbreds was significantly correlated with that of their respective parents. Significant differences were found also for a number of agronomic characters, including yield of hay, basal diameter, vigor of recovery and leaf width, and here again the selfed progenies were highly correlated with their parental clones. Similar results were reported for heat resistance, drought resistance and β-carotene content. Spectographic analysis of the 36 clones also indicated differences in Mg and K.

Harlan (114) concluded that *B. carinatus* should be classed as facultatively cleistogamous, since both chasmogamous and cleistogamous florets were produced on the same plant. Optimum flowering conditions favored the former and adverse conditions the latter.

2. *Species Hybrids*

Cugnac (83) and Cugnac and Camus (86), in an extended series of papers, described natural hybrids of brome species and conducted artificial hybridizations to verify their conclusions. A wide range of types was secured but mostly with European species which have little agronomic value in this country. The hybrid *B. hordaceus*×*B. mollis* was found by Nilsson (234) to be as fertile as its parents; the range of segregation in F_2 and later generations suggested that the two parents should be regarded as a single species. Additional interspecific hybrids were reported by Ullman (328), Hertzsch (123) and Knowles (172). The last named author attempted crosses with 13 species, which were representative of 5 sections

of the genus. Hybrids were obtained from five combinations and the extent of chromosome pairing in the F_1 plants paralleled the extent of morphological similarity of parent species. The hybrids were characterized by a large proportion of univalent chromosomes.

Stebbins and Tobgy (302) obtained hybrids between four 8*n* strains of *B. carinatus* and also between three of these and 6*n* *B. catharticus*. The hybrids were vigorous in growth but they showed varying degrees of sterility, which was apparently chromosomal rather than genic. Agronomic value depends in part on the elimination of this sterility in later generations. Another hybrid was reported by Stebbins, Tobgy, and Harlan (303) between the 12*n* *B. arizonicus* and *B. carinatus;* it was completely sterile with trivalents, bivalents and univalents at meiosis. A general conclusion from all this work is that the parents were allopolyploids.

3. *Breeding Investigations*

It was suggested by Popravko (259) that the easiest and quickest method of building up new varieties in *B. inermis* was mass selection. Family selection can be applied later, but inbreeding was regarded as necessary for final refinement. Hübner (137) showed that dark color in the leaves was positively correlated with protein content. The importance of selfed lines was emphasized in the work of Hayes and Schmid (119) and Tsiang (319). The former authors found that 11 I_2 lines of *B. inermis* yielded about the same, on the average, as the commercial check, and about half of the lines were significantly more resistant to leaf spot than the commercial variety. Several crosses between I_1 clones yielded from 126.5–220.9% of the commercial check. The work of Cook (73) suggested that the more drought resistant strains had greater "total axial root length."

Several distinct strains of *B. inermis* have developed in the United States, following the importation of different seed lots. It was suggested by Newell and Keim (224) that these could be placed into two general groups, called the northern and southern types; the former probably arose from Russian introductions and the latter from Hungarian. Under Nebraska conditions, the southern type strains produced more vigorous seedlings, were more tolerant of drought and heat, possessed more vegetative vigor and were more productive than the northern types. Since the southern types produced a larger proportion of their total forage in early spring, they were designated as "early" in contrast to the "late" northern strains. Farther north, where winterkilling is a factor, the northern types may be more satisfactory, but here it has been noticed that the sod-bound condition of brome grass may be troublesome. The Parkland strain, the

origin of which was described by Kirk (166) and Stevenson (304), was selected mainly for bunch-type growth, which, in turn, helps to prevent the sod-bound condition.

The extent of variation in *B. mollis* was studied by Knowles (171). Strains from a number of sources were included but the only strain difference which he found was between an early interior ecotype and a later coastal ecotype. Within collections, however, wide differences in morphological appearance and physiological behavior were noted.

VII. Ryegrasses and Fescues

1. *Cytological and Genetical Investigations*

In 1938, Jenkin and Thomas (143) distinguished six species of Lolium, all with $2n=14$. Previously meiotic behavior had been studied by Peto (255) in two plants of *L. perenne* and one F_1 plant of *L. perenne* × *L. perenne* var. *multiflorum*. At IM, all chromosomes were associated as bivalents, and chiasma frequencies for the three plants were 1.81, 1.62 and 1.59 per bivalent, respectively.

Chromosomal behavior at meiosis was studied by Myers (205) in 19 plants of *L. perenne*. Significant differences were found in total number of chiasmata, number of terminal chiasmata and number of open bivalents per sporocyte. Also at IM, varying proportions of the cells showed univalents, non-orientated bivalents and loosely attached bivalents. There were significant negative correlations (a) between total chiasma frequency and per cent of IM sporocytes with univalents and (b) between both total and terminal chiasma frequency and per cent of sporocytes with loosely attached bivalents. The univalents and loosely attached bivalents at IM gave rise to laggards at IA. Four plants had no laggards at IA, 13 others showed varying per cents up to 2.5, whereas one other had laggards in 41.6% of the cells. In 633 sporocytes in which lagging did not occur, seven chromosomes were seen in all IA groups. Varying per cents of the quartets had micronuclei; most of the micronuclei arose apparently from IM univalents and loosely attached bivalents, but in certain plants micronuclei arose from some other source in addition. The presence of dicentric bridges and acentric fragments indicated that 13 of the 19 plants were heterozygous for inversions. Pollen abortion varied from 4.3% to 78.8% in 11 plants, but factors in addition to the meiotic irregularities studied were apparently conditioning this behavior.

Colchicine treatment of *L. perenne* seeds was found by Myers (204) to be successful in inducing tetraploidy. The most satisfactory treatments from the standpoint of seedling survival and incidence of tetraploidy were for 24 hours with 0.2 and 0.4% solutions; these resulted in 12 out of 29

and 11 out of 28 plants, respectively, with 4*n* tissue. Hill and Myers (125) reported considerable difficulty in isolating pure 2*n* and 4*n* sectors from chimeras induced in this way. Mixtures of the two types of tissues persisted in some clones through eleven vegetative generations. There was no observable tendency for either 2*n* or 4*n* tissue to be eliminated by the other. In chemical analyses of a few of these sectoral chimeras, Sullivan and Myers (314) and Sullivan (313) found that several environmental factors greatly influenced the results and in no case were the differences of great magnitude. In general, however, the higher chromosome number was associated with higher moisture, higher soluble constituents and lower structural constituents. The meiotic behavior of 12 of the same 2*n*–4*n* pairs growing in the field and two others growing in the greenhouse was studied by Myers (215). In the 4*n* sectors there was a delay of chromosome contraction relative to onset of IM and, in two clones, absence of orderly orientation on the equatorial plate prior to IA. In some pairs, chiasma frequency in the 4*n* sectors was lower than in the 2*n* while in other pairs the difference was not significant. Three major types of meiotic irregularity occurred: (a) unequal disjunction of quadrivalents, (b) incomplete disjunction of quadrivalents, resulting in laggards, and (c) IM univalents, which tended to lag at IA and form micronuclei at I and II T. Variations in behavior of the 4*n* sectors were not significantly correlated with those of the 2*n*. Tetraploid plants were obtained also by Shalygin (282) in both *L. perenne* and *L. multiflorum*. The tetraploids were characterized by much lower fertility, more compact growth habit, darker green color and later heading than the diploids.

The cytological behavior of 3*n* *L. perenne* and its progeny also was reported by Myers (212, 214). In the two 3*n* plants studied, the distribution of the unpaired chromosomes at IA was apparently at random. One of the plants showed evidence of non-homologous association at prophase and of improper disjunction of trivalents at IA. As judged by the chromosome numbers of progeny plants, it appeared that haploid eggs functioned in excess over the aneuploid types, particularly those with three or more extra chromosomes.

Complementary factors for red color at the base of the tiller sheath were reported by Hills (127), and it was suggested that the non-red types would be useful in measuring natural crossing.

Several reports have been made on self-fertility in Lolium and Festuca, but most of these were reviewed in the Yearbook (329).

2. *Interspecific and Intergeneric Hybrids*

The genera Lolium and Festuca have proven especially amenable to both interspecific and intergeneric crosses. Much of this work was sum-

marized by Vinall and Hein (329), but several contributions have been made since then.

In 1938, Jenkin and Thomas (143) described 11 of the 15 possible hybrids between the six species of Lolium, all $2n=14$. Some failure of chromosome conjugation was observed in all hybrids except one, and inversion bridges were noted in six hybrids, indicating the importance of these chromosomal aberrations in species delimitation. Later, Jenkin and Thomas (144) gave a more detailed report of the cytological behavior of two hybrid plants, one $2n$ and one $3n$; these were obtained from the cross between *L. loliaceum* and *L. rigidum*, both of which occur naturally in Wimmera ryegrass. It was concluded that there is little structural difference between the chromosomes of these two species.

Although intergeneric hybrids of Lolium and Festuca have been known for many years, the first intensive cytological investigations of such plants were those of Nilsson (232) and Peto (255). Nilsson found from 7 to 12 bivalents in a natural, 28-chromosome hybrid of *F. rubra* ($2n=42$) and *L. perenne;* this was considered evidence that pairing took place not only between Lolium and Festuca chromosomes but also between chromosomes in the Festuca genome. *F. rubra* was regarded as both an auto- and allopolyploid, with one of its 7 chromosome genomes homologous with the Lolium genome. Peto (255) studied the hybrids *L. perenne* ($2n=14$)$\times$*F. pratensis* ($2n=14$), *F. pratensis*$\times$(*L. perenne*$\times$*L. perenne* var *multiflorum*), *L. perenne*$\times$*F. arundianacea* ($2n=42$), and supposed natural hybrids (*F. loliacea*). The diploid Festuca-Lolium hybrids were unique in that there was perfect pairing between parental chromosomes in the F_1. Some autosyndesis between the *F. arundinacea* chromosomes was observed in hybrids involving this species. Additional Lolium-Festuca hybrids have been reported by Winkler (347), Åkerberg (5), Bulaševič (52) and Jenkin (141). Certain of the segregates from these crosses appear superior to their parents in such characters as early development, yield and winter-hardiness. Because of the close relationship between Lolium and Festuca, as evidenced by crossing experiments, Jenkin (141) concluded that they probably were derived from a common prototype.

Nilsson (236) described the hybrid *F. arundinacea*$\times$*F. pratensis*, which formed $7_{II}+14_{I}$ in most cells.

3. *Breeding Investigations*

The breeding work with Lolium and Festuca has resulted in several improved types, principal of which are the Aberystwyth (142) strains. The value of inbreeding in *F. elatior* was emphasized by Hayes and Schmid (119); one inbred line yielded significantly more than the check in both I_1 and I_2 generations, and some of the lines were highly winter-hardy

despite the reduction of vigor. Eight strains of *F. elatior*, 8 of *F. rubra*, 2 of *F. ovina*, 8 of *L. perenne* and 2 of *L. multiflorum* have been included in the Uniform Grass Nurseries (121, 122); one of these strains, Alta fescue, is registered (134), and considerable seed is now being produced.

VIII. Southern Grasses

1. *Cytological Investigations*

Most of the grasses, both native and introduced, now included in improved pastures in southeastern United States belong to the tribe Paniceae (58); comparatively few of these species have been studied cytologically. The chromosome numbers for five species of Paspalum were listed in the Yearbook (329). Burton (57) reported the numbers for seven additional species, and he concluded that 10 is the basic number, with four species $2n$, six $4n$, one $8n$ and one $16n$. All the most promising pasture species are $4n$. From a study of eight genera of the Andropogoneae, Paniceae and Chlorideae, Krishnaswamy (177) concluded that although the basic numbers 9 and 10 prevail in these families, they probably were derived from forerunners with the basic number of 6 or 12. Later Burton (58) listed numbers for eight more species of Paspalum and 19 other collections including species of Panicum, Pennisetum, Digitaria and Axonopus. Among the Paspalum species were one $3n$ and one $10n$, as well as two species with 24 and 48 chromosomes, respectively. The latter species confirmed Krishnaswamy's (177) idea of a basic number of either 6 or 12. Additional chromosome counts in Paspalum were reported by Saura (273, 274).

In Panicum also Burton (58) reported a polyploid series, ranging from $2n=18$ to $2n=72$, with 9 as the basic number; intraspecific races were demonstrated for two species. Church (68) reported additional chromosome numbers in Spartina, Andropogon and Panicum: an Atlantic coast $4n$ *Panicum virgatum* was contrasted with the western $4n$ and $8n$ forms.

2. *Species Hybrids*

Hybrids within the genus Paspalum were described in 1943 by Burton (61). Five unusual plants were obtained from a single individual of *P. urvillei* (V) which had been grown about 50 feet from a planting of *P. malacophyllum* (M). The five plants turned out to be very similar to controlled V×M hybrids which were made later. All five plants were male sterile and when pollinated with *P. urvillei* and *P. dilatatum* (D) pollen gave rise to 57 (V×M)×V hybrids, 100 (V×M)×D hybrids, and several hundred plants identical with the V×M parent. All hybrids

were vigorous and, when cut only once, in the fall, they produced over twice as much dry matter as their parents. Several morphological and physiological characters showed varying degrees of dominance in the hybrids. Of principal interest from the breeding standpoint was the ergot resistance, which was complete in the V×M hybrids and partial in certain (V×M)×D hybrids. Cytological examination of the (V×M)×D hybrids showed 60 or 60+ chromosomes, indicating the presence of one set from each of the three parent species. All of the hybrids were so highly sterile that propagation by common seeding methods was not feasible. It was observed, however, that the few progenies obtained from the V×M and the (V×M)×D hybrids were remarkably uniform and similar to their hybrid parents.

The hybrid between Napier grass (*Pennisetum purpureum*) and Merker grass (*P. Merkeri*) was studied by Parris and Ripperton (253) in relation to the eye-spot disease caused by *Helminthosporium sacchari*. Napier grass is susceptible to the disease and Merker grass is resistant. Both resistant and susceptible types were found among the open-pollinated progeny of Napier and among the species hybrids, but all plants from Merker selfed were resistant.

Hybrids in the genus Pennisetum also were described by Burton (63). A total of 134 F_1 hybrids was obtained using the perennial Napier grass, *P. purpureum*, as female, and two late maturing strains of annual cattail millet, *P. glaucum*, as male. Forty-nine of the hybrids were small and chlorophyll deficient; all others were normal green and resembled the Napier grass parent. Many of the green hybrids yielded more, and eight of them branched more, than their Napier parent. All hybrids were highly sterile, with shrivelled anthers which failed to shed pollen. All those examined cytologically proved to be triploids with 21 chromosomes, the combined *n* numbers of the two parents. The hybrids were not as winter-hardy as Napier grass, but their increased vigor suggested that some may have superior value in the tropics.

3. *Improved Strains*

The superiority of certain strains in several Southern grass species has been recognized during the past few years and some of these strains are being increased rapidly. Vegetative propagation is used with some of the sterile types. For example, in the Yearbook (329) Tift Bermuda grass is listed as a vigorous, fine-stemmed type selected by J. L. Stephens in Georgia. Recently a hybrid clone has been named Coastal Bermuda (59), and because of its superiority in several regards, increase plantings are maintained at the Georgia Coastal Plain Experiment Station for supplying farmers with stolons. Likewise, eyespot-resistant and winter-hardy

strains of Napier grass were selected both in Florida (267) and Georgia (54).

Burton (65) described a strain test of 24 types of Dallis grass coming from South Africa, Uruguay, Australia, and the principal seed producing areas in the United States. Significant differences between strains were observed in forage yield, anthracnose resistance, self-fertility, heading date, ergot resistance and longevity. None of the strains was outstanding in all characteristics but some appeared worthy of recommendation.

IX. Prairie Grasses

1. *Cytological and Genetical Investigations*

The chromosome numbers for many of the species which are common to the Great Plains and intermountain regions of North America were listed in the Yearbook (329). Since that time, several important additions have been made by Nielsen and Humphrey (229), Nielsen (226), Church (68), Stebbins and Love (301), and Fults (103).

A thorough analysis of chromosome complements in Bouteloua was reported by Fults (103) for 114 plants from 85 seed sources; the plants represented 18 biotypes belonging to seven species. The numbers ranged from $2n=21$ to $2n=98$. The two species investigated most completely were blue grama, *B. gracilis*, and side-oats grama, *B. curtipendula*. The former had $2n$ numbers of 28, 35, 42, 61, and 77; the latter had $2n$ numbers of 28, 35, 40, 42, 45, 56, 70, and 98. Six biotypes of blue grama were distinguished, but these types showed no definite relation to the chromosome complements; one biotype often had two or more complements. A similar lack of relationship was noted for five biotypes of side-oats grama.

Diploid and tetraploid races of *Agropyron cristatum* were first distinguished by Peto (256). He found that the 28 chromosome type tended to be taller and more variable than the 14. One aneuploid plant with 29 chromosomes also was found. Likewise Myers and Hill (218) found one plant out of six with approximately 31 chromosomes, while the rest had the normal $2n=28$. Meiotic irregularities, similar to those in orchard grass, were described by Myers and Hill (218). Only one plant out of 33 was found to be aneuploid by Murphy (203). Three intra-specific races with $2n=14$, 28 and 42 were found by Araratian (13) in *Agropyron cristatum*. When this species grew with others, there were also found $2n=48$ intermediate forms, which possibly were natural hybrids. A giant wheatgrass from Nevada was described by Robertson and Weaver (268) as a tetraploid form of *Agropyron spicatum*.

Johnson and Rogler (145) made a cyto-taxonomic study of the natural hybrids between *Oryzopsis hymenoides* and *Stipa viridula*. Chromosome numbers of the two parents were 48 and 82, respectively, and the hybrids

had 65 as expected. Normal pairing and disjunction was observed in both parents, but the hybrids invariably showed laggards at IA and micronuclei at telophase. The hybrid pollen was essentially 100% sterile, and the plants set no seed. A large number of morphological characters were measured in the hybrid, and some showed distinct dominance.

The variation in *Panicum virgatum* was reported by Cornelius and Johnston (81) and by Nielsen (227). The former authors recorded the variation in several agronomic characters for 34 accessions collected throughout the Great Plains region. Rust caused by *Uromyces graminicola* appeared to be a major factor in limiting growth, but some strains appeared to be completely free from it. Under Kansas conditions the accessions from the southern areas appeared to be superior to those from northern areas in regard to such characters as height of stem, lateness of maturity, forage yield and resistance to rust. Nielsen (227) studied 59 isolates from seed and clonal stocks taken from an area extending from Wisconsin and Montana south to Arkansas and Arizona. Previously Church (68) had described $4n$ and $8n$ races of *P. virgatum*, but Nielsen (227) reported additional numbers to make up a polyploid series of 18, 36, 54, 72, 90, and 108. No regional segregation of races on the basis of chromosome number was observed. A detailed statistical study of 28 of the 59 isolates was made for 15 quantitative characters. About the same per cent of the comparisons were significantly different when making comparisons (a) between isolates with the same chromosome numbers (58.6%) or (b) between isolates with different chromosome numbers (66.4%). By use of linear regression, plant height was shown to be positively related to size of the aerial vegetative organs but there was no relation between plant height and size of floral or underground parts. Normal frequency distributions were calculated for each of the 15 characters of four isolates; for some characters wide differences separated certain of the lines, whereas for others there was almost complete overlap.

Olmsted (248) reported wide variation among 12 geographic strains of side-oats grama in respect to photoperiodic response, as manifested in morphological characters.

2. *Breeding Investigations*

A study of the effect of selection on variability in little bluestem, *Andropogon scoparius*, was reported by Anderson and Aldous (12). Leaf area was found to be the best single measure of quality and yield of forage. Wide variations between plants in total leaf area were noted, and it was concluded that selection, even in open-pollinated populations, tended to increase uniformity. Similar conclusions were reached for basal diameter, plant height and date of maturity. Seed set was reduced by selfing,

but the amount attributable to genetic causes was not determined definitely. Vigor was not affected seriously, if at all, by inbreeding. Significant correlations were found between most of the characters studied.

A similar study for big bluestem, *Andropogon furcatus*, was reported by Law and Anderson (178). Here also a marked increase in vigor and a decrease in variability were obtained by selection in open-pollinated lines. Inbreeding was accompanied by a marked and progressive loss of vigor, but some lines showed no reduction in comparison with their open-pollinated sibs. Seed set was greatly reduced following inbreeding, probably due to genetic factors. Significant inter-annual correlations were found for each of the characters studied, and leaf area was significantly correlated with both number of culms and plant height.

The early work in Canada on crested wheatgrass, *Agropyron cristatum*, gave rise to several improved types. The strains Grazier, Mecca, Fairway, and Fyra were listed in the Yearbook (329). Several breeding technics had been utilized in the development of these lines, but in 1937 it was reported (168) that selection within inbred lines had been largely abandoned in Canada because of the enormous loss of vigor brought about by inbreeding. A similar conclusion was reached by Murphy (203) as a result of a two-year study of variability and the effects of self-fertilization. He found that both the 4*n* forage type and the 2*n* Fairway type were highly unfruitful when selfed. Only one plant appeared to be self-fertile, but its selfed progeny in turn were unfruitful. Significant differences in yield, plant height and root rot injury were found among plants of the forage type. Plants of the Fairway type also differed significantly in yield. The latter type was superior to the forage type in yield and resistance to root rot. Knowles and Horner (174) also obtained a low seed set, less than 0.10%, from selfing *A. cristatum* under kraft or glassine bags; somewhat more seed was obtained under cotton cages. Hot water emasculation at 48° C. for one minute gave satisfactory results.

A wide range of variability in buffalo grass, *Buchloe dactyloides*, was described by Wenger (333); selection for yield, quality, seed productivity and disease resistance appeared promising.

Improved strains of Russian wild-rye, *Elymus junceus*, developed mainly by mass selection, were described by Rogler (269); variations in self-fertility and in reduction in vigor due to inbreeding also were noted.

Rogler (270) investigated the response of geographical strains of several grasses to low temperatures. In field tests at North Dakota, the northern races of *Bouteloua gracilis*, *B. curtipendula*, *Panicum virgatum* and *Andropogon furcatus* were more winter-hardy than the southern races. No significant differences in winter-hardiness were observed between races of *Agropyron cristatum*, *A. Smithii* and *Bromus inermis*. Similar results

were obtained from artificial freezing tests of seedlings, except in the case of *A. Smithii*, where significant differences were obtained although they had not been observed in the field.

Different degrees of dormancy were found by Coukos (82) among accessions of several native grasses.

X. Sudan Grass

All chromosome counts in normal Sudan grass have shown $2n=20$. The only deviations from this have been the colchicine-induced tetraploids found by Salomon (272) and others. Salomon (272) obtained a large number of seeds on both of his $4n$ plants; these seeds were larger in size than those from diploids, and they gave rise to exclusively $4n$ progeny. Meiosis in the $4n$ plants appeared to be normal. In the colchicine-induced tetraploids obtained by Randolph (unpub.), fertility was reduced in comparison with the diploids. Randolph was successful in crossing $4n$ Sudan with Johnson grass, $2n=40$, and the hybrids were highly fertile, giving rise to many segregating types in F_2. Meiosis was studied by Atwood (unpub.) in some of Randolph's tetraploids. At diakinesis, averages of 4.69 quadrivalents and 0.21 trivalents per cell were observed for all chromosomes except the nucleolar chromosome; the latter formed quadrivalents in 89% of the cells examined. Triploid plants were obtained from the cross between $2n$ and $4n$ plants, and in two of the triploids averages of 8.17 and 8.39 trivalents per cell were observed at IM. Using some of this same material, Garber (104) found that at most temperatures, the roots of $4n$ Sudan elongated faster than those of $2n$.

A total of 13 characters, each determined by single recessive genes, were described by Rangaswami Ayyangar and Ponnaiya (264). Three of the mutants were rare but were common to Sudan and *Sorghum Dochna*. This was considered evidence that Sudan contributed to the ancestry of the cultivated sorghums, particularly *S. Dochna*. Garber and Chilton (106) observed several types of leaf spot on Sudan grass; the inheritance of the tendency to spot was not simple, and no causal organisms were isolated.

A principal drawback to the use of Sudan grass for forage is its high content of the cyanogenetic glucoside, durrin, which on hydrolysis releases HCN, making the forage poisonous to ruminants under certain conditions. In 1938 Coleman and Robertson (72) reported that differences between various lines of Sudan in amount of HCN were heritable. Some correlations were noted between high HCN and both non-glossy leaves and purple-tipped seedling leaves. Differences between inbred lines in HCN also were described by Schieblich (275), and he suggested that low HCN was recessive. Likewise in sorghum, Franzke, Puhr and Hume (101)

found that low HCN was recessive and that the F_2 and F_3 segregations indicated only one or two major genes, with perhaps a number of modifiers. The low HCN sorghum lines had about the same amount of HCN as Sudan grass. The inheritance of HCN in Sudan grass was investigated further by Hogg and Ahlgren (130). Eleven crosses between Sudan plants, high and low in HCN, were made in the field during the summer. When the F_1 plants were tested in the greenhouse during the following winter, they were found to be intermediate between the parents in HCN. When the F_2 seedlings likewise were tested in the greenhouse, some plants in most of the families approached, if not surpassed, their parents in HCN content. The total F_2 population of 890 plants had a distribution approaching the normal curve, with no tendency to be bimodal. In four crosses between parents low in HCN, the F_2 distribution was essentially the same as that of the parents. The increased vigor of F_1 and F_2 plants was not accompanied by increases in HCN. All these data suggested that HCN content was definitely heritable but that more than a single pair of genes were involved.

A marked reduction in vegetative vigor and, still more, in seed production accompanied inbreeding in 20 inbred lines tested by Hogg and Ahlgren (130). By repeated selection, however, it was possible to develop strains low in HCN, while retaining a high degree of vegetative vigor. Natural crossing was found by Hogg and Ahlgren (130) to average 6.7%, but the results of Garber and Atwood (105) indicated that under some circumstances it might be much higher. In three successive years averages of 76.4, 18.2 and 34.4% crossing were obtained. In the third year, when 3,999 progeny plants were tested, highly significant differences were found between the 6 crosses and the 4 pollination periods during the season.

A recently introduced selection of Sudan grass, named Tift, was described by Burton (60) in Georgia. A single individual which combined the disease resistance of Leoti sorghum and the vegetative characters of Sudan was selected in a population of about 30,000 (Sudan×Leoti)× Sudan F_2 plants. This gave rise by selfing to the Tift strain, which is disease resistant and high yielding, both as hay and pasture. Tift Sudan has about twice the HCN content of common Sudan, but in the preliminary grazing trials no cases of poisoning were encountered. Quinby and Karper (261) described another new strain, Sweet Sudan, which also arose from crosses with Leoti sorghum followed by selection and repeated backcrosses to Sudan. The sorghum characters, sweet stalk, juicy stalk, non-shattering seed habit, distinctive sienna glume color and resistance to bacterial diseases, were combined with the growth habit and production of common Sudan.

XI. Miscellaneous Grasses

Twelve genera of arctic grasses, particularly those from Spitsbergen, were examined cytologically by Flovik (98). In his summary of the chromosome counts for all arctic grasses, it was shown that 80% were polyploids and this, together with the factors of hybridity and extreme environmental conditions, was considered a basis for the common occurrence of vivipary in the arctic types. Most of the evidence from these studies suggested 5 as the basic chromosome number for the Gramineae. Further investigations of Deschampsia and Aira by Hagerup (110) showed that the tetraploid species had wider geographical and ecological ranges than the diploids, they were more vigorous and they tended to show more vivipary. Cytological studies were made by Strelkova (311) on the species of Alopecurus in the U.S.S.R. This information was related to the taxonomic classification of the species and to their geographical distribution. The Mediterranean region was considered the primary center of origin for the genus, with the mountains of the Caucasus and of Central Asia representing secondary centers. Johnsson (149) also investigated cytological relations in Alopecurus; among the 12 species dealt with, 7 were diploids, 3 tetraploids and the other two high polyploids with 112–122 chromosomes. In the hybrids between the different species, pollen sterility due to irregular meiosis was the rule in all except one cross. In the species *A. myosuroides* Johnsson (150) found that ordinarily the seven chromosome pairs behave regularly in meiosis, but following inbreeding the following aberrations sometimes occurred: absolute asynapsis, partial asynapsis, premature centrosome division, stickiness, hypercontraction, retarded meiosis, polymitosis, syncyte formation, male sterility and female sterility. Relatively simple factorial inheritance was found for most of these aberrations.

It was shown by Simonet (284, 285) and Pardi (252) that *Agropyron junceum* consisted of two races, an Atlantic form with $2n=28$ and a Mediterranean form with $2n=42$. Ostergren (249, 250) found that natural hybrids between *A. junceum*, $2n=28$, and *A. repens*, $2n=42$, had either $2n=35$ or $2n=49$. The 35 chromosome hybrids were intermediate, whereas the 49 chromosome plant resembled *A. junceum*, presumably because it received two gametic sets from this parent. All hybrids were highly sterile. Meiosis in the pentaploid hybrids showed pairing ranging from $9_{II}+17_{I}$ to $13_{II}+9_{I}$. The hybrid *Elymus riparius*×*Agropyron caninum* was found by Cugnac (84) to have heads with a mixture of both multiple and single spikelets, characteristic respectively of the two parents, as well as heads with only single spikelets. The carbohydrate reserves of the two parents were shown by Cugnac and Belval (85) to be respectively elymoside and triticine; the hybrid had only the latter. Many papers

have been published recently on the intergeneric hybrids between Triticum and Agropyron; the objective in most of this work has been the development of a large seeded forage crop. So far the practical results appear promising, and the more theoretical aspects may have a wide application. A review of this work in Russia, Canada and the U.S.A. was presented in 1943 by Smith (291).

From a cytotaxonomic survey of the genus Agrostis, Sokolovskaya (297) concluded that the Mediterranean region was probably the original center of origin for the genus and that Eastern Asia was a secondary center for forms with the higher number of chromosomes. Breeding populations of *A. alba* and *A. tenuis*, studied by Stuckey and Banfield (312), showed wide variations with many intermediate types. Chromosome counts ranged from $2n=28$ to $2n=42$, but there was no correlation between morphological type and chromosome number.

Genetic studies of *Setaria italica* by Li, Meng and Li (187) demonstrated three genes for seed coat color, two for endosperm characters and two for earhead types. Later Li, Pao and Li (185) found a constant association of $9_{II}+9_{I}$ in the triploid hybrids from the crosses *S. faberii* × *S. italica* and (*S. italica* × *S. viridis*) × *S. faberii*. Sterility in the hybrids was very high. In the hybrid *S. italica* × *S. viridis* Li, Li and Pao (186) found nine pairs of chromosomes, the same as in the parents, but the F_1 pollen was 70% sterile. The F_1 resembled the *viridis* parent in all except one of the qualitative characters studied. In the F_2 generation, 15 genes and 3 linkage groups were identified.

XII. Alfalfa

Alfalfa is generally regarded as one of the most important forage crops. The literature on it has been extensive, but not until recently have there been many papers describing work in alfalfa cytology, genetics and breeding. The important contributions which were published prior to 1937 were reviewed by Tysdal and Westover (326); more recently additional summaries were presented by Tysdal (321) and Tysdal, Kiesselbach and Westover (325). Since the crop is of major importance, however, and since the principals which have been evolved with it may have application to other forage crops, it seems worthwhile to present brief reviews of the more pertinent recent literature and to indicate present and possible future trends in the research development.

1. *Cytological Investigations*

The sequence through normal macrosporogenesis, embryo sac development, fertilization and embryo development was described by Cooper (75) for *Medicago sativa*. Cooper also observed that less than

half the ovules showed signs of fertilization despite an abundance of pollen tubes. A similar account of normal development was presented by Farley and Hutchinson (96) for plants descended from crosses involving *M. media* and *M. falcata*. Further studies of seed failure were made by Cooper, Brink and Albrecht (78), using ten plants of alfalfa, five of which were high seed producers and five of which were low seed producers. When the flowers were selfed by tripping, two distinct causes for seed failure were noticed. In the first place, only part of the ovules became fertile, 35% for the high seed producers, and 25% for the low. The fertile ovules tended to be at the stylar end of the ovary. The second cause was embryo abortion. Among the high seed producers, an average of 3.1 ovules per flower was fertilized, whereas only 1.25 seeds per flower were found at maturity. Likewise among the low seed producers, the average number of fertile ovules was 2.5, but only 0.07 seeds per flower matured. Much of the embryo abortion was at an early stage of development. When histological studies indicated that embryo and ovule collapse followed an abnormal growth of somatic integument tissue adjacent to the embryo sac, the term somatoplastic sterility was proposed by Brink and Cooper (43) to describe this condition. Later, Brink and Cooper (44) gave a more detailed description of this phenomenon, pointing out that the first sign of abnormal development in collapsing ovules was the excessive growth of cells of the inner integument adjacent to the megagametophyte on the funicular side of the seed in the region of the vascular bundle. The next step was breakdown of the endosperm and the ovule eventually aborted. The critical feature of this type of seed failure was the inability of the endosperm to keep pace with growth in surrounding somatic tissues of the young seed.

Histological studies by Brink and Cooper (42) showed that still another type of behavior, partial self-incompatibility, was involved in reduced seed set. By observing pollen tube growth following comparable self- and cross-pollinations on seven plants, it was found that the male gametophytes, while not impotent on the individual from which they arose, were less effective in accomplishing fertilization than were unrelated male gametophytes. Complete data from this study were presented later by Cooper and Brink (77). Following selfing, only 14.6% of the ovules became fertile, as compared with 66.2% following crossing. The seven plants showed significant differences in proportion of fertile ovules and less variability was observed following crossing than was observed following selfing. The lower fertility of ovules following selfing was conditioned by (a) slower growth of pollen tubes, (b) fewer basal ovules being fertilized, and (c) a greater tendency for the pollen tubes to frequently pass directly by the micropyles. It was shown also (44, 77) that, following

selfing, 34.4% of the fertile ovules collapsed during the first 144 hours after pollination as against only 7.1% collapse following crossing. These two factors, proportion of ovules becoming fertile and proportion collapsing, meant a net fertility six times as high in the crossed as in the selfed series at the end of 144 hours, and they account for most of the difference in seed production following selfing and crossing.

The chromosome number for *M. sativa* has been reported many times as $2n=32$ (see review by Senn (281)), and the same number has been found for most other species used in breeding work in this country. The numbers 16 and 14 have been reported for other species in the genus, suggesting that the types with $2n=32$ are tetraploids. Sinskaya (286) considers *M. hemicycla* as the progenitor for the whole group. This species is found in the Caucasus and in Afghanistan; it includes both $2n=16$ and $2n=32$ forms.

By means of heat treatment during early embryo formation, Cooper (76) obtained a 64 chromosome plant of *M. sativa*. It was similar to 32 chromosome plants in general habit but had somewhat stouter stems and larger leaves and flowers. From three to six quadrivalents were seen at diakinesis and laggards were present during both the I and II divisions. The 64 chromosome plant was highly sterile, but on selfing three 64 chromosome progeny were obtained.

Colchicine-induced tetraploids ($2n=64$) and triploids ($2n=48$) were reported by Nilsson and Andersson (237, 238). The tetraploids from pure *sativa* showed increased growth compared with the diploids, but tetraploids from Ultana alfalfa, derived from the cross *M. sativa*×*M. falcata*, did not show increased growth. Both triploids and tetraploids were derived from crossing flowers on supposed tetraploid branches. The triploids showed very luxuriant growth, and pollen fertility in both $3n$ and $4n$ plants was high but seed setting in both was low. A similar study of $2n$, $3n$ and $4n$ types was described by Julén (154); in this case the $3n$ showed optimum vegetative vigor. About the same frequency of univalents and multivalents was observed in the three types. Further comparisons of $2n$ and $4n$ alfalfa were described by Schröck (279). The $4n$ clones were almost sterile, they yielded less and had fewer shoots than the $2n$, but they were much superior to the $2n$ in winter hardiness.

Aneuploidy was found by Skovsted (288) among twin plants of *M. sativa*. In one pair, both members had $2n=33$, whereas in another pair, the two plants had 31 and 32 chromosomes, respectively.

2. *Genetical Investigations*

In the segregating generations from the crosses *M. falcata*×Hairy peruvian and *M. falcata*×Hardigan, Burton (56) observed that most of

the characters studied gave normal distributions, indicating multifactorial inheritance. When correlations were computed between several of the characters, it was found that high yield was associated with the following characters: large leaflets, high seed yield and branched root system. The prostrate growth habit of *M. falcata* was recessive in the F_1 and no prostrate individuals were recovered among the 285 F_2 plants. In other F_2 populations, yellow flowered plants were present in the frequency of 3 out of 100 and 10 out of 461; this was taken as evidence that yellow flower color was controlled by three factors. Flower color also was studied by Lepper and Odland (181) in crosses involving *M. falcata*. The following factors were suggested: *P*, dominant factor for purple; *C* and *A*, supplementary dominant factors for color; and *Y*, dominant factor for yellow. In the absence of both *C* and *A*, the flowers were white, and purple was found to be epistatic to yellow. Another character, crinkled leaf, was found by Odland and Lepper (246) in the same cross. The parents and F_1 had normal leaves, but a segregation of 244 normal to 174 crinkled was found in the F_2. Three interacting dominant factors were postulated to explain this behavior.

Armstrong and Gibson (15) studied flower color inheritance in a cross between *M. glutinosa* and an autogamous selection from Grimm. To explain their different F_2 ratios of 3:1, 15:1 and 63:1, they proposed three factors for purple, P_1, P_2 and P_3, and a factor for cream, *C*, allelomorphic to P_1. The same authors suggested one or two factors to explain the inheritance of sticky hairs on the pods, but multiple factors apparently conditioned growth habit, stoloniferous habit and leaf shape and size.

It was pointed out by Tysdal, Kiesselbach, and Westover (325) that certain of the complex genetic ratios which have been obtained may be interpreted on the basis of tetrasomic inheritance.

Rudorf (271) found 22 *M. media* plants with undivided leaves among a progeny of 114, which had come from a plant treated with 0.1% colchicine. The unifoliata types showed various floral anomalies and were both male and female sterile, but all had 32 chromosomes in their root tips. A number of other characters in *M. media* were found by Schröck (278) to show simple Mendelian inheritance. The characters studied were: yellow petal color, anthocyanin in veins of the standard, pigmentation pattern on inner region of the keel, flower size, pigmentation of the calyx teeth, folded leaf, dark green leaf color and stunting.

The resistance of alfalfa to bacterial wilt disease was studied by Peltier and Tysdal (254). They suggested that three major factor pairs might explain their results with inbred lines, though they did not consider this conclusive. On the other hand, Brink, Jones and Albrecht (45) concluded that resistance behaved as an intergrading character and

probably rested upon a complex genetic basis. No factorial explanation appeared possible. It was also suggested that wilt resistance bears no relationship to the external form of the plant or to winter-hardiness.

Koepper (175) tested several species and varieties of alfalfa for resistance to the rust caused by *Uromyces striatus*. The highest degree of resistance was found in *M. ruthenica*, $2n=16$, but some very resistant plants were found in other types.

Some heritable resistance to aphids was noted by Albrecht and Chamberlain (11), but the difference between two years results suggested that resistance was not a stable character.

3. *Species Hybrids*

In addition to the papers cited above (56, 181, 246, 15), a few others have considered the problems involved in species hybrids. Ledingham (179) studied the cross *M. sativa*, $4n \times$ *M. falcata*, $2n$. Ordinarily in this cross, fertilization is delayed, and embryo development ceases after the second day, so that no viable seeds are obtained, but in one series, two $3n$ plants were found. At IM in these $3n$ hybrids, there were usually $8_{II}+8_{I}$; this resulted in poor pollen, but some female gametes were functional in backcrosses to the parents. In the $3n \times$ *falcata* progeny, 16, 17 and 18 chromosome plants were found; in the backcross to *sativa*, numbers from 30 to 36 were obtained, but most were 32. Likewise, in the reciprocal cross, *M. falcata*, $2n \times$ *M. sativa*, $4n$, most of the developing ovules aborted, but a few $4n$ hybrids were obtained. At IM in these $4n$ hybrids, usually 16_{II} were formed; fertility was high. These results were contrasted with the cross between *sativa* and $4n$ races of *falcata*, which has given rise to highly polymorphic forms, in which species differentiation appeared impossible.

A number of other species hybrids were described by Sinskaya (287). Spontaneous hybrids between species of different chromosome number in general were more fertile than those produced artificially. The most promising method of hybridizing for certain combinations appeared to be to sow a few plants of one species in a bed of the other species and allow selective fertilization to take place. Crosses between *M. sativa* and *M. glutinosa* were considered promising since the hybrids were highly fertile, they had many of the characters of the cultivated type and they were disease resistant.

Hybrids between *M. media* and *M. lupulina* were reported by Schröck (277). The F_1 plants had 24 chromosomes and were intermediate between the parents in most characters. The F_2 plants usually had 24 or 32 chromosomes, and showed continuous variation in morphological characters and fertility. An amphidiploid was produced by colchicine, but it proved

to have weaker vegetative growth than the F_1 hybrid. In spite of producing a high per cent of viable pollen it was less self-fertile than the hybrid.

4. *Breeding Investigations*

It has been known for many years that alfalfa was cross-pollinated by insects, and it was generally presumed that the per cent of natural crossing was high. Among the recent results on natural crossing are those of Burkart (53), who found individual plants varying from 67 to 98% with an average of 85%. Studies by Tysdal, Kiesselbach, and Westover (325) showed that under Nebraska conditions the average crossing for several years was 89%; in Saskatchewan, Knowles (173) found 94.2% crossing.

It has also been known that self-fertilization generally resulted in much less seed per flower than did cross-fertilization, but the evidence on the necessity of tripping has been conflicting. Armstrong and White (16) concluded that, in the process of tripping, the stigmatic surface was ruptured, which in turn initiated pollen germination. In tripped flowers pollen germination was 84%, in comparison with less than 1% in untripped flowers. On the other hand, Brink and Cooper (41) pointed out that during a period of exceptionally hot, dry weather in Wisconsin, only 12% of the flowers were tripped, whereas 34% set pods. This was contrary to the results which they had observed under greenhouse conditions in the winter, when tripping was practically indispensable to seed setting. The dry climate of Utah was considered by Carlson (67) to be a principal cause of good seed setting there, but tripping was not essential to the process. Even so 44% of the flowers tripped artificially and 54% of those artificially cross-pollinated formed seed pods, whereas only 27% set seed under natural conditions. Tysdal (320) concluded from a study of seed setting in Nebraska and in several other states that, in general, alfalfa flowers must be tripped to form seed. The most effective pollinating insects were the Megachile and Nomia bees; seed setting in the field was closely correlated with their visitation. The honey bee was not an effective tripper of alfalfa. Likewise Knowles (173) found in Saskatchewan that there was a close correlation between the number of Megachile bees and the amount of tripping and seed setting. Similar observations on the relative value of wild and honey bees were reported by Koperzinskii (176).

Many diverse breeding methods have been employed in the improvement of alfalfa and some superior types have resulted from most of them. Opinions on the relative value of each method are not unanimous, however, and several methods are being employed in current breeding programs. Present methods were classified by Tysdal, Kiesselbach, and Westover (325) and a thorough discussion of each was included. The recently

released wilt-resistant varieties, Ranger and Buffalo, were developed, respectively, as a synthetic variety and as a straight selection. These varieties have been registered (133), and it was reported that over 30,000 and 1,000 pounds, respectively, of certified seed were produced in 1944.

Fryer (102) advocated the use of maternal-line selection, especially for improving seed-setting capacity, and results for the new variety, Ferax, which was developed by this method, were presented. On the other hand, a mass of evidence has been compiled by Tysdal, Kiesselbach and Westover (325) and by Tysdal and Kiesselbach (324) to suggest that ultimately the most improvement can be expected from either recombination of selected inbred lines as synthetic varieties, or by hybridization of selected clones for use as single or double cross hybrids. In either case self-sterility is regarded as a highly desirable character.

It has often been noted that both forage and seed productivity are reduced as a result of inbreeding. For example Tysdal, Kiesselbach and Westover (325) report that the average of 54 I_1 lines in yield of forage was 68% of that of the original open-pollinated varieties; the corresponding seed yield was 62%. Both forage and seed yield declined continuously through seven or eight generations, at which point they seemed to level off. In contrast to these yields, those of controlled hybrids often exhibited considerable heterosis. The forage yield of 28 hybrids showed a wide range, but the yield of the highest producing hybrid exceeded the average yields of the checks by 39%. The average of the top ten hybrids was 15% above the yield of the checks. The utilization of this hybrid vigor through controlled crosses was considered important because both actual and theoretical yields (325) of synthetics indicated that there may be a yield ceiling for varieties developed in this way. Tysdal and Kiesselbach (324) presented much additional data to support the concept of controlled hybridization; the main subjects considered were evidence of hybrid vigor, differences in combining ability, amount of self-sterility, relation of self-sterility to forage yield and testing for combining ability. All this work has led to theoretical calculations (325, 324) on the feasibility of producing single or double crossed seed. As an example, it was shown that two isolated 25-acre natural-crossing fields, each planted with cuttings from two clonal lines, should produce annually sufficient F_1 seed to plant 10,000 acres of F_1 crossing fields. In turn the 10,000 acres should produce sufficient double cross seed to plant 200,000 acres of hybrid alfalfa, so that on a five-year rotation basis, the original fields should maintain one million acres of the commercial double cross.

In all this breeding work, there is usually some question as to the best nursery technics to follow. Tysdal and Kiesselbach (323, 324) presented considerable information on this subject, and it has also been

investigated by Weihing and Robertson (331, 332) in Colorado. Many observations have been made on such factors as size and shape of plot, border effect and inter-plot competition, so that many of the procedures appear now to be fairly well standardized.

A correlation, which might prove useful in plant breeding, was reported by Smirnova (290). He found that crude protein content in leaves and stems of light green forms was respectively 21.23 and 7.75%, whereas in the leaves and stems of dark green forms it was respectively 29.43 and 9.13%. Yield of both stems and leaves also was higher in the dark green forms. Schröck (276) reported similar correlations between protein content and certain other characters, but he considered their value to be too low to be of much use in practical breeding.

Problems related to breeding for insect resistance in alfalfa have been reviewed recently by Packard (251), Snelling (296) and Blanchard (39). The principal insect pests appear to be leafhoppers, aphids and Lygus, although several others have been studied. Recently Ragonese and Marcó (262) have demonstrated resistance to stem nematode. Apparently no strains of alfalfa have thus far been bred especially for insect resistance but a great deal of resistant material is known. Breeding for insect resistance holds promise and probably will be emphasized in the future.

Peltier and Tysdal (254) reported heritable differences in cold resistance, and Brink, Keller and Eisenhart (46) described significant differences between strains in survival under an ice sheet. In this latter experiment the average mortality of the individual strains ranged from 12 to 96%, and several were more resistant than either the Grimm or Hardigan checks. Recently Jones (151) described the several factors responsible for reduced longevity in clones of unselected plants of four wilt-resistant varieties of alfalfa. The diseases bacterial wilt and downy mildew, together with winterkilling, appeared to have killed only about half of the dead clones. Other parasitic diseases and injurious insects were relatively unimportant in causing death. Most of the remaining deterioration and death appeared to have resulted from winter injury, as distinct from winterkilling. The winter injury assumed many forms, but it was distinct enough to be used as a basis of selecting for longevity.

In much of the work involving controlled crossing, the suction method of emasculation, described by Kirk (164), has been used. Another method was suggested by Tysdal and Garl (322). The standards were clipped at full bloom, and the flowers were then tripped to expose the anthers. The entire raceme was immersed in 57% ethyl alcohol for 10 seconds and then rinsed by transferring to a beaker of water for a few seconds. Any adhering water or dead anthers were blown off, and pollen was applied immediately or any time up to one hour later.

XIII. Red Clover

1. *Cytological Investigations*

When Senn (281) reviewed the work on chromosome numbers in the Leguminosae, the reports agreed in general that *Trifolium pratense* is a diploid with $2n=14$. Since that time polyploids have been induced by colchicine treatment and they have been studied from several aspects. The first reports describing polyploids were those of Levan (182, 184). Colchicine was applied both to seeds and to growing points of seedlings and about 400 affected plants were obtained. Many plants appeared to be chimeras of $4n$, $8n$ and even $16n$ tissues. Some pure $4n$ plants were found and cuttings were made to isolate others. In general, the $4n$ plants had larger and coarser leaves than the $2n$ and usually were of the gigas type. Pollen fertility was 77% as compared with 93% for the controls and seed set was good. When three populations and cuttings from a $2n$–$4n$ chimera were tested in the field (184), the $4n$ groups showed an increase in green weight of 28, 36, 40 and 35% respectively. The per cent of dry matter was somewhat lower, however, in the $4n$, and the differences between $2n$ and $4n$ in crude protein were small. Levan (184) emphasized that Trifolium may be a genus in which the optimum chromosome number may be higher than $2n$ and that practical results may be expected if sterility is not too great a difficulty. Further reports on this material by Müntzing (199, 200) pointed out that the $4n$ material was higher not only in green weight but also in average weight per plant and in total dry weight. Although fertility was low when the plants were isolated, 100% seed set was obtained either by artificial pollination or by pollination with insects.

2. *Genetical Investigations*

The genetic studies up to 1937 were reviewed by Pieters and Hollowell (258); since then the research on genetics has progressed along several lines. Nijdam (230), in studying flower color, postulated the following factors: *G*, a dominant factor for anthocyanin, *gg* individuals having yellowish-white flowers; *B* and *E*, together producing purple red in the presence of *G*, *bb* flowers being pink, *ee* blue, and *bbee* pink with a dull blue shade, not easily distinguished from pink. All colors may fluctuate in shade from very light to very dark, and this was attributed to a series of polymeric factors which acted on all colors in the same way. The factors *B* and *E* were linked, with about 40% crossing over. The factor *G* appeared to be independent of *B* and *E*. Pure white flowers, without any admixture of yellow, such as were found earlier by Williams (338), were not encountered in these cultures.

Nijdam (231) also found evidence for a recessive factor which

conditions the formation of a brownish-black spot on the seed coat. No evidence of linkage between this factor, *a*, and *g* was obtained, but linkage between *a* and *e* seemed fairly certain. The pigment causing this spot was melanin, which was insoluble in water, acid, alkali, alcohol and ether. This is in contrast to the water-soluble flavonol- and anthocyanin-pigments, which are conditioned by *G* and which bring about the yellow and purple tints found in most red clover seed.

Certain phases of the extensive genetic investigations conducted at Aberystwyth were summarized by Williams (340, 341, 342). He reported that 152 recessive chlorophyll deficiencies had been found, and that most of them appeared to be conditioned by different recessive factors inherited on a simple Mendelian basis. Of these deficient types, 57 were lethal in the seedling stage, while the remaining 95 survived to maturity. The abundance of these factors was illustrated by the fact that of 22 plants in a commercial population, 13 were found to be heterozygous for chlorophyll deficiency, and 22 different mutants were obtained from them. In the heterozygous condition, these factors had no apparent deleterious effect upon the plants, except possibly to make them shorter lived. Other lethal types have been identified, including 8 dark green types and 30 dwarfs. In addition there were found 13 factors affecting leaf growth, one factor governing stem development and 6 factors responsible for flower development. The effect of many of these factors was to alter the whole appearance of the plant. Numerous deleterious recessives likewise were found by Wexelsen (335). On the other hand, Williams (340) showed that there were many other recessive factors, such as those conditioning leaf-markings, anthocyanin pigmentation, pubescence and flower and seed color, which had no apparent effect on plant growth. For example, normal purple-red flower color was considered to be due to the interaction of at least 14 dominant genes, but only one was known to have any possible effect on growth. The linkage relations of 37 factors were established, and five of the seven possible linkage groups were found. The largest group consisted of 21 factors and the smallest of four.

3. *Fertility Relationships*

Red clover has been used in studies of incompatibility for many years, and it appears to be one of the best examples of inheritance according to the diploid personate type of multiple oppositional alleles (310). The first reports of incompatibility in red clover were by Williams (336) and Williams and Silow (345). Subsequently Williams (340, 344) confirmed these results and reported much additional data. In the first reports, it was shown that almost all plants of red clover were self-sterile and that intra-sterile, inter-fertile classes occurred within families, obtained

by mating pairs of self-sterile individuals. This behavior was explained by a series of multiple oppositional alleles which functioned in such a way that whenever the same allele was present in both pollen and style, the growth of the pollen tube was inhibited. Silow (283) showed that this inhibition occurred after the incompatible tubes had grown through about half of the style. It was shown, however, by Williams and Silow (345) that occasionally a few seeds were set on self-sterile plants and these seeds may give rise to plants homozygous for the sterility alleles. Williams and Silow (345) also found a single self-fertile plant and, on crossing, it was shown that a non-inhibitory self-fertility allelomorph, S_f, conditioned this behavior. In a recent report, Williams (344) stated that only three fully self-compatible plants carrying the completely dominant S_f allele had been found, and in each case the heterozygous S_fS_a plants appeared as mutations in crosses between self-incompatible parents. The factor S was shown by Williams (344) to be located in linkage group 2, where it was linked with two factors for flower color and seven for chlorophyll deficiency.

The number of alleles conditioning incompatibility was shown by Williams (340, 344) to be very extensive. By intercrossing F_1 plants all having one allele in common, it was demonstrated that 24 plants of English late variety, and 20 plants of English broad red variety, taken at random from commercial populations, carried 41 and 37 different S alleles out of a possible 48 and 40 respectively. The majority of the alleles studied appeared to be fully incompatible when present in both pollen and pistils, but some were less incompatible in so far as they were capable of effecting pseudo-fertilization under certain conditions. Evidence for a large number of sterility alleles also was obtained by Wexelsen (335).

A self-fertile line of red clover, which was isolated in Minnesota, was described by Rinke and Johnson (266). It continued to be highly self-fertile through 10 generations of inbreeding, but it was much less vigorous than commercial red clover. In crosses between the self-fertile line and commercial red clover, self-fertility was dominant in the F_1, and only two out of 182 F_2 plants appeared to be definitely self-sterile. A plan for utilizing the self-fertility gene in a breeding program was outlined.

Numerous investigations have been made on the environmental factors influencing seed set in red clover. An example of this type of work is the paper by Bird (38), in which his recent results were presented together with an extensive review of the literature. Bird (38) concluded that the number of bumblebee visitors was a much more important factor in seed setting than number of honeybee visitors or number of florets per head. Consequently, aftermath crops of red clover brought into full bloom during late July or early August in eastern Canada have a much better chance of receiving satisfactory pollination than does the first growth blooming

earlier in the season or aftermath crops brought into full bloom at a later date.

Fedorčuk (97) described abnormalities which were initiated at various stages of embryo development and which led to dying off of ovules and partial sterility. This may be comparable to the situation found in alfalfa (see above).

4. *Breeding Investigations*

It was pointed out by Pieters and Hollowell (258) in 1937 that many of the improved strains of red clover in the United States have developed as a result of natural selection. These strains have distinct value in regard to such characters as winter-hardiness or disease resistance in the region where they arose. In the case of some other strains this process has been aided by a conscious mass selection, particularly for disease resistance. A coöperative program, involving several state experiment stations and the U. S. Department of Agriculture, was initiated in 1928 to extend the range of adaptation of this material and facilitate its increase. Three strains, from Kentucky, Tennessee and Virginia, were composited in equal proportions to form Cumberland; four other old strains, from Illinois, Ohio, Indiana and Iowa, were blended in equal proportions to form Midland. These composite strains were registered (132) on the basis of their superior performance over several years at several locations, and the seed is now being increased rapidly.

According to Nilsson-Leissner (239) a similar procedure has been found useful in Sweden. The best among old local strains, grown in different parts of the country and found to have gradually become specially adapted to the climate and soil by natural selection, are multiplied and distributed. A description of the origin and performance of the types of red clover used in Great Britain was presented by Williams (346). The early strain S.151 and the late strain S.123, both bred at Aberystwyth, were recommended in place of their commercial counterparts.

Many difficulties have been encountered when more intensive plant breeding methods were applied to red clover, and principal among these difficulties was the reduction of vigor following inbreeding. Williams (339, 340) found a progressive reduction in both productivity and longevity from the first to the third generation. The loss was most pronounced in the first generation and became less in each subsequent generation. For example, in one series of plants, all derived from a common ancestry, the I_1 generation averaged 62.1 in productivity, and the I_2 38.2, as compared with the cross-bred population classed as 100. On the other hand, the fertility of cross-compatible plants apparently was not depressed by inbreeding and the inbred seeds were just as viable and showed just as

vigorous germination as the outbred seeds. Similarly, Wexelsen (335) found that on inbreeding there was a decided loss in vigor; it was especially noticeable in such characters as seedling growth, stem number, plant height, internode length, plant weight and seed per plant. He concluded that inbreeding should be avoided for most short-time practical results.

Although the average loss in vigor has made selection difficult, Williams (340) reported that certain lines retained their vigor remarkably, and these lines in turn were strongly prepotent in regard to yield when outcrossed. This difference was more pronounced in the third year than the second. Wexelsen (335) also found a high degree of hybrid vigor in F_1.

In connection with evaluating strains in a breeding program, there usually arises a question as to the best plot technic. Recently Torrie and Allison (318) have shown that with small plots, if a separation of clover and timothy is made, the strains react the same in forage yield whether seeded in rows or broadcast, and either with or without timothy. When the yield of timothy was included, the differences between strains were masked and a significant strains×methods interaction was obtained.

XIV. White Clover

White clover has sometimes been cited as a species with intraspecific races differing in chromosome number. All recent counts, however, confirmed the number $2n=32$, and there is reason to believe that the early accounts of other numbers were incorrect. Meiosis in *T. repens* was found by Atwood and Hill (30) to be very regular in both pairing and disjunction of the chromosomes. Only five of the 610 cells observed in 11 plants varied from the regular occurrence of 16_{II}, and in none of the cells was polyvalent pairing observed. Chiasma frequency was low, with an average of 16.7 per cell. Counts of 912 IA or IIM groups were made, and in only three cells was there other than a 16–16 distribution. Likewise, all 93 groups counted at IIA had 16 chromosomes. It was concluded that white clover is probably an amphidiploid and probably shows mostly disomic inheritance.

Colchicine-induced polyploids were obtained by Atwood (25) in the Kent strain of white clover. Ten pairs of 32- and 64-chromosome cuttings, each pair derived from a single treated seedling, were planted in the field in three replications. An analysis of the vigor notes showed highly significant differences due to chromosome numbers, plants and the chromosome number×plants interaction. The 32-chromosome cuttings average 8.38 in vigor, whereas the 64 averaged only 4.53. This greater vigor for the 32 held for all 10 plants and suggested that the practical usefulness of the 64-chromosome material for forage may be limited. In the chemical analyses of some of this material, reported by Sullivan (313), the 64-

chromosome sectors were significantly lower in crude fiber and also lower in carotene in September but not in July.

Cyanogenetic glucosides have been known in white clover since 1912, but the first report on inheritance was by Williams (343) in 1939. He found monohybrid ratios regularly in backcross, F_2 and F_3 generations, and he postulated a single recessive factor, *ac*, to explain the acyanogenetic condition. The glucosides from white clover which react to the picric acid test were isolated by Melville and Doak (191), and the enzyme capable of hydrolyzing these compounds was identified by Coop (74). Using purified preparations of these compounds, Corkill (79) found some plants containing enzyme alone, some glucoside alone, some both and some neither. Subsequently Corkill (80) found that the presence of enzyme and glucoside were each determined by single dominant factors and that these genes segregated independently. Similar results were obtained by Atwood and Sullivan (33) from a cross between two inbred lines. No linkage was observed between the factor for white marking on the leaf blade and the factors for either glucoside or enzyme. These results on inheritance of HCN have been of practical interest since Doak (89) showed in New Zealand that the better white clover strains contained more glucoside than did the more poorly adapted strains. The glucoside test was then applied to seed certification, according to Foy and Hyde (100), since it was found that the types giving the higher tests were superior in regard to such characters as total production, seasonal distribution and persistence under competition. This relationship did not hold, however, in the populations examined by Williams (343) and by Atwood and Sullivan (33).

The phenomenon of sterility has been known in white clover for many years, but in 1931 Williams (337) first pointed out (a) that unrelated plants generally were reciprocally cross-compatible, (b) that sister plants were either reciprocally cross-incompatible (*ca.* 26% of his crosses) or reciprocally cross-compatible, and (c) that compatible F_1 sister crosses on the average produced as many seeds as crosses between unrelated plants. Further evidence on the inheritance of cross-incompatibility was obtained by Atwood (18) from a cross between two self-incompatible plants. The 13 F_1 plants consisted of four intra-sterile, inter-fertile groups of five, four, three and one plant, respectively, and all F_1 plants were reciprocally compatible with both parents. These results were best explained by multiple oppositional alleles, where the parents differed in both factors. This hypothesis was confirmed when 39 F_2 plants from three F_1 intercrosses were found to consist of only expected types on backcrossing to the two parental and four F_1 groups. The four possible homozygous types were identified by appropriate tests. The cytological basis for incompatibility was found by Atwood (19) to be an inhibition of the incompatible

tubes after they had grown through the first three-fourths of the style, although there was also a distinct inhibition on the stigma.

The ability of a plant to set some seeds per head following selfing has been termed pseudo-self-compatibility. This character was found by Atwood (21) to be heritable and it appeared to be conditioned by several genes, which were additive in effect and heterozygous in the parents. Additional evidence for oppositional alleles was found in this study and, since plants of both high and low pseudo-self-compatibility were found in each of the six genotypes involved, it was concluded that little relationship existed between the S factor and those conditioning the ability to set some selfed seed after manipulation.

A genetic basis for true self-compatibility also was desrcibed by Atwood (22). Using a cross between a female plant of low self-compatibility and a male that was the only one out of 615 to average over 100 seeds per head on selfing, it was found that the 14 F_1 plants consisted of two intra-sterile, inter-fertile groups of five and six, respectively, together with three other plants that were self-compatible like their male parent and cross-compatible in all combinations. This behavior was explained by postulating a self-compatibility factor, S_f, which is a member of the multiple-allelic series conditioning self- and cross-incompatibility. The theory was confirmed when 48 F_2 plants from four F_1 intercrosses were backcrossed to the parental and F_1 groups. In a further study of this self-compatibility gene, Atwood (28) obtained data on the functioning of S_f gametes in relation to other alleles, and he found that homozygous S_fS_f individuals were obtained easily for use in an inbreeding program.

The amount of natural crossing under bee cages was found by Atwood (24) to vary from 85 to 100%, depending in part on the pseudo-self-compatibility of the plant tested. With a clone heterozygous for S_f, however, only 19% crossing was obtained. It had been found previously by Atwood (20) that differences obtained under bee cages in both self- and cross-compatibility were similar to those obtained by hand pollination.

The number of oppositional alleles in four populations was tested by Atwood (23, 26) using diallel crosses between F_1 plants which had one S factor in common. In two breeding populations, supposedly of unrelated plants, 25 out of 26, and 34 out of 41 of the alleles, respectively, were different. In two natural populations, estimated at a few thousand and a few hundred thousand individuals, respectively, 36 out of 49 and 39 out of 49 of the alleles were different. These latter frequencies of different alleles were somewhat lower than the former but, even so, only about 1% of all matings were incompatible, so that under most field conditions it appeared highly improbable that cross-incompatibility would be a major factor in lowering seed set.

When the behavior of the oppositional alleles was studied in 64-chromosome plants, Atwood (27) found that the sectors with doubled chromosome number were self-incompatible like the 32 chromosome plants from which they arose. A cross between two such 64-chromosome plants gave rise to an F_1 of both self-incompatible and self-compatible individuals. A hypothesis to explain the self- and cross-compatibilities which were obtained was based on the assumption that pollen bearing two different alleles depended sometimes on the interaction between the stigma and all pollen placed upon it rather than on a specific oppositional effect.

White clover is commonly recognized as a highly polymorphic species. This has given rise to many strains both by natural selection and by the process of mass selection, similar to the breeding of red clover. Wide differences in the performance of these strains depends upon the local conditions under which they are tested. The types of white clover adapted to Great Britain were discussed by Williams (346). In most cases the Aberystwyth strains S.100 and S.184 proved superior under English conditions. The range of variability in white clover was surveyed by Ahlgren and Sprague (1), and its relation to breeding was discussed. One of the most difficult problems has been the evaluation of individual plants in regard to their yield under clipping when grown in association with grass. The technic of growing cuttings of selected plants in plots overseeded with bluegrass was tested by Atwood and Garber (29), and highly significant differences between plants in yield were obtained.

Recently a virus-like disease of white clover was found by Atwood and Kreitlow (31) to be determined by two complimentary factors; the disease was not transmissible by inoculating or grafting.

XV. Sweet Clover

1. *Cytological and Genetical Investigations*

According to Senn's (281) review of chromosome numbers, the genus *Melilotus* is unique in its complete uniformity of $2n=16$. Apparently the only deviations from this have been the induced polyploids described by Atwood (17), Weichsel (330) and Johnson and Sass (148). The two exceptional 24 and 32 chromosome plants described by Atwood (17) were found following heat treatments, which were applied at the time of the divisions in the zygote and proembryo. The 32-chromosome plant showed regular meiotic divisions, whereas the 24-chromosome plant had a number of irregularities. Johnson and Sass (148) observed that the different $4n$ lines obtained by colchicine treatment showed marked differences in irregularities of IA disjunction.

A mottled condition of the seed coat was found by Stevenson (305)

to be dependent upon a single factor pair, but the character was expressed only if factors for pigment were present. Other undetermined factors influenced the character since the same plant sometimes produced both non-mottled seed and seed varying greatly in the degree of mottling. Fowlds (99) investigated a green-seeded plant of sweet clover and found that the translucency of the seed coat made it necessary to remove the seed coat to determine accurately the color of both seed coat and embryo. He found a single recessive gene, *y*, which determined green color in both seed coat and embryo, and noted a pale green type of seed which resulted from either green coat plus yellow embryo or yellow coat plus green embryo. Another type of seed, pale-yellow, conditioned by a white seed coat, was found to be recessive to yellow. When green was crossed with pale-yellow, the F_1 hybrid plants had yellow seed coats and their segregation indicated complementary factors for basic color and yellow. The F_2 of this cross, green × pale yellow, was studied by Swenson (315), and the hypothesis of a basic color gene, *C*, together with the factor *y* for yellow versus green, was confirmed. The independence of these two genes was demonstrated by the χ^2 test. In contrast, Hartwig (116) found that cotyledon color in F_1 seed always resembled the color of the maternal parent, and he suggested an inhibitor gene causing green pigment to fade at maturity.

It was shown by Elders (91), Clarke (70), Kirk (165) and Stevenson (305), that the dwarf-branching types of sweet clover were recessive in F_1 and showed 3:1 segregation in F_2 when crossed with normals. Clarke (70) crossed the Alpha type with his dwarf-bunch type and from the F_2 segregation of 9 tall : 6 dwarf, he postulated two recessive genes, with the double recessive lethal. Hartwig (116) found two recessive dwarf types, which appeared by observation and crossing tests to be different from those studied by Clarke (70). On the other hand, Swenson (315) found the F_1 plants intermediate in a dwarf × normal cross and a 1:2:1 segregation in the F_2. The gene for growth habit in this material was independent of the two genes determining seed color.

The characters unifoliolate-leaf and cup-leaf were found by Hartwig (115, 116) to be conditioned by single recessive genes. The gene for cup-leaf was shown by linkage tests to be independent of the factor for green cotyledons and one of the factors for dwarf growth habit.

An annual mutant from Alpha was crossed with biennial forms by Stevenson and White (307), and it was shown that the annual habit was produced by a single dominant factor identical with the one in Hubam. Johnson (146) investigated the cross-fertility relationships between Golden Annual and *M. alba*, *M. officinalis* and *M. suaveolens*. On the basis of seed setting and seed weight, it was concluded that the variety is an annual form

of *M. suaveolens*, with the annual form dominant over biennial in the F_1 of the same species.

2. *Fertility Relationships*

The inheritance of self- and cross-sterility in *M. officinalis* was studied by Gettys and Johnson (107). Two plants, which averaged 7.6 and 10.0% self-fertility, were crossed and the average self-fertility of the F_1 generation was 11.1%, or not greatly different from that of the two parents. This was in contrast to an average fertility of 62.7% for all compatible crosses. When 19 F_1 progeny were reciprocally backcrossed to both parents, 9 were found to be cross-compatible with one parent and cross-incompatible with the other, while the other 10 plants were cross-compatible with both plants. In addition five plants in each F_1 group were found to be cross-incompatible in crosses within their groups and cross-compatible with plants of the other group. These results were explained by the oppositional factor hypothesis, where the parents have one sterility allele in common.

Gettys and Johnson (107) also crossed a self-sterile plant with a self-fertile plant, and they found that all seven F_1 plants and all 139 F_2 plants were self-fertile like their male parent. Thus it appeared that an S_f allele was transmitted to all F_1 plants, where in turn only the S_f pollen was effective in self-fertilization.

In colchicine-induced tetraploids of *M. alba*, Johnson and Sass (148) found that among 20 $4n$ plants self-fertility ranged from 0.8 to 18.8% and averaged 7.8%, whereas among 10 comparable $2n$ plants, tested at the same time, self-fertility ranged from 27.2 to 68.7% and averaged 43.7%. Cross- and self-fertility were then compared in eight of the $4n$ plants, which differed significantly in self-fertility. A total of 32 crosses were made among the eight plants, and in four cases the per cent of cross-fertility was significantly higher than the corresponding per cent of self-fertility. In the F_1 generation from crosses between parents differing in self-fertility, significant differences were noted between plants, with the highest self-fertility being found in plants from the higher fertility parents. This work indicated the possibility of selecting for higher levels of self-fertility.

3. *Studies of Coumarin*

A serious drawback to the use of *M. alba* and *M. officinalis* as forage plants has been their high content of coumarin. This compound is largely responsible for the bitter taste of sweet clover used as pasture and for the formation of a poisonous substance in sweet clover hay and silage. This poison may occur in sufficient amounts to cause severe and often fatal internal hemorrhages in animals feeding on the spoiled hay or silage.

Several investigators have studied the possibility of breeding types free of, or relatively low in, coumarin.

Through continuous selection in inbred lines, Stevenson and White (308) developed strains with only about 1/10 normal coumarin content; they also selected types for increased coumarin content. A plant that produced progeny averaging 0.41% coumarin was crossed with one that produced progeny averaging 0.01% coumarin. The F_1 was intermediate, and the F_2 had a bimodal distribution, with only a few plants being intermediate. This F_2 segregation gave a satisfactory fit to a 3:1 ratio, indicating a single dominant factor for high coumarin. When Horner and White (135) crossed several low-coumarin lines with high-coumarin lines, the F_1 progenies were high. In this material, the low F_2 plants tested less than 0.05% coumarin and their F_3 progeny usually bred true with no segregates above this amount. The high F_2 plants, however, tested over 0.10% coumarin, and one-third of them bred true in F_3 for this high amount while the other two-thirds segregated. The variation between plants was explained by one or more modifying factors, which influenced coumarin content, in addition to the major factors differentiating high and low. No evidence of linkage was found between the major factor pair for coumarin and the factors for dwarf growth habit or white sepals.

Another cross between inbred lines relatively low and high in coumarin was studied by Rinke (265), but in this case the F_1 plants averaged low in coumarin. Approximately 77% of the 418 plants in the F_2 population were in the range of the selfed progeny from the low coumarin parent, while the remaining 23% were in the range of the selfed progeny from the high parent. The F_2 distribution was not bimodal, but it was suggested that a single dominant factor for low coumarin was involved.

Several other species, particularly *M. dentata*, have been shown by Brink (40), Brink and Roberts (47), and Smith and Brink (295) to be non-bitter and to produce non-toxic hay when spoiled. Crosses between *M. dentata* and other species were reported by Stevenson and White (308) and Smith (294), but the hybrid seedlings usually survived for only a few weeks. A technic was developed by Smith (294) whereby the chlorophyll-deficient hybrid seedlings were grafted on vigorous shoots of *M. alba* and *M. officinalis*. Three such grafted hybrids produced flowers, but all were completely self-sterile. Meiosis appeared to be regular, with no laggards at IA. When pollen from *M. alba* was applied to the hybrids, one of them bore seven seeds, which gave rise to two mature plants. It may be possible in this way to transfer the coumarin-free character of *M. dentata* to other species.

4. *Breeding Investigations*

The results on fertility and inbreeding, which were summarized up to 1937 by Pieters and Hollowell (258), showed considerable variation, depending mostly on the varieties used and conditions of the test. Further results on the effects of inbreeding were reported by Hartwig (117). Plant height decreased progressively through three generations of inbreeding in *M. alba* and *M. officinalis*. There was also a reduction in fertility and a tendency for the inbreds to be more susceptible to black-stem disease.

Recently three of the newer varieties of sweet clover, Spanish (formerly called Madrid white), Madrid (formerly called Madrid yellow) and Evergreen (a mass selection made in Ohio) have been registered (131). Another variety, Emerald, has been superior in Texas.

In a greenhouse study of open-pollinated seed lots from four $2n$ and four $4n$ first-generation selfed lines of Iowa Late White sweetclover, Evans and Johnson (94) found a significantly greater vigor of the $4n$ plants in the early stages of growth but a trend toward equalized height and weight at later stages. The seedling differences were attributed to the larger seed size of the $4n$ lines.

XVI. Miscellaneous Legumes

From a study of the cytological relationships in Lespedeza, Pierce (257) concluded that the basic chromosome number for the American species is 10. For the rest of the genus, the basic number is probably 11, although $n=9$ has been reported for certain Japanese species. Hanson (112), in his studies of cleistogamy in *Lespedeza stipulacea*, found that the apetalous flowers were highly fertile whereas the petaliferous flowers were highly variable in fertility, depending on the conditions under which the flowers were formed. The proportion of the two types of flowers appeared to be determined largely, if not entirely, by environmental factors, with temperature very important. Six clones of *L. cuneata* were found by Stitt (309) to be significantly different in tannin content, suggesting that considerable improvement might be effected through breeding.

A single dominant gene, which gave tetrasomic F_2 ratios for cyanogenesis, was found by Dawson (88) in *Lotus corniculatus*. From this it was concluded that the species is an autotetraploid with $2n=24$, despite the fact that quadrivalent formation was rare. On the basis of evidence from morphology, genetics, cytology and wild populations, the species was considered to have arisen from *L. tenuis* or its prototype.

In *Trifolium subterraneum*, Hills (128) observed that resistance to *Uromyces trifolii* was recessive. Resistance ordinarily was associated with early maturity, but in certain F_3 lines it was combined with lateness.

Hills (129) also reported that varieties of *T. subterraneum* differed in their tendency to produce dormant seed but not in hard-seededness.

The chromosome number in nodules was found by Wipf (348) and Wipf and Cooper (349, 350) to be double the somatic number. This held true for all 31 diploid species investigated as well as for various polyploid races in three genera. The tetraploid cells were present before the infection by the nodule bacteria, and their presence appeared to be a necessary condition for nodule development. Uninfected cells of the nodular cortex had the normal number of chromosomes, and no mitotic irregularities were observed.

Using new varieties of vetch developed in Alabama, Albrecht (9) found that earliness of maturity was a major factor conditioning high seed production. Not only were the early strains more prolific, but they also matured their seed before adverse weather and insect infestations occurred.

XVII. Summary

The recent researches in cytogenetics of forage species have advanced particularly in the fields of polyploidy, apomixis and sterility. Many of the results have been translated directly to breeding operations, so that as the limits of improvement through simple selection are reached, technics probably will be available for many specialized breeding procedures. Basic knowledge is still needed in many lines, however, since the forage crops, as a group, present an array of complex problems unequalled in most other crops.

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Cytogenetics and Speciation in Crepis

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CONTENTS

I. SPECIATION

In general, the origin of species is the result of the interaction of a complex of internal and external factors. In the first place, it involves several different genetic processes which may be going on simultaneously. These processes of genetic change either provide new hereditary material or set up new internal conditions which make speciation possible. These

genetic processes furnish the basis for discussion in the present paper. But, to avoid the impression that speciation is a relatively simple matter dependent merely on one or another genetic process, it is desirable to note in passing some of the other factors involved in organic evolution. In addition to the genetic processes, such internal conditions as the natural method of reproduction, the existence of syngamy, the occurrence of self- or cross-sterility and the limits of tolerance of a species to environmental changes also have a bearing on speciation. Furthermore, the advent of species in nature depends not only on certain internal conditions but also on conditions external to the organism which play an important role in determining the characteristics of new species as they develop. These external conditions include: spatial isolation of populations; reunion of populations; size of population; and changes in the environment resulting from migration, gene-flow or other causes. Any extreme variation in the external conditions affecting a species may upset the existing balance within it, result in the elimination of individuals through natural selection or in a change of genotype through random fixation, and tend to establish a new genetic balance which may be one step toward a new species. Through natural selection, adaptation may accompany speciation as a gradual readjustment of existing species to a changing environment. The origin of a small group of related species in a common center and the gradual spread of their descendants over the earth is a time-consuming series of events. Hence, the evolutionary history of any large, widely distributed group of related organisms is apt to be long and difficult to trace.

II. The Genus Crepis

This group of 196 closely related species is certainly no exception to the foregoing generalization. In an attempt to trace the evolutionary history of this genus (1), one line of approach to the problem has been the genetic, cytologic and cytogenetic research on various species and interspecific hybrids carried on by the author and numerous collaborators during the past 25 years. It is the purpose of the present paper to review the evidence on speciation brought to light by this research. But it should be understood that the conclusions which have been reached concerning the phylogeny and systematics of these species are based not on the cytogenetic evidence alone, but also on comparative morphology, geographic distribution, paleontology and the floristic history of Eurasia. On this broad basis it has been concluded that the genus is monophyletic and that the center of origin was in northern Central Asia. Because of the thoroughness with which the phylogeny and systematics of the genus have been worked out, it is hoped that this truly remarkable group of related

species, as well as the unsolved problems they present, will challenge the efforts of future cytogeneticists, taxonomists and students of evolution.

III. The Genetic Processes of Speciation in Crepis

The genetic processes involved in speciation in this genus have been recognized as belonging to two categories: *primary*, those that have been of chief importance in the history of the genus; and *secondary*, those that have played a less important role. The primary genetic processes are reciprocal translocation between nonhomologous chromosomes and gene mutation. The secondary genetic processes are interspecific hybridization, polyploidy and apomixis. The remainder of this paper will discuss the roles that have been played in Crepis speciation by each of these genetic processes and will review the evidence on which these conclusions are based.

1. *Reciprocal Translocation between Nonhomologous Chromosomes*

That some kind of chromosomal transformation must necessarily have been important in the evolution of Crepis was concluded by Babcock and Navashin (10) and by Hollingshead and Babcock (24) in their reviews of the evidence available in 1930. Three years later reciprocal translocations between nonhomologous chromosomes were reported in maize by McClintock (28). Babcock (3) then proposed that this type of chromosomal transformation would explain the aneuploid series of diploid chromosome numbers in Crepis. But research had to be continued ten years longer before satisfactory proof was obtained of the truth of this proposition. The evidence that reciprocal translocations between nonhomologous chromosomes have played a primary role in Crepis speciation may be reviewed under two general heads, namely, karyotype evolution and genesis of intraspecific sterility.

A. Karyotype Evolution in Crepis

The conception that successive changes occurred in the *number* of chromosomes present in a diploid species and that these changes were accompanied by changes in the *size and shape* of the chromosomes was forced upon the early students of Crepis cytology. This conviction grew stronger with the mounting evidence that the genus is truly natural, *i.e.*, monophyletic. The evidence on karyotype evolution will be summarized under five heads: *a*, chromosomes and phylogeny; *b*, progressive changes in the karyotype; *c*, reduction in chromosome number; *d*, experimental evidence; and *e*, karyotype analysis.

a. Chromosomes and Phylogeny in Crepis. Of the 113 species studied cytologically, 98 are diploid. Of these diploid species, 3 have 7 as the

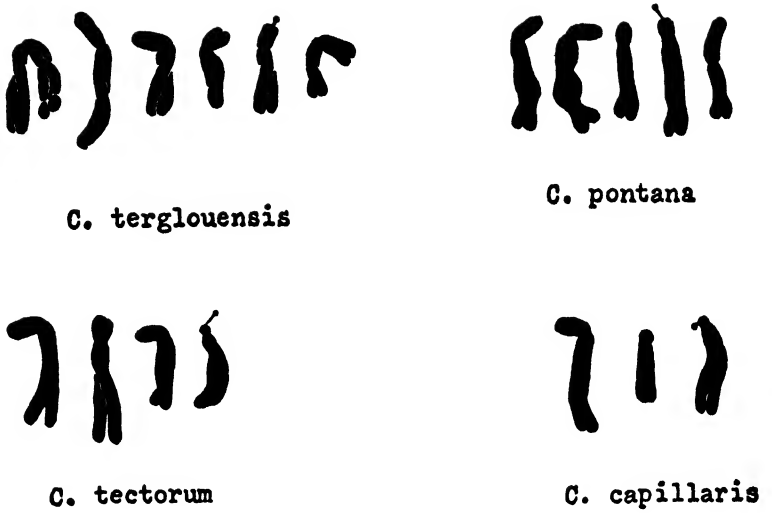

FIG. 1
Haploid Chromosomes of Four Species of Crepis.
Reproduced from *Amer. Nat.* 78, 388 (1944).

haploid number, 14 have 6, 19 have 5, 59 have 4, and 3 have 3. The three species with $n=7$ are unique in their morphological characteristics and require a special hypothesis, involving intergeneric hybridization, to account for their origin. Also they are not so primitive morphologically as any of the 6-paired species. The other 94 species comprise a series, ranging from most primitive to most advanced, in which the phylogenetic progression is marked by reduction in size of the plant and its parts, by reduction in length of the life cycle and by specialization of certain parts, particularly the involucre and the achenes. Of these 94 species, the most primitive have $n=6$ and 5, whereas the most advanced have $n=4$ and 3. The 59 species with $n=4$ range from fairly primitive to the most advanced type in the genus, and all of the 3-paired species are very advanced. Thus there is close correspondence between chromosome number and phylogeny as determined on the basis of morphology and life cycle. Furthermore, along with reduction in chromosome number, there have been definite changes in both size and shape of the chromosomes themselves (27, 9, 24, 4, 8). Fig. 1 shows *camera lucida* drawings of one member of each pair of chromosomes found in root-tip cells from 4 species: *Crepis terglouensis*, a high alpine perennial found only in the European Alps and one of the most primitive species; *C. pontana*, another alpine perennial of the European Alps and the most primitive 5-paired species; *C. tectorum*, a wide-

spread annual of Asia and Europe; and *C. capillaris*, a widespread European annual and well known as a weed in lawns.

b. Progressive Changes in the Karyotype. The salient facts concerning number, symmetry (relative length of the arms), and relative total length of the chromosomes in the diploid species of Crepis (excluding the 7-paired species for reasons stated above) are shown in Fig. 2. The idiograms were drawn from measurements made on *camera lucida* drawings of uniform

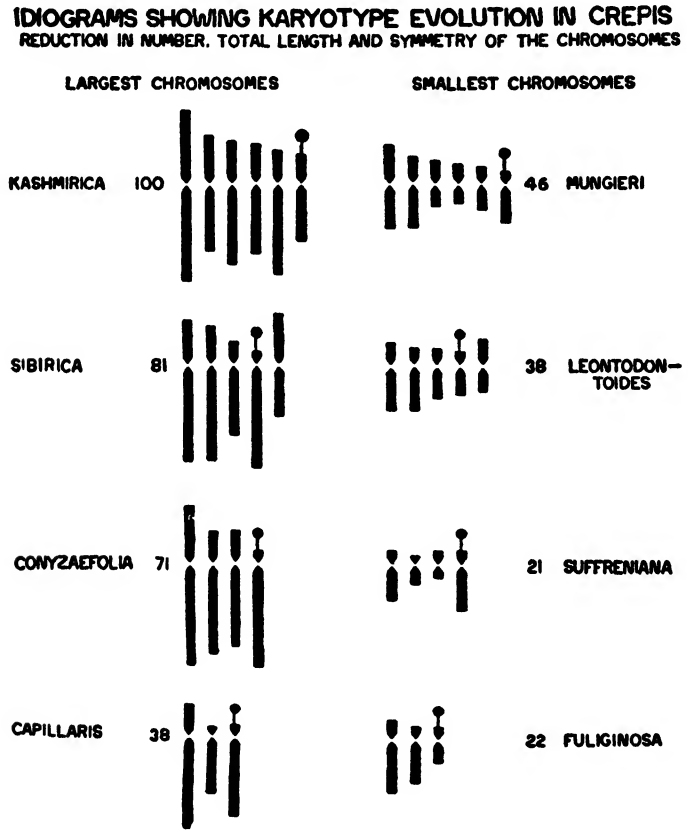

FIG. 2

These diagrammatic representations of the actual chromosomes show the karyotype of the species with longest and shortest total length in each of the four number classes, $n=6$, 5, 4 and 3. Using the total length of the *kashmirica* chromosomes as a base of 100, the proportional total length of each idiogram is shown by the number between the name of the species and its idiogram. These idiograms approximate the absolute size of the chromosomes sufficiently for purposes of this discussion.

Reproduced from *Amer. Nat.* 76, 345 (1942).

magnification of mitotic metaphase figures in root-tip cells. The degree of accuracy is sufficient to depict faithfully the trends of karyotype evolution. *C. kashmirica* has the greatest total length of chromosomes of all the species studied cytologically. Like several other primitive 6-paired species, it has relatively longer short arms than the other species shown, with the exception of the next. The most advanced 6-paired species is *C. Mungierii*, a small perennial endemic on Crete. Its total chromosome length is only 46% of that in *C. kashmirica*, but its degree of symmetry happens to be higher owing to the nearly equal arms of the 3 smallest chromosomes, a rather unusual feature in Crepis. The other 6-paired species comprise a fairly continuous series between these two extremes (4, 8). Similar comparisons hold in general for the species with greatest and least total length of chromosomes in the 5-paired species, the 4-paired species and the 3-paired species (Fig. 2). It will be noted that, in the two 5-paired species, the karyotype has one equi-armed member. This is characteristic of all 5-paired species; whereas absence of an equi-armed member is characteristic of most of the 4-paired and all of the 3-paired species. But the other members of the karyotype have a general similarity throughout the 3 number classes, 5, 4 and 3. Such a degree of similarity suggests that the important steps in reduction in chromosome number have been infrequent or rare events.

c. Reduction in Chromosome Number. Although there is a certain degree of parallelism between reduction in chromosome number and reduction in size and in the life cycle of the plant, yet the causes of these two trends are different. The essential facts are as follows: all of the 6-paired species are primitive perennials; most of the 5-paired species are less primitive, and a few are annuals; a few of the 4-paired species are fairly primitive perennials, but more of them are less primitive and many are annuals; while all of the 3-paired species are reduced or specialized annuals. Most of the species, however, are either 5-paired or 4-paired; and *within each of these two groups* there is a general trend toward reduction in size and life cycle of the plants. Therefore, the parallelism between reduction in chromosome *number* and reduction in the plants does not indicate a causal relation between the two. On the contrary, as will be shown below, these trends are the results of entirely different processes of genetic change. The structural changes leading to reduction in chromosome number — namely, reciprocal translocations between nonhomologous chromosomes — seem to have occurred only rarely, whereas morphological and physiological differentiation is due to gene mutations, which have gone on continually.

d. Experimental Evidence. Interspecific hybridization in Crepis has been carried out on an extensive scale (1, 31, 20). Between 1920 and 1939

my own coworkers obtained seed from 206 interspecific crosses involving 59 species. In general, hybrids between species considered to be less closely related, on the basis of comparative morphology, tend to be weak and sterile or, if vigorous, to be sterile or of low fertility; whereas hybrids between more closely related species tend to be vigorous and more or less fertile. But in the latter the fertility is low, even when the two species have the same chromosome number, if they differ much in the structure of their chromosomes. It is, in fact, the existence of structural hybridity in certain species, combined with karyotype analysis, that provides conclusive evidence concerning the primary cause of reduction in chromosome number in Crepis. But, before that evidence is presented, mention should be made of the only successful attempt thus far to change the chromosome number artificially.

(*1*) *Experimental Reduction of Chromosome Number in Drosophila.* Dubinin (19), from an X-ray experiment on *D. melanogaster*, obtained a reciprocal translocation between the fourth and the Y chromosomes. Flies homozygous for it were viable and fertile. Detailed genetic analysis showed that this aberration could serve as the first step toward reduction from 4 to 3 pairs of chromosomes. Next the arm of the Y chromosome with the fourth chromosome attached was transferred to the X chromosome by means of crossing over. In this way a complex chromosome was built up, consisting of an X, part of one arm of the Y and all of the genetically active material of the fourth. This complex chromosome had only one kinetic body or centromere. By a special breeding procedure, females homozygous for this complex chromosome, and having the second and third pairs in normal condition, were obtained. By mating these with males carrying the aberrant Y that had lost part of one arm in the original translocation, a strain with only 3 pairs of chromosomes was produced. Dubinin was also able to obtain a 5-paired strain of this species by starting with a different reciprocal translocation; *i.e.*, one between an already abnormal X chromosome (having a small hyperploid region) and the fourth chromosome. In discussing these experiments Dubinin points out: "Both the reduction and augmentation in number of chromosome pairs were accomplished by the method of inducing aberrations within the inert region of the chromosome. This proves that the role played by the inert region (generally located near the kinetic bodies) is particularly important for the karyotype insofar as the evolution of the number of chromosomes seems to be closely linked with the fate of the kinetic bodies. We do not know of any experimental demonstration of chromosome fragmentation with *'de novo'* formation of kinetic bodies, or of chromosome fusion. The aberrations which occur in the inert material in the vicinity of the kinetic bodies may exclude from the karyotype the kinetic bodies

of a given chromosome pair without disturbing the gene balance or, on the other hand, they may free the kinetic body which, hence, can receive active sections of other chromosomes, forming a 'place of anchorage' for new chromosome pairs." Thus Dubinin has provided the all-important experimental proof that chromosome number *can* be reduced through reciprocal translocation between nonhomologous chromosomes, together with a reasonable hypothesis as to the roles played by the kinetic bodies or centromeres and adjacent inert regions in the process.

(*2*) *Structural Hybridity in Crepis.* Müntzing (29) studied meiosis in a hybrid between *C. divaricata* and *C. Dioscoridis*, both 4-paired species but classified in different sections of the genus (1). An average of only 1.8 bivalents was found at first metaphase in pollen mother cells, which indicates considerable lack of homology between the chromosomes of the two species. But chromatin bridges and fragments were observed in many cells, from which Müntzing inferred that the chromosomes of the two species have certain homologous segments. He suggested that the existence of such homologous segments in the chromosomes of different species provides a mechanism capable of producing chromosomal alterations of evolutionary value. Since interspecific hybridization is known to occur naturally in Crepis, at least to some extent (1), the origin of new chromosome types through the operation of this mechanism in natural hybrids has doubtless played a role in karyotype evolution and in the origin of species in this genus. This is a secondary process, however, as compared with the genesis of major chromosomal alterations within a species. Thus the most important question is: How did the first chromosomal alterations originate? Direct evidence is given below (p. 78) showing how this occurs within a species.

(*3*) *Crepis Kotschyana and its Close Relatives.* More convincing evidence concerning the relation between partial chromosome homology and reduction in chromosome number was discovered by Marta Sherman Walters (34). *C. Kotschyana* has only 4 pairs of chromosomes, but, on the basis of close morphological similarity, it had been classified in the same section as 6 other species, all of which have 5 pairs (1). The karyotypes of these seven species are shown in Fig. 3. Meiosis was regular in all seven species; but, in all of the F_1 hybrids between *C. Kotschyana* and the other six species, strong indications of partial chromosome homology were found. At first metaphase in the pollen mother cells of these hybrids, univalents, bivalents, trivalents, quadrivalents and higher polyvalents were observed. Also, at first and second telophase all 6 hybrids showed one or two chromatin bridges and one or more fragments in a certain proportion of the cells examined. By a careful analytical study of the paired homologous sections of chromosomes in the hybrids, indirect evi-

FIG. 3

Haploid Chromosomes of Seven Crepis Species, All of Which Belong in the Same Section on the Basis of Comparative Morphology of the Plants.

Reproduced from original photograph of Marta Sherman.

dence was obtained on the reciprocal translocations involved in the reduction from 5 to 4 pairs of chromosomes. Like most 4-paired species, *C. Kotschyana* lacks the short equi-armed member, designated as the *E* chromosome, which is present in all 5-paired species. It is the right-hand member of each 5-paired karyotype shown in Fig. 3. Evidence was found that parts of the *E* chromosome are present in three of the four chromosomes of *C. Kotschyana*, and that both arms of the *E* chromosome are represented. It was inferred, therefore, that, in the 5-paired ancestor of *C. Kotschyana*, three reciprocal translocations were involved in the eventual disappearance of the *E* chromosome and the reduction from 5 to 4 pairs. Because of the complicated history of the reducing process, this evidence

is not so clear as that which is presented below. But the evidence does strongly confirm the assumption that the 4-paired *C. Kotschyana* descended from the same 5-paired ancestral species as its six 5-paired relatives; and it appears highly probable that the process involved the disappearance of the *E* chromosome through reciprocal translocations between it and other chromosomes in certain plants of the common 5-paired ancestral species (34).

(*4*) *Origin of a 3-Paired Species from a 4-Paired Species.* H. A. Tobgy (39) investigated *Crepis neglecta* ($n=4$), *C. fuliginosa* ($n=3$) as well as F_1 and F_2 hybrids and certain forms found in nature. Fig. 4 shows, on the

FIG. 4

Crepis neglecta (right), *C. fuliginosa* (left), and reciprocal F_1 hybrids. Reproduced from original photograph of H. A. Tobgy.

right, a plant of *C. neglecta* and, on the left, one of *C. fuliginosa*, with reciprocal hybrids between them. These hybrids were extremely sterile, producing very few viable seeds, even though the two species are very closely related, owing to the difference in chromosome number of the parents. The chromosomes of the two species and of an F_1 hybrid are shown in Fig. 5, with the haploid set of *C. neglecta* at upper right and that of *C. fuliginosa* at upper left. It will be noted that the *neglecta* chromosomes are definitely wider than those of *fuliginosa* and that this difference persists in the hybrid, facilitating karyotype analysis. The *A* and *D* chromosomes of *neglecta* are structurally similar to the *A* and *D* chromosomes of *fuliginosa*, except for an unequal reciprocal translocation. The *B* chromosome of *fuliginosa* is mostly homologous with the *B* of *neglecta*, but it also contains the essential material of the *C* of *neglecta*. One arm of the *C* chromosome and its centromere present in *neglecta* are absent from

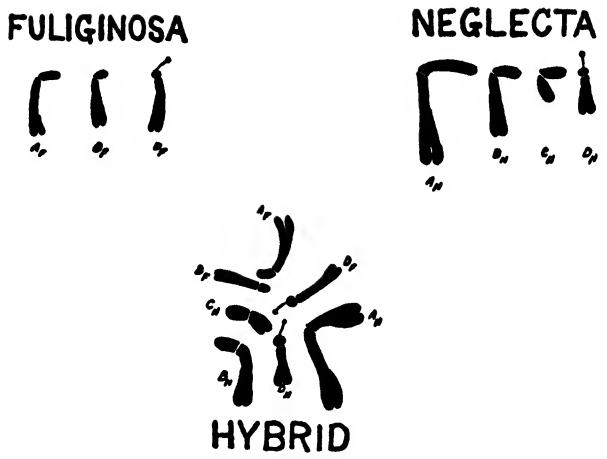

FIG. 5

Haploid chromosomes of *C. fuliginosa* and of *C. neglecta*. Diploid chromosomes of the F_1 hybrid.

Reproduced from original photograph of H. A. Tobgy.

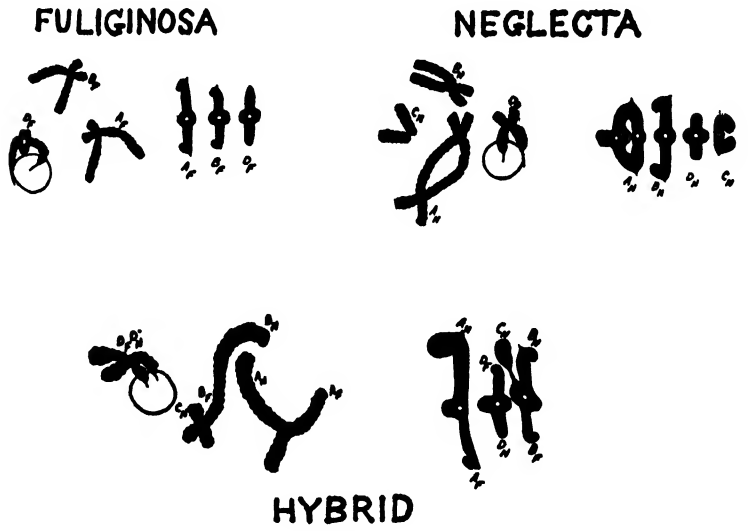

FIG. 6

Diakinesis and first metaphase in *Crepis fuliginosa*, *C. neglecta* and an F_1 hybrid.

Reproduced from original photograph of H. A. Tobgy.

the *fuliginosa* complement. These factual statements are based on a wealth of supporting evidence from Tobgy's analysis of meiotic figures in parents and hybrids (39). The convincing nature of this evidence is shown by Fig. 6, which illustrates diakinesis and first metaphase in *C. fuliginosa* and *C. neglecta*, in comparison with the same meiotic stages in an F_1 hybrid. In the last the trivalent consists of *neglecta B*, *fuliginosa B*, and *neglecta C*, and the pending disjunction of such a trivalent is shown on the right. These facts indicate that two chromosomes in *C. neglecta* are represented by just one chromosome in *C. fuliginosa*. Phylogenetically it may be assumed that the *B* and *C* chromosomes of *neglecta* gave rise to the *B* of *fuliginosa* through a reciprocal translocation between a distal segment of the long arm of the *B* and one arm of the *C*. Of the two chromosomes resulting from this translocation, the one with the *C* centromere is genetically inactive and is lost, while the other with the *B* centromere corresponds to the *B* chromosome of *C. fuliginosa* (39). Thus the direct dependence of reduction in chromosome number upon intraspecific chromosomal interchange has been experimentally proved in Drosophila and clearly demonstrated in Crepis. This most striking aspect of karyotype evolution, reduction in chromosome number, is certainly caused by structural changes in the chromosomes. But it should be emphasized that reduction in chromosome number *per se* is not a cause of speciation. The importance of structural changes in the chromosomes in the origin of species is due to the fact that they initiate intraspecific sterility, thus making it possible for other genetic processes to induce the morphological and physiological differentiation essential for speciation. This induction of intraspecific sterility by reciprocal translocations between nonhomologous chromosomes must be recognized as a primary isolating factor in the genus Crepis, and probably in other plant genera in which the diploid species include a progressively reducing aneuploid series. In such genera, therefore, geographic isolation is *not* the only isolating factor of primary importance in speciation (25, 30).

B. Genesis of Intraspecific Sterility

Gerassimova (21) discovered, among the progeny from X-rayed seeds of *Crepis tectorum*, two plants that were homozygous for different reciprocal translocations. One involved the *A* and *D* chromosomes and the other the *B* and *C* chromosomes. Both were morphologically identical with normal *C. tectorum* and equally fertile. By crossing these two plants, F_1 progeny were obtained in which each of the 4 chromosome pairs differed structurally but the plants differed from normal *tectorum* only in lowered fertility. Selfing these F_1 plants produced both normal plants and plants with all possible combinations of heterozygous and homozygous trans-

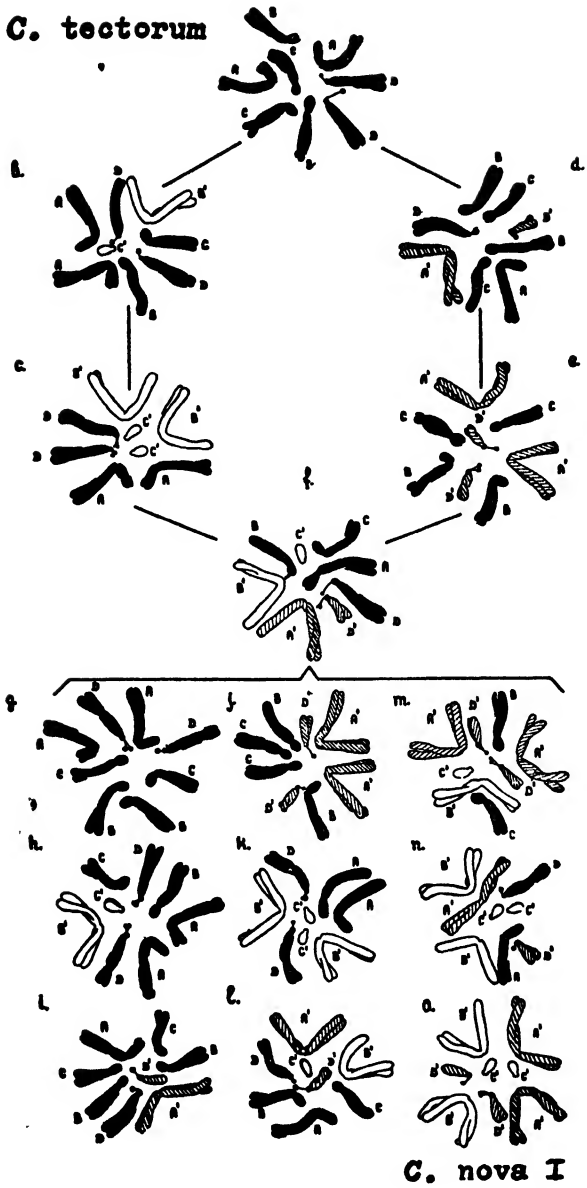

FIG. 7

Somatic chromosomes of *Crepis tectorum*, *C. nova I* and intermediate stages in chromosomal transformation (see text). Reproduced from a photograph kindly provided by Miss Gerassimova, the original of Fig. 1 in *C. R. Acad. Sci. U.R.S.S.* **25**, 150 (1939).

locations, including one which was homozygous for translocations in all 4 chromosome pairs and which was called *C. nova I.* This karyotypically new form was morphologically identical with normal *tectorum* and was equally viable and fertile. But, when *C. nova I* was crossed with normal *tectorum*, the F_1 hybrids were only 30% fertile when selfed and but slightly more fertile when open-pollinated. The salient features of this outstanding achievement are shown in Fig. 7. Meiosis in these hybrids agreed fully with expectations; and it is assumed that all gametes except those resulting from alternating distribution of the chromosomes to the poles are inviable. It is suggested, however, that part of the sterility may be due to other causes. At any rate, there certainly exists in these hybrids a highly efficient mechanism causing genetic isolation between two constant forms of the same species, *i.e.*, "a situation characteristic of a hybrid between two genuine species." And, although *C. nova I* is still morphologically indistinguishable from normal *C. tectorum*, "accumulation of mutational changes should undoubtedly lead in future to such distinction." That morphological and physiological differentiation between *C. tectorum* and *C. nova I* would inevitably result from gene mutations and that such mutations would also tend to increase the sterility of hybrids between them will be shown in the following section.

2. Gene Mutations

That gene mutations are of primary importance in the origin of Crepis species is shown by the roles they play in morphological and physiological differentiation and the accumulation of interspecific sterility. (It is also possible, if not probable, that gene mutations have played a role in the progressive reduction in *size* of the chromosomes which tends to accompany evolutionary advancement in this genus. See Babcock, Stebbins and Jenkins, 13.) The tremendous amount of differentiation that has actually occurred during the evolution of this genus can be rather definitely indicated by a brief characterization of the most primitive and most advanced species. The most primitive species are robust, rhizomatous, mesophytic perennials with large but short-lived achenes. They have existed since early Tertiary and have changed very little since Mid-pliocene. The most advanced species are small, delicate, extremely precocious, desert annuals with an ephemeral fibrous taproot and tiny long-beaked achenes which remain viable for several years. They are young species, having originated probably in late Pleistocene (1). These two groups of species, so strongly contrasted, both morphologically and physiologically, are few in number. The other species in the genus have been arranged in a phylogenetic series connecting the most advanced with the most primitive (1). They constitute living evidence that the desert annuals actually

evolved from the moisture-loving perennials. The evidence that gene mutations made possible the differentiation which, under favorable conditions, led to all this speciation may be summarized under *a*, interspecific lethal genes, *b*, groups of closely related species, and *c*, interspecific genic homology.

a. Interspecific Lethal Genes. The effect of an interspecific lethal was observed in the first experimental hybrids between Crepis species (6). But it was only after a thorough analysis that Hollingshead (22) proved the existence in *C. tectorum* of a Mendelian factor that is lethal in hybrids with *C. capillaris*. Since meiosis in normal *tectorum* is always regular, it may be assumed that this factor is not a structural difference but a gene. Evidence was also found that a lethal, presumably the same one, is effective in hybrids between *C. tectorum* and *C. bursifolia* and between *C. tectorum* and *C. leontodontoides;* but when *C. tectorum* plants known to carry the lethal were crossed with *C. vesicaria* and with *C. setosa* the lethal was not effective in the hybrids. Other data on interspecific hybrids indicate that similar lethals may exist in other species of Crepis, but these have not been investigated. Some of the strains of *C. tectorum* that carried the lethal were obtained from wild plants which had been collected in widely separated localities. Evidently the mutation involved must have occurred either long ago or more than once. Since the closest relatives of *C. tectorum* are *C. Bungei* and *C. ircutensis* (1), it is very desirable that hybrids between these three species be investigated. *C. tectorum* is the most advanced of the three species and, if the lethal is effective in hybrids between it and the other two, the probability of its importance in the evolution of *tectorum* would be great. However, the role played by such lethals in Crepis seems to have been insignificant, compared with the great mass of gene mutations, affecting all parts of the plant, which provides most of the material for intra- and interspecific differentiation.

b. Groups of Closely Related Species. Certain species of Crepis are polymorphic, consisting of distinct geographical races, or subspecies, which have identical karyotypes but which have been shown to differ from one another in certain genes (1, 2). That such species are in process of differentiation, which may lead to speciation when isolation of some kind prevents syngamy, is certainly indicated by two groups of very closely related Crepis species. In the first group, the 3 species studied — *viz.*, *C. foetida*, *C. eritreënsis* and *C. Thomsonii* — are widely separated geographically (5). Although similar morphologically, they exhibit marked differences in their involucres, flowers and fruits as well as other parts; and genetic evidence shows that they differ from one another in numerous genes. They have closely similar karyotypes, and in F_1 hybrids between them meiosis is highly regular. It happens that *C. foetida* is a polymorphic

species comprising 3 subspecies. The fertility of the hybrids between these subspecies and the other two species varies from high in some to low in others. Furthermore, both of the other two species show more morphological resemblance to *C. foetida vulgaris* than to the other two subspecies, but subspecies *vulgaris* is farther removed from them geographically, which indicates a phylogenetic connection fairly remote in time. The second group (26) consists of three endemic species, isolated on the Madeira and Canary Islands, *C. canariensis*, *C. Noronhaea* and *C. divaricata*, together with two subspecies of *C. vesicaria* — *viz.*, *andryaloides* and *taraxacifolia*. Just as in the three species considered above, these five entities are, in general, similar morphologically but exhibit many differences affecting all parts of the plant; their chromosomes are similar and both parents and hybrids have a high degree of meiotic regularity; and they differ in many genes affecting both quantitative and qualitative characters. In experimental F_1 hybrids the average fertility, indicated by the proportion of open-pollinated seed to the potential set, was 25–50%. The least fertile hybrids had only 1–2%, and the most fertile 50–75%, compared with nearly 100% in all the parents. Thus interfertility between all four species has been definitely though not completely reduced. It is inferred that this has been the result of gene mutations, because the available cytological evidence certainly indicates that the five entities have a similar arrangement of genes in all their chromosomes. Detailed pachytene studies have not been made, however, and it is possible that minute structural differences are also present in these species. Nevertheless, it is a fair inference that much of this interspecific sterility is due to gene differences, since many such differences are known to exist between the species in both of these groups. Other evidence (10) reveals similar partial sterility between certain morphologically diverse variants in *C. capillaris* and *C. tectorum*. In both morphological and physiological differentiation, within and between species of Crepis, there can be no question that gene mutations are of major importance; and it is highly probable that they have been of equal importance in building up interspecific sterility.

c. Interspecific Genic Homology. Comparative genetics has not been a major feature of the Crepis investigations, but evidence has been summarized (1) on segregation in hybrid populations, indicating genic homology between different species, which involves such features as leaf outline, anthocyanin, chlorophyll reduction, erect *vs.* nodding flower-buds, paleae on the receptacle, self-incompatibility and plant stature and other size differences. It has also been demonstrated (1) that genes can be transferred between various Crepis species and that, when meiosis is regular and the fertility of the hybrids sufficient to produce numerous

progeny, either ordinary Mendelian or multiple-gene inheritance occurs. Hence, it may be inferred that the important genetic process involved in differentiation between such species is gene mutation. Furthermore, a statistical study (1) of the degree of fertility observed in 195 hybrids involving 55 species, representing 17 of the 27 sections into which the genus has been divided, shows strong correlation between degree of hybrid fertility (or sterility) and degree of taxonomic relationship of the parental species, as determined primarily on the basis of comparative morphology and secondarily by comparing the chromosomes. Only 17.5% of the intrasectional hybrids, as compared with 100% of the intersectional hybrids, were of low fertility or sterile. This certainly indicates that the 17 sections represented in these crosses contain species that are more closely related genetically to one another than to the species of other sections. That this relationship is based on gene homology is definitely indicated by studies of meiotic chromosome pairing in eleven interspecific hybrids (7). The results of these studies indicate that the genic complements of the 14 species involved are all more or less homologous. This inference is consistent with the evidence on chromosome morphology in the genus (27, 9, 24, 4, 8), and on geographic distribution (1), and with the results of genetic investigations on small groups of closely related species (5, 26). The evidence on the meiotic pairing of the chromosomes supports the conception that the species of Crepis had a common origin and are still more or less similar in genic constitution. At the same time, the variation in number of bivalents counted at first metaphase in these hybrids indicates marked differences in the degree of genic homology between the parental species. In view of the evidence summarized above on groups of closely related species, it seems inevitable that these specific differences in genic homology have developed largely through gene mutations.

3. Interspecific Hybridization and Speciation

With regard to the number of species of Crepis and their mode of origin, interspecific hybridization has been of much less importance than either intraspecific chromosomal alterations or gene mutations. That it has played a certain role, however, is shown by several lines of evidence, which are summarized under the following heads: *A*, experimental hybrids; *B*, natural hybrids..

A. Experimental Hybrids

Among the experimental crosses (1) that have been made between different Crepis species, 55 produced viable seeds, most of which developed into plants, and 65 failed to produce any progeny even though the

number of seeds obtained in many cases was large (ranging from 35 to 95). Crossability, however, is not a dependable index of relationship, since it is well known that many species have been crossed experimentally only after repeated attempts, using various strains. But the fact that hybrids have been obtained by crossing so many species, some of which belong in widely separated sections of the genus (1), certainly indicates that many species may have hybridized naturally during the evolution of the genus. And, if the species involved were closely related, the probability that the hybrids were partly fertile is good. This serves to emphasize the importance of some sort of isolation, either spatial or physiological, as a factor in speciation. In this connection, the suggestion of Müntzing (29) that interspecific hybridization must tend to augment the process of chromosomal alteration finds added significance. In addition to these general implications of the results obtained by experimental hybridization, there are certain specific products of this research which will be considered under the following heads: *a*, potential new species; and *b*, artificial amphidiploids.

a. Potential New Species. One of the earliest products of experimental hybridization in the genus was *Crepis artificialis* (16, 17, 18), which was derived from the cross, *C. setosa* ($n=4$)$\times$*C. biennis* ($n=20$) as a result of the peculiar distribution of the chromosomes to the gametes in these hybrids. Autosyndesis among the 20 *biennis* chromosomes caused each gamete to receive 10 from that species, whereas the 4 *setosa* chromosomes were distributed at random. Through chance recombination, an F_4 segregant was obtained that had 24 chromosomes, consisting of 20 *biennis* and 2 pairs of *setosa* chromosomes. Selfing this plant produced nearly uniform progeny; and after the first few generations had been found to be fairly uniform it was planned to test the "new species" under natural conditions. Later, however, considerable variation was observed among progenies from selfed plants, and, as a result of testing selected individuals, it was possible to obtain strains with diploid numbers ranging from 20 to 36. Some of the strains thus established appear to be fairly uniform morphologically; but cytogenetic studies have not been made and the cause of the breaking up of *C. artificialis* into these new types has not been ascertained. In any event, the mode of origin of the original *C. artificialis* is not likely to have been duplicated in nature, because the polyploid species of Crepis are few and mostly isolated. Even *Crepis biennis*, which is widespread in Europe, seems to have been involved in very few natural hybrids (p. 87). It is possible, however, that partly fertile natural hybrids between diploid species may have provided new starting points for speciation. Certain hypothetical cases of this sort are discussed below (p. 88). In Tobgy's research (39) on *C. neglecta*$\times$*C. fuliginosa* (p. 78) it was possible

to obtain 64 F_2 and backcross plants from the highly sterile F_1 hybrids. Among them were found both parental and F_1 types, as well as numerous new forms with chromosome numbers ranging from 6 to 11. One of the F_2 segregants was similar in morphology and karyotype to a plant found in a culture grown from seeds collected in the wild in northeastern Greece, where it is known that the two species have come into contact (1). This particular form is about 70% fertile. It has a *neglecta* karyotype, but one or more chromosomes carry *fuliginosa* segments, which explains the presence of certain *fuliginosa* characters. The occurrence of a duplicate of this wild form among the F_2 segregants indicates the method of origin of intergrades occurring in nature. In addition to the above form, which was produced by two successive crossovers in the same chromosome arm, several new forms with changed karyotypes, resulting from single crossing over, have been obtained among the progeny. Although it is not known how extensively these new types occur in nature, the fact of their existence does indicate the possibility that new species could originate as the direct result of interspecific hybridization, provided that some kind of isolation favored their perpetuation.

b. Artificial Amphidiploids. Amphidiploids have arisen spontaneously from three different interspecific hybrids: *Crepis capillaris*×*C. Dioscoridis* (10); *C. capillaris*×*C. tectorum* (23); and *C. rubra*×*C. foetida* (32, 33). The first two were sterile. The third produced some seed but the most fertile second-generation plants obtained were not over 10% fertile. It was concluded that stable races are not likely to be derived from this amphidiploid. But that amphiploidy has been of importance in the origin of certain Crepis species is shown below.

B. Natural Hybrids

The evidence on natural hybrids in Crepis will be presented under three heads: *a*, named hybrids; *b*, assumed hybrid origin of other Old World species; *c*, hybrid origin of the American species.

a. Named Hybrids. Latin names have been given to at least ten natural interspecific hybrids in Crepis. Some of the original descriptions have not been available on account of war conditions; but, so far as is known, all except two of them are hybrids between diploid species. One of these, ×*C. turicensis* Bruegg. (1), is said to be a hybrid between *C. biennis* and *C. vesicaria* subsp. *taraxacifolia*.

b. The Assumed Hybrid Origin of Other Old World Species. (1) *C. syriaca* was reported by Cameron (15) to have a basic complement of 5 pairs of chromosomes and a variable number of supernumeraries. He suggested that most probably it originated through natural crossing of two different races of its close relative, *C. alpina* ($n=5$), followed by

chromosomal alterations in the progeny. (2) *C. biennis*, an octoploid with the base number $x=5$ (17), was assumed (12) to have originated as an amphidiploid hybrid between two species with $n=5$ and to have doubled its chromosome number a second time. The assumption of an amphiploid rather than an autoploid origin is consistent with the data on geographic distribution, since *C. biennis* is a widespread species, whereas its close relative, *C. ciliata*, which may be an autoploid, has a comparatively limited distribution. (3) *C. paludosa* has long been recognized as a unique species, in that it resembles Hieracium in its achenes and pappus although otherwise it is Crepis-like. Since it is a primitive species, it may represent a product of the differentiation processes that separated Crepis and Hieracium, or it may be the result of hybridization between a species of Crepis and one of Hieracium at a time when they were still partly interfertile. The chromosomes of *C. paludosa* are morphologically typical of 6-paired Crepis species. If a 6-paired Crepis species did cross with a species of Hieracium having 8 or 9 pairs, the only functional F_1 gametes would probably be those containing all or mostly either Crepis or Hieracium chromosomes; but there might have been an interchange of one segment between a Crepis and a Hieracium chromosome, which provided *C. paludosa* with the genes conditioning its Hieracium-like achenes and pappus. (4) Section 12, Ixeridopsis, and section 18, Pyrimachos, as classified by Babcock (1), are assumed to have originated through intergeneric hybridization at a time when the species involved in the crosses were still somewhat interfertile. Three of the species in section 12 are known to have $n=7$ chromosomes (p. 72) and it is safe to predict that their four close relatives will be found to have the same number. On the basis of plant morphology, however, these seven entities are not among the most primitive species of the genus. It has been suggested (14) that there is a close genetic connection between these 7-paired species and the 7-paired *Ixeris alpicola* as a result of hybridization when Ixeris and Crepis were in a formative period, a suggestion compatible with the chromosome number, plant morphology and geographic distribution of the two groups. Section 18 presents a similar problem. None of the five species comprising it has been studied cytologically, but morphologically they exhibit some evidence of relationship, particularly in their achenes, with Youngia and Ixeris, both of which genera occur in the same general region. It is not improbable that they have been derived from a common ancestral stock through interspecific hybridization (1).

c. The Hybrid Origin of the American Species. There are 12 native American species of Crepis. Two of these belong in section Ixeridopsis and have 7 pairs of chromosomes; the probability that they originated through interspecific hybridization has been mentioned. The other 10

species are unique as to chromosome number, since they all have the base number $x=11$. It was suggested by Babcock and Navashin (10) that these species arose as amphidiploids from hybrids between Asiatic or extinct American species with lower chromosome numbers, probably $n=4$ and 7. This hypothesis was strongly supported by the later monographic work on the group (11, 37). One of the ten species, *C. runcinata*, consists of 7 subspecies, all with the same chromosome number ($2n=22$). There is no close connection between it and the other 9. The latter include 7 diploids with the somatic chromosome number $2n=22$. Although these diploid forms are entirely distinct from one another, they are connected by several series of intergrading polyploid forms, which are partly or wholly apomictic, with somatic chromosome numbers ranging from 33 to 88. These heteroploid complexes will be mentioned again. Here it is sufficient to point out that the ten American species with $x=11$ must have originated through interspecific hybridization and amphidiploidy. Probably they were not all derived from the same 4-paired×7-paired hybrid, however, since each of the 7 diploids shows more or less resemblance to *different* species of eastern Asia with $n=4$ or $n=7$. Two of the species that have been recognized in this section are assemblages of polyploids. Reviewing the above evidence on natural interspecific hybrids in Crepis, we find the following for which a hybrid origin has been assumed: the 10 American species; the 10 Old World species which have been given Latin names; and 15 other species, including those in sections 12 and 18. This makes a total of 35, or 18% of all the species in the genus. It will be shown below that polyploidy and apomixis have been responsible for the origin of still fewer species of Crepis. Interspecific hybridization certainly ranks next in importance to intraspecific reciprocal translocations and gene mutations in the history of this genus.

4. *Polyploidy*

Only 15 of the 113 species of Crepis that have been studied cytologically are polyploids; and from their morphology it appears likely that most of the other 83 species are diploids. In fact, section 18, with 5 species, is the only group among those not yet studied cytologically that stands out as very probably composed of polyploid species. Of the 15 known polyploids, 10 are American species; and of the other 5 it is possible that 3 or 4 originated as autoploids, but this is very uncertain. The situation in regard to *C. biennis* and *C. ciliata* was mentioned on p. 88. The great difference in their distributional area suggests that the former originated as an amphiploid and the latter as an autoploid, but sufficient cytogenetic evidence to establish this hypothesis is lacking. In the 10 American species with $x=11$, however, amphiploidy and alloploidy have

been of great importance in speciation. The basic somatic number, $2n=22$, must have originated through interspecific hybridization and amphiploidy. The polyploids found in these heteroploid complexes are of two sorts: a few, judging from their morphology, are autoploids since they are identical with the diploids except in their larger size; by far the most are alloploids since they combine the characteristics of two or more diploids. The autoploids usually do not occur outside the province occupied by the corresponding diploid, whereas the alloploids show by their distribution the combination through hybridization of the physiological characteristics that determine their distribution (11). Briefly, the history of this small but extremely differentiated group of species was presumably as follows. The original amphiploids ($2n=22$) originated in eastern Asia and migrated during Miocene or Pliocene to northeastern North America. Then two processes began. First, they hybridized to produce more or less sterile progeny, and at the same time they produced some autoploid offspring. Second, by means of chromosome doubling in the F_1 ($2n=22$) hybrids, or by hybridization of autoploids of different species, or between the autoploid of one species and the diploid of another, the various intermediate alloploids were produced. In other words, differentiation and speciation, in so far as speciation has actually occurred, have been determined by hybridization, polyploidy and apomixis, along with the selective effects of the environment. But speciation, in the sense of divergent evolution, has been at a standstill; this will be discussed further in the following section.

5. *Apomixis*

One of the most striking features of these 10 American species is the prevalence of apomixis. Following the production of autoploids and alloploids from the 7 original 11-paired species, the complex began to break up into "microspecies," the individuals of which are identical with one another but are separated from most nearly related "microspecies" by small differences. In their method of reproduction and their polymorphism, these apomictic forms resemble those of the Hieracium subgenus Pilosella (37). In this process of formation of microspecies, somatic apospory is followed by parthenogenesis (38). There is no direct evidence as to how the polyploid forms became apomictic; but the fact that some of the forms which are autoploid (judging from their morphology) are strongly apomictic indicates that apomixis was not caused by hybridization. As the various apomictic forms appeared, they must have been subjected to rigid selection, and only those forms that were able to occupy the ecological niche where they occurred were able to survive. Since most of these can produce a small percentage of sexual offspring (38), they

can still hybridize with each other to produce new apomictic forms. The ultimate fate of such an agamic complex in which the ancestral forms have become very restricted can be foreseen; it will flourish as long as conditions remain favorable, but it will be unable to meet new changes in the environment and will therefore gradually become more restricted and eventually die out. In Crepis, apomixis has been an unimportant cause of speciation. In addition to the 8 species based on the original 11-paired diploids, it has only been necessary to recognize as "species" two other assemblages of polyploid apomicts — namely, *C. intermedia* and *C. barbigera* — in order to arrive at a practical solution of this taxonomic problem. The only other group of Crepis species in which apomixis is suspected to occur is section 18, Pyrimachos, of southeastern Asia. This consists of only 5 species at present, and it is probable that the situation is comparable to that known to exist in the American species. It is clear, therefore, that apomixis is not a major cause of evolution, however important it may be in increasing polymorphism and geographic distribution (35).

IV. Summary

Speciation is a very complex aspect of organic evolution, in which 5 different processes of genetic change play more or less important roles. In Crepis, a monophyletic genus, cytogenetic research has thrown new light on the relative importance of these processes of genetic change. (1) *Reciprocal translocations* have been of fundamental importance by initiating interspecific sterility leading to genetic isolation and hence to speciation. They have also been primarily responsible for karyotype evolution, resulting in the series of haploid chromosome numbers, 6, 5, 4, 3, and in progressive asymmetry of the chromosomes. Through the genesis of hybrid sterility, resulting from the primary steps in reduction in chromosome number, reciprocal translocations have contributed directly or indirectly to the evolution of the whole genus. (2) *Gene mutations* have made possible the morphological and physiological differentiation within and between species which was essential to speciation throughout the genus. They have also contributed to the accumulation of interspecific sterility, and possibly to the reduction in absolute size of the chromosomes. Both gene mutations and reciprocal translocations, therefore, have been of primary importance in the evolution of this group of species. (3) *Interspecific hybridization* has been involved in the origin of certain Crepis species, perhaps to the extent of 1/5 of all the species in the genus. Undoubtedly it has also played a certain role in the development of further chromosomal alterations following the initial step of reciprocal translocation. Compared with the first two processes, however, interspecific hybridization has been

of secondary importance in this genus. (4) *Polyploidy* has been involved in less than 8% of the species of Crepis. But, in certain small groups of species, it has been an important cause of differentiation and extension of distributional areas. (5) *Apomixis* has been of practically no importance in speciation, in the sense of divergent evolution; although the partial sexual fertility of many of the apomictic forms has resulted in a multitude of "microspecies" and of intergrading forms, which complicate the taxonomic treatment of some of the American species.

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Speciation in Fishes

Distribution in Time and Space of Seven Dominant Multiple Alleles in *Platypoecilus maculatus*

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CONTENTS

I. INTRODUCTION

In a preliminary report on the genetics of speciation in the Mexican swordtail-platyfish group, Gordon (1943) indicated that the large series of color patterns in *Platypoecilus maculatus* are inherited and they may be used as indices in the analyses of the genic composition of the natural fish populations in the four great river systems of southern Mexico and Guatemala.

Gordon (1931) described the morphological and histological features of four heritable patterns of *P. maculatus* which appear in the posterior

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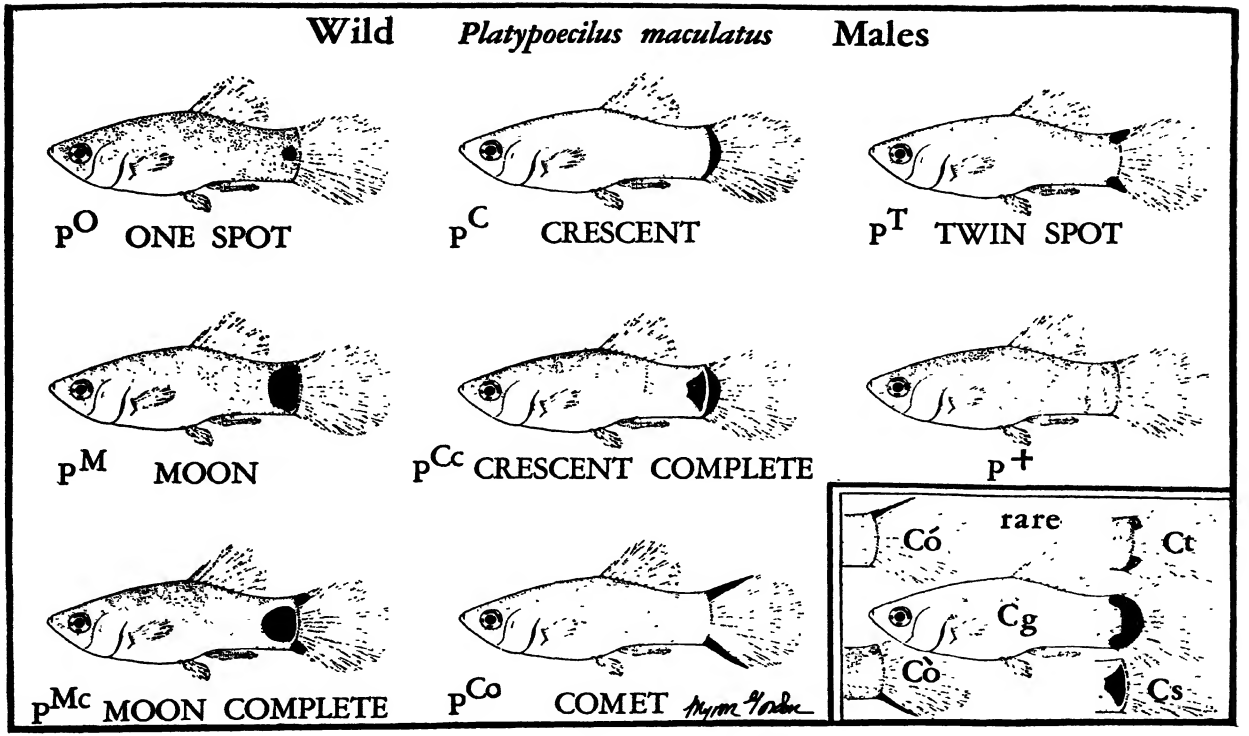


FIG. 1

The Seven Tail Patterns and Their Genes Make Up a Multiple Allelic Series in Wild *Platypoecilus maculatus*.

All are dominant to the universal recessive which is indicated as P^+. The five patterns shown in the lower right hand corner are rare; most of them are known only from preserved material. *Cg* represents the Guatemala crescent pattern, a counterpart of which is apparently present in domesticated platyfish. These figures represent individual or single patterns. No more than two of the first seven patterns were found in any fish.

part of the fish's body, partly on the caudal peduncle and partly on the anterior portion of the caudal fin. The pattern names and the genetic symbols now used for them are as follows: one-spot, P^{O}; twin-spot, P^{T}; crescent complete, P^{Cc}; and moon complete, P^{Mc}.

Gordon and Fraser (1931) showed that the four genes constituted a dominant multiple allelic series. Three more patterns may now be added to the group since they, too, appear at the same region of the body and recent genetic experiments have shown that the corresponding genes belong to the same allelic series. They are crescent, P^{C}; moon, P^{M}; and comet, P^{Co}. All are illustrated in Fig. 1.

All seven patterns are formed by precise groupings of many small melanophores. Fortunately the melanic patterns so produced are unaffected by preserving fluids. As a result it has been possible to utilize and examine collections of *P. maculatus* in various museums and classify the fish with respect to this and other series of heritable patterns. In this way a comparison of their gene frequency distributions at various time levels has been made of populations from the same general area; some collections were taken 70 years apart.

II. Material and Methods

Platypoecilus maculatus was first described by Gunther in 1866 on the basis of two female specimens sent to the British Museum by La Salle from "Central America." In his original definition of the species Gunther described the patterns, in part, as follows: "with a roundish black spot on the middle of the root of the caudal" (perhaps this was P^{O}); "dorsal sometimes densely spotted with black" (this probably is the sex-linked pattern factor *Sd*); "the lower margin of the anal and the upper and lower margin of the caudal black" (the marking of the anal is being studied — but the marginal markings of the caudal is probably the pattern gene P^{Co}). These variable markings probably account for Gunther's choice of the word "maculatus" for the name of the species. His specimens were not examined personally but these details are mentioned here to show the platyfish was a marked species from the day of its discovery.

In 1867 Francis Sumicrast, collecting for the Smithsonian Institution of Washington, obtained 13 variously marked *P. maculatus* from the Rio Papaloapan at Cosamaloapan in the State of Veracruz. This extremely important population was studied through the courtesy of the officials of the National Museum in Washington. The list of single patterns and their frequencies in this collection, and in those to follow, are indicated in Table II.

In 1902 Seth E. Meek collected a series of 68 *P. maculatus* in the vicinity of El Hule, Oaxaca (now Papaloapan, Oaxaca) from the Rio

TABLE I

Genetic Evidence for Multiple Allelic Series for Seven Genes in Platypoecilus maculatus

No.	Cultures	P_1 ♀♀	P_1 ♂♂	F_1 ++	O+	M+	Mc	C+	Cc+	Co+	T+	OM	OMc	OC	OCc	OCo	OT	MC	MCo	MT	McT	McCo	CCo	CT	CcCo	CcT	CoT	Totals	χ^2
1	39–28	*OC*	*Co+*	..	21	..	..	25	..	..	..	..	..	..	..	17	..	..	..	..	..	..	21	..	..	..	..	84	1.52
2	39–38	++	*OC*	..	17	..	..	17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	34	0.00
3	39–36	++	*OC*	..	37	..	..	33	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	70	0.23
4	39–29	*OCc*	*Cc+*	..	12	..	..	..	18	..	..	..	..	..	10	..	..	..	..	..	..	..	..	..	..	..	..	40	0.60
5	39–19	*OCo*	*O+*	..	29	..	..	..	..	19	..	..	..	..	..	11	..	..	..	..	..	..	..	..	..	..	..	59	2.19
6	39–20	*OCo*	*O+*	..	20	..	..	..	..	10	..	..	..	..	..	8	..	..	..	..	..	..	..	..	..	..	..	38	0.32
7	39–32	++	*OCo*	..	15	..	..	..	..	11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	26	0.62
8	39–71	++	*OCo*	..	16	..	..	..	..	19	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	35	0.26
9	39–34	++	*OT*	..	18	..	..	..	..	..	25	..	..	..	..	..	1*	..	..	..	..	..	..	..	..	..	..	44	1.46
10	39–45	++	*OT*	..	31	..	..	..	..	..	25	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	56	0.64
11	39–13	*CCo*	*O+*	..	..	..	..	12	..	14	..	..	..	8	..	10	..	..	..	..	..	..	..	..	..	..	..	44	1.82
12	39–22	*CCo*	*T+*	..	..	..	..	10	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	..	8	32	0.75
13	39–73	++	*CCo*	..	..	..	..	31	..	38	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	69	0.71
14	39–26	*CT*	*O+*	..	..	..	..	14	..	..	12	..	..	12	..	..	10	..	..	..	..	..	..	..	..	..	..	48	0.67
15	39–17	*CcCo*	++	..	..	..	..	..	23	28	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	51	0.49
16	32–3	*MC*	++	..	..	9	..	12	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	21	0.43
17	32–5	++	*MCc*	..	..	15	..	..	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	29	0.03
18	32–9	*C+*	*OM*	..	6	7	..	..	..	..	..	..	..	7	..	..	..	3	..	..	..	..	..	..	..	..	..	23	1.08
19	32–11	*M+*	*O+*	3	4	6	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	16	1.50
20	32–23	*Co+*	*Mc+*	13	..	..	14	..	..	10	..	..	..	..	..	..	..	..	..	..	..	..	9	..	..	..	..	46	1.48
21	32–2	++	*CcCo*	..	..	..	..	..	15	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	29	0.03
22	32–7	*OCo*	++	..	9	..	..	..	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	16	0.25
23	32–14	*OCo*	++	..	12	..	..	..	..	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	26	0.15
24	32–18	*CCo*	*O+*	..	..	..	..	5	3	..	..	..	..	8	8	..	..	..	..	..	..	..	..	..	..	..	..	24	3.00
25	32–15	*CoT*	....	..	..	..	..	..	..	13	11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	3	31	....
26	32–4	*Co+*	....	3	..	10	..	..	..	5	..	..	..	..	..	..	..	..	7	..	..	..	..	..	..	..	..	25	....
27	32–5	*OT*	....	..	7	..	..	..	..	..	7	4	..	..	..	4	..	..	..	4	..	..	..	..	..	..	3	29	....
28	32–8	*CCo*	....	..	..	..	..	7	..	6	..	..	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	16	....
29	32–10	*T+*	....	..	8	..	..	..	..	..	4	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	..	15	....
30	32–13	*OCc*	....	..	6	..	..	..	5	5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..	20	....
31	39–21	*O*	....	..	109	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	109	....
32	39–24	*C*	....	..	3	..	..	8	..	..	..	..	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..	18	....

33	39–25	*Cc*	....	1	2	..	..	2	..	..	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	7	....
34	39–14	*Cc*	....	30	3	..	..	..	23	..	..	..	..	..	5	..	..	..	..	..	..	..	..	..	..	..	..	61	....
35	39–15	*Cc*	....	3	3	..	..	..	5	..	..	1	..	..	7	..	..	..	..	..	..	..	..	..	..	..	..	19	....
36	39–16	*Cc*	....	8	2	..	..	..	9	2	..	..	..	..	3	..	..	..	..	..	..	..	..	..	1	..	..	25	....
37	39–12	*Co*	....	20	23	..	..	..	..	25	..	..	..	..	..	19	..	..	..	..	..	..	..	..	..	..	..	87	....
38	39–11	*CoT*	....	..	..	..	..	..	..	14	11	..	..	..	13	11	..	..	..	..	..	..	..	..	..	..	..	49	....
39	39–18	*CoT*	....	..	..	..	..	..	..	22	11	..	..	..	5	..	..	..	..	..	..	..	..	..	..	..	13	51	....
40	39–23	*CoT*	....	..	..	..	..	..	..	1	3	..	..	..	..	1	12	..	..	..	..	..	..	..	..	..	..	17	....
41	495, 522, 522.1	*O+*	++	63	65	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	128	0.31
42	494, 535	*T+*	++	62	..	..	..	..	..	..	61	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	123	0.01
43	Below	*Mc+*	++	347	..	..	268	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	615	10.16
44	492	*McMc*	++	..	..	..	95	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	95	0.00
45	709, 840, 971	*OO*	*OO*	..	182	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	182	0.00
46	1222	*Mc+*	*OO*	..	29	..	..	..	..	..	..	..	27	..	..	..	..	..	..	..	..	..	..	..	..	..	..	56	0.71
47	611	*OMc*	*OO*	..	59	..	..	..	..	..	..	..	79	..	..	..	..	..	..	..	..	..	..	..	..	..	..	138	1.45
48	546, 598	*OMc*	*O+*	..	78	..	52	..	..	..	..	..	48	..	..	..	..	..	..	..	..	..	..	..	..	..	..	178	2.90
49	547, 548, 566	*McT*	*O+*	..	..	..	21	..	..	..	30	..	30	..	..	..	19	..	..	..	..	..	..	..	..	..	..	100	4.08
50	604	*OMc*	*OT*	..	10	..	..	..	..	..	..	..	16	..	..	..	12	..	..	..	10	..	..	..	..	..	..	48	2.00
51	602, 610	*OMc*	*OMc*	..	34	..	32	..	..	..	..	..	75	..	..	..	..	..	..	..	..	..	..	..	..	..	..	141	0.74
52	462, 588	*OT*	++	..	23	..	..	..	..	..	18	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	41	0.61
53	Below	*OMc*	++	..	141	..	140	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	281	0.04
54	550, 715	*McT*	++	..	..	..	43	..	..	..	58	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	101	2.23
55	463.1, 2	*Mc+*	*McMc*	..	..	..	75	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	75	0.00
56	Below	*Mc+*	*Mc+*	107	..	..	271	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	378	2.21
57	524	*McMc*	*T+*	..	..	..	..	..	..	..	5	..	..	..	..	..	..	..	..	..	5	..	..	..	..	..	..	10	0.00
58	462.1	*T+*	*T+*	2	..	..	..	..	..	..	16	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	18	2.85
59	567	*Mc+*	*O+*	23	15	..	21	..	..	..	..	..	24	..	..	..	..	..	..	..	..	..	..	..	..	..	..	83	2.35
60	594	*T+*	*O+*	12	7	..	..	..	..	..	16	..	..	..	..	..	18	..	..	..	..	..	..	..	..	..	..	53	5.35
61	575, 599, 578	*OMc*	*T+*	..	34	..	25	..	..	..	..	..	..	..	..	..	23	..	..	..	17	..	..	..	..	..	..	99	5 91
																											4482	

43 463.1.1–475–489–490–496–507–523–525–526–527–465.1–573–617–630–722
53 532–576–577–579–593–596
56 450.1–463.1–492.1–492.2–492.3

Matings listed from 1 to 15 and 31 to 40 indicate test of wild platyfish from the Río Jamapa. Matings number 16 to 30 refer to tests of wild platyfish from the Río Papaloapan. Data for matings numbered 41 to 61 were furnished by Dr. A. W. Bellamy of the University of California at Los Angeles; these represent "domesticated" platyfish.

*Phenotypically *OT*; when tested proved to be *O*.

The author wishes to acknowledge with thanks the aid of Miss Jo Miller and Mr. Hugh Gordon in the preparation of this table and those that follow.

Papaloapan. Later (1904) he said that this species had a remarkable series of color markings — more so than in any other he had ever seen. Gordon and Fraser (1931) analyzed Meek's specimens (through the courtesy of The Chicago Natural History Museum) and suggested a genetic basis for the variability.

In 1932 the author, assisted by Joseph Whetzel and John Ross of Ithaca, New York, on an expedition made possible by funds provided by the Heckscher Research Foundation of Cornell University and a group of aquarium societies, collected 101 *P. maculatus* in a tributary of the Rio Tonto, a main branch of the Rio Papaloapan about 10 miles from the village of Papaloapan, Oaxaca.

In 1939 the author assisted by Evelyn Gordon and James Atz of the New York Aquarium, on an expedition made possible by a John Simon Guggenheim Memorial Foundation Fellowship, made a number of large collections of this species (see Gordon 1940). One series of 860 specimens was taken from the Rio Jamapa, near the city of Veracruz at the northernmost limit of its range. Six series, totaling 3,492 adult specimens, were caught in various parts of the Rio Papaloapan system but mostly from the vicinity of Papaloapan, Oaxaca, the same general locality from which previous collections in 1867, 1902 and 1932 were made. These four collections, made at various times, made possible an analysis of the temporal factor in gene frequency distributions.

A collection of 115 specimens from the Rio Coatzacoalcos was made in 1939 by Señor Salvador Coronado, formerly of the Mexican Government fisheries staff. This is the first series ever collected for scientific purposes from this river.

The last group, consisting of 552 specimens, was taken in 1935 from the great Rio Usumacinta system, chiefly in Guatemala at the eastern and southernmost limit of the species' range. These specimens were made available by the Museum of Zoology at the University of Michigan and I wish to express my thanks to the collectors: Doctors Carl L. Hubbs, Henry van der Schalie and Josslyn Van Tyne.

Most of the specimens collected by our expedition were preserved in formalin at the site of the capture. Later, in the laboratory, they were transferred to 70% alcohol and at this time a detailed study of the pattern frequencies was made. Since the patterns are melanic, and genetic experiments in the past have shown all of them to be dominant, the pattern or combination of patterns could be read at a glance from the specimen. Some of the difficulties in determining the genetic constitution of a few pattern combinations accurately will be mentioned later.

In 1932 a shipment of living *P. maculatus* was successfully made to our laboratory then at Cornell University, Ithaca, New York, from a

tributary of the Rio Tonto of the Rio Papaloapan system, about five miles from Papaloapan, Oaxaca. The results of genetic analysis of this fish population are given in Table I, items 16–30.

In 1939 another shipment of living *P. maculatus* was successfully made to our New York Aquarium laboratory from a tributary of the Rio Jamapa at Plaza de Agua (El Tejar), a few miles from the city of Veracruz. The results of genetic analysis of this fish population are given in Table I, items 1–15 and 31–40.

III. Genetic Analysis

1. *Introduction*

Platyfish are viviparous; fertilization is internal. After mating, a complement of ripe eggs, consisting of about 40 to 60, is fertilized while other sperms are stored within the folds of the females' oviducts. The sperms are viable for a long time and are capable of fertilizing successive complements of eggs as they ripen. Isolated females, once mated, often produce broods about every month for about four months.

A large number of living mature female platyfish brought to the laboratory were already mated and gravid. Many of them apparently carried sperms contributed by more than one male. This was determined by study of the variety and frequency of patterns represented in the young of a given brood.

2. *The Rio Jamapa Platyfish*

Some female platyfish from the Rio Jamapa having dual caudal patterns were each placed in a separate aquarium. Young females of mating 4, 5, 6, 11, 12, 14 and 15 of Table I did not produce a brood for a period of two months; they were mated with suitable males to test their genetic constitution. One female, born of wild parents, having a dual pattern, was reared separately and mated later to a double recessive male; this is shown in mating number 1. Females in matings 2, 3, 7, 9 and 10 were of a domesticated stock, recessive for the caudal patterns; they were mated with wild males having dual patterns. Females in matings numbered 8 and 13 were *Xiphophorus hellerii;* these were mated with platyfish having dual patterns. The intergeneric hybrid offspring displayed the same type of gene segregation as the offspring of intervarietal matings except that the intergeneric hybrids having the gene for *comet* showed a modified pattern, called the "black wagtail." Gordon (1946a) has described the complementary action of the *Co* gene and its specific modifier, *E*. None of the other members of the allelic series is similarly affected by crossing with the swordtail.

The results are consistent in illustrating the genetic behavior of a

series of multiple alleles. This may be expressed in the following simple terms: If one parent has two dominant alleles of the series, for example, *AB* and the other parent is double recessive ++, then half of the offspring will be genotypically *A*+ and the other half will be *B*+. If one of the parents is *AB* and the other has a third allele, *C*, in the heterozygous phase, then there will be four phenotypes among the offspring: *A*+, *B*+, *AC* and *BC*, each appears approximately with the same frequency. If the same gene is represented in both parents like *AB* and *A*+, the number of offspring with *A* (*AA* plus *A*+) will be twice the number of *AB* or *B*+ or equal to their sum. Finally, if one parent is *AB* and the other is *CD*, the offspring will be *AC*, *AD*, *BC*, *BD* in equal numbers.

Matings listed under 31 through 40 represent some of the wild Rio Jamapa females which were gravid when brought to the laboratory. They do not necessarily represent females which were mated in the wild state, since the entire lot of fish spent two months in a common pool at a New Orleans fish hatchery awaiting warmer weather for final shipment to New York City. While multiple matings occurred, some of the females produced offspring having patterns in such proportions that it was possible to deduce the genetic constitution of their mates with some accuracy. For instance, in mating 37, the female was phenotypically P^{Co} and it produces P^{O} P^{Co}, P^{O}, P^{Co} and + offspring in equal proportions. It would seem that she was heterozygous $P^{Co}P^{+}$ and the male was $P^{O}P^{+}$. In the offspring of mating 34 (39–14) the ratios are so far off any definite ratio that one must assume that double matings had taken place. Similarly, the female indicated in mating 36 (39–16), a $P^{C+}P^{+}$ fish produced young of six phenotypes: P^{+}, P^{O}, P^{Cc}, P^{Co}, $P^{O}P^{Co}$ and $P^{Cc}P^{Co}$, an improbable performance unless aided by two or more males.

It is evident from the breeding behavior of the fish from the Rio Jamapa region as given in Table I, that the new patterns, the single crescent (P^{C}) and the comet (P^{Co}) belong, genetically, to the same series of alleles as P^{O}, P^{Mc}, P^{Cc} and P^{T} previously analyzed by Gordon and Fraser (1931). The seventh unit in the series, single moon (P^{M}) was not represented in the Rio Jamapa population. The P^{M} gene was found in the fishes of the Rio Papaloapan of the 1932 collection and unpublished data are available of its genetic association. These may now be presented.

3. *The Rio Papaloapan Platyfish*

Matings 16 through 30 represent the breeding performances of females originally collected from the Rio Papaloapan and analyzed at the Zoological Laboratories of Cornell University. The fish from this region are indicated by the number 32 preceding their individual culture number. The results of tests of females indicated under mating 16 (32–3), 17 (32–5),

18 (32–9) and 20 (32–23) show that the single moon pattern, P^M definitely belongs to the same multiple allelic series as P^O, P^{Mc}, P^C, P^{Cc}, P^{Co} and P^T. From matings 16 through 30 further evidence is obtained that P^{Co} and P^C of the Rio Papaloapan and of the Rio Jamapa are essentially similar in their genetic behavior.

Again there are several instances where, in mating 27, for example, the number of phenotypes among the offspring of a given female (32–6) is greater than four; this indicates the probability of multiple mating.

4. *Domesticated Platyfish at Los Angeles*

Matings listed from 42 through 61 were conducted and analyzed by Dr. A. W. Bellamy at the aquarium of the Department of Zoology of the University of California at Los Angeles. The stocks used represent domesticated fish and they are, therefore, comparable to those originally used by Gordon and Fraser in 1931. This group, involving only the genes P^O, P^{Mc}, P^T and the recessive P^+, furnishes an important confirmation of the reality of the multiple allelic series. The fact that the tests were conducted independently and with unrelated stocks, gives them a special significance. Mating listed under 43, and possibly the one listed under 49, do not quite conform to the expected ratios but, in the light of the rest of the data, the present interpretation of the multiple allelic series appears most logical. I wish to express my thanks to Dr. Bellamy for making these data available for this paper.

IV. Gene Frequency Distributions

In all, over 8,000 *P. maculatus*, including 5,019 adults, were classified and arranged according to collection station, date of collection, sex, maturity and tail pattern types. In addition to the seven patterns mentioned in the introduction, P^O, P^M, P^{Mc}, P^C, P^{Cc}, P^{Co} and P^T, there are a number of lesser tail patterns which at present do not lend themselves to this kind of study because they are quite infrequent (see Fig. 1).

In further addition to the tail and caudal peduncular patterns an important series of black body markings were found in the species. A preliminary note on these patterns and their heredity was presented by Gordon (1944). The five patterns are formed by large melanophores (macromelanophores) and are controlled by sex-linked genes *Sp*, *Sd*, *Sr*, *Sb* and *N*. A report on the genetics of these patterns will be presented at another time (Gordon, 1946b, 1947).

The original tabulations (according to Gordon 1939) showed over 125 different patterns and pattern combinations with respect to melanic markings only. Finally, these fish have a series of red patterns on various parts of their bodies: the dorsal fin, the belly area and anal fin. Owing to

the fact that the xanthophores and erythrophores that produce these patterns are destroyed in formalin and alcohol, no data on the red series have been recorded.

Since the seven tail patterns are inherited independently of other markings, they may be analyzed separately for purposes of gene frequency distributions. In addition to the seven individual tail patterns, seventeen double, but no triple nor more complex combinations were recorded. The presence of double, but no more complex combinations, suggested the operation of seven dominant multiple alleles and evidence from genetic experiments supports the supposition. The following tabulation lists all the possible types and indicates in parenthesis those combinations which have not been detected:

	O	*M*	*Mc*	*C*	*Cc*	*Co*	*T*
O	*O*						
M	*O M*	*M*					
Mc	*O Mc*	(*M Mc*)	*Mc*				
C	*O C*	*M C*	(*McC*)	*C*			
Cc	*O Cc*	*M Cc*	*Mc Cc*	(*C Cc*)	*Cc*		
Co	*O Co*	*M Co*	*Mc Co*	*C Co*	*Cc Co*	*Co*	
T	*O T*	*M T*	(*Mc T*)	*C T*	*Cc T*	*Co T*	*T*

All the tail patterns, both singles and doubles, are listed in Table II and in some of the following tables. But for purposes of these analyses only the single tail patterns will be evaluated. This will account for the fact that, when all the numbers representing the various patterns for a particular river or year are summed, the result is not necessarily equivalent to the total number of specimens in that population. For the same reason the percentage values do not necessarily add up to 100.

The decision to use the numbers representing single tail patterns alone was made because it was thought they could bring to light the major principles involved more simply and clearly, and errors which may have been made in determining the exact pattern combinations may be avoided. Even with the elimination of dual patterns there still remains a small source of potential errors in recording single patterns and for the purposes of evaluating these the following notes are in order.

Theoretically, with seven dominant alleles in operation there should have been 21 dual patterns observed $\left(\frac{n^2-n}{2}\right)$ in the natural populations. Missing from the expected combinations were the following: *M Mc*, *Mc C*, *Mc T* and *C Cc*. It is likely that these were unrecorded because they cannot be told apart phenotypically from *Mc*, *MC*, *MT* and *Cc* respectively. Since the *MC* and *MT* patterns are dual combinations, they

need not concern us at this time. The errors may have occurred in listing the frequencies of *Mc* and *Cc* and these may have had values added to them rightfully belonging to *M Mc* and *C Cc*. These errors cannot be large. From the drawings, it might appear that there might be some difficulty in separating combinations of *O* with *M*, *Mc* or *Cc*, but Gordon (1931) has pointed out that in *O* pattern micromelanophores lie in the superficial muscle areas while the other patterns are found in deeper layers. The result is that *O* in dual patterns may be seen superimposed upon the others occupying somewhat similar areas.

The use of single pattern frequencies may be justified if the sample is large enough. In the case of the 1867 collection, where only 13 specimens represent the entire population, a serious error may be made by using single patterns only. An instance of this may be seen by listing the complete constitution of all the members of 1867 or Sumicrast's 13 specimens as determined by their phenotypes with respect to the caudal region. These are as follows: 4 +; 2 *C*; 2 *Cc*; 2 *T*; 3 *O Cc*. Thus, if the doubles were not counted, there would be no representative of *O* listed for evaluation. Since *O* was present, but always with *O Cc* in 3 instances, the value of *O* in per cent is 23.1, at least. Undoubtedly, statisticians may require additional adjustments in estimating gene frequencies distributions from the complete data presented in these tables.

In the present preliminary analysis based upon single patterns, it is hoped that the restricted data may still be sufficient upon which to base limited conclusions.

1. *Methods of Presenting the Data*

First the initial analysis of the platyfish population was made for each local unit and time of capture according to the sex and pattern of the individual. For instance, Table II contains analyses of 11 populations in the Rio Papaloapan Systems. A description of some of these collection stations is given elsewhere, and Figs. 2 and 3 show some of the points where the collections were made. A similar pattern analysis for the platyfish at three points in the Rio Coatzacoalcos is presented in Table III; and the patterns of the species at 21 stations in the Rio Usumacinta are given in Table IV and the localities are indicated in Fig. 4.

The analysis of the *P. maculatus* population of the Rio Jamapa is given in Table V. The data originally presented in Tables II, III and IV, representing the populations in the Rio Papaloapan, Rio Coatzacoalcos and Rio Usumacinta, are summed up and the results entered in Table V together with those of the Rio Jamapa.

Finally, the data on single patterns in males and females from each of the four great river systems were combined and presented in Table VI. In

this table the percentage values were derived for the comparative study of geographical variation. The graph, Fig. 5, was constructed from the data contained in this table.

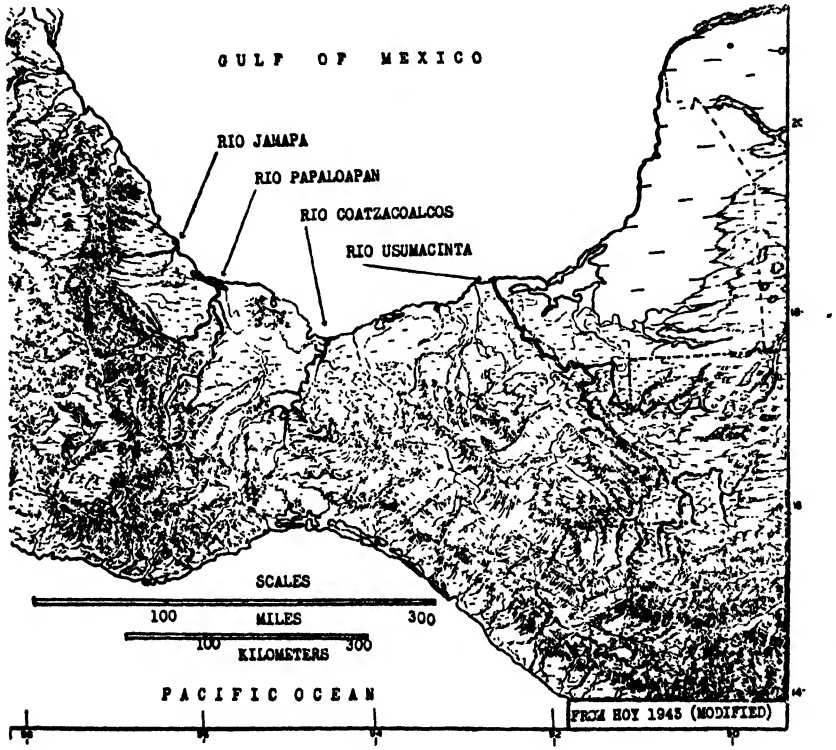
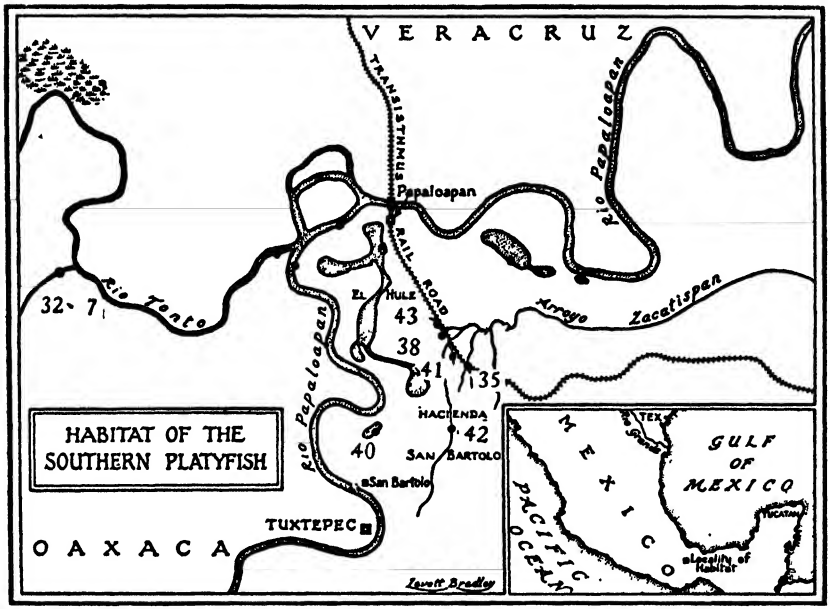


FIG. 2

Part of Mexico and Guatemala Showing the Four Great River Systems in which *Platypoecilus maculatus* are Found.

Each river population may be distinguished on the basis of its frequencies of the pattern genes indicated in Fig. 1.

It must be remembered that the number of individuals indicated as *P+* may have had a pattern other than a tail pattern. Thus, if a fish carried only one of the sex-linked patterns, *Sp*, *Sd*, *Sr*, *Sb*, *N* or any dual combinations of some of these, it would be classified in this study as P^+. Undoubtedly a small number of tail patterns were obscured by some of the sex-linked ones but again, the number of tail patterns missed cannot be great.



NATURAL HISTORY, FEB., 1940

FIG. 3

The Collection Stations in the Vicinity of Papaloapan, Oaxaca, in which *Platypoecilus maculatus* Were Taken in Large Numbers.

Xiphophorus hellerii alone were found at station 42, but none was found at station 41. Both species were taken together elsewhere. No hybrids were found.

V. GEOGRAPHICAL VARIATION

Of the four river populations, the Mexican platyfish of the Rio Papaloapan are the most variable with respect to the number of different kinds of patterns which they display — the entire series of seven is represented. The Rio Jamapa platyfish population lacks the *M* and *Mc* genes and this feature distinguishes them from all the others. The platyfish populations from the Rio Coatzacoalcos and the Rio Usumacinta do not contain *C* and *Co* genes and this fact sets them off from the two more northern river populations. The platyfish from the Rio Coatzacoalcos may be distinguished from those of the Rio Usumacinta by a number of quantitative differences in gene frequencies; the platyfish from Guatemala (southernmost river system) have a much higher proportion of the *O* and *M* genes and a much smaller proportion of the *Mc* and *T* genes. Summaries may be found in Tables V and VI.

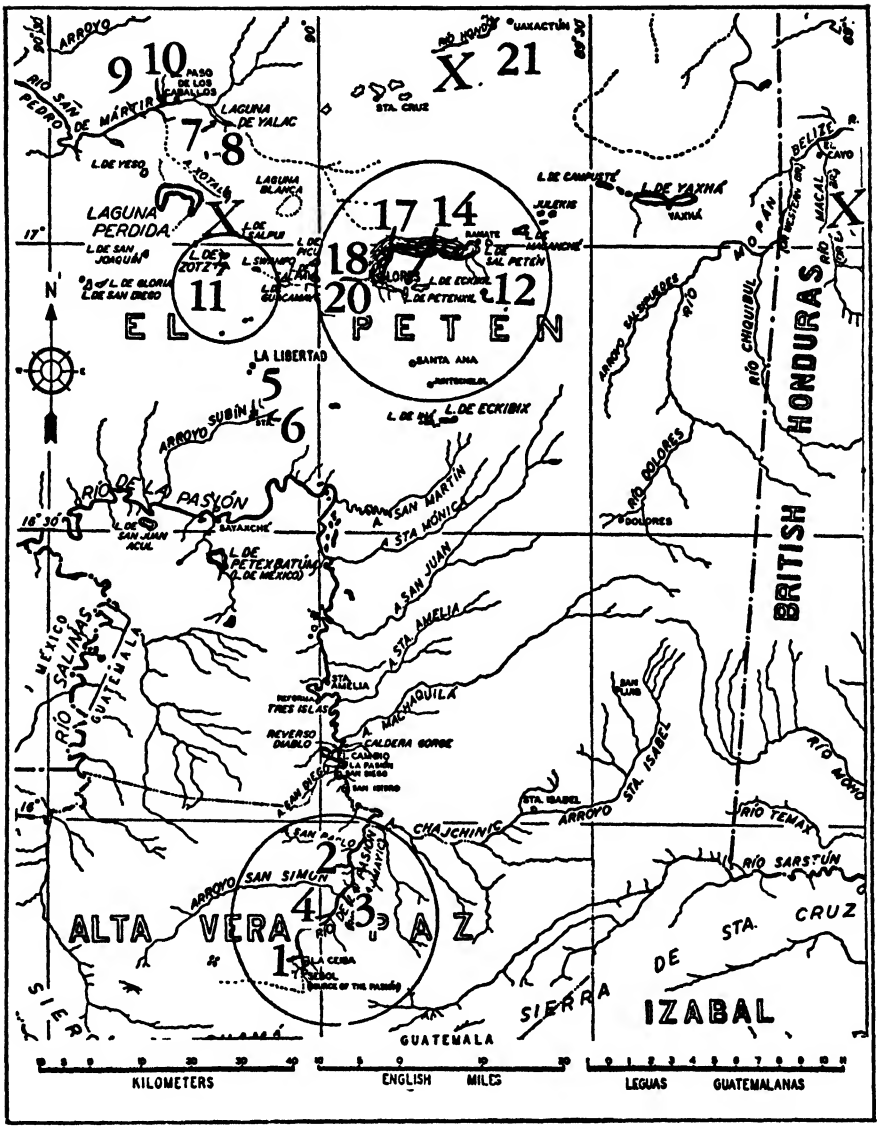


FIG. 4

Collection Stations in the Guatemala and British Honduras Area for *Platypoecilus maculatus* (indicated by 1 to 21) and *Xiphophorus hellerii* (indicated by *X*).

The three circles indicate genetically distinct platyfish populations based upon details of their gene frequencies. This map has been modified from one compiled by Carl L. Hubbs and Henry van der Schalie, 1937.

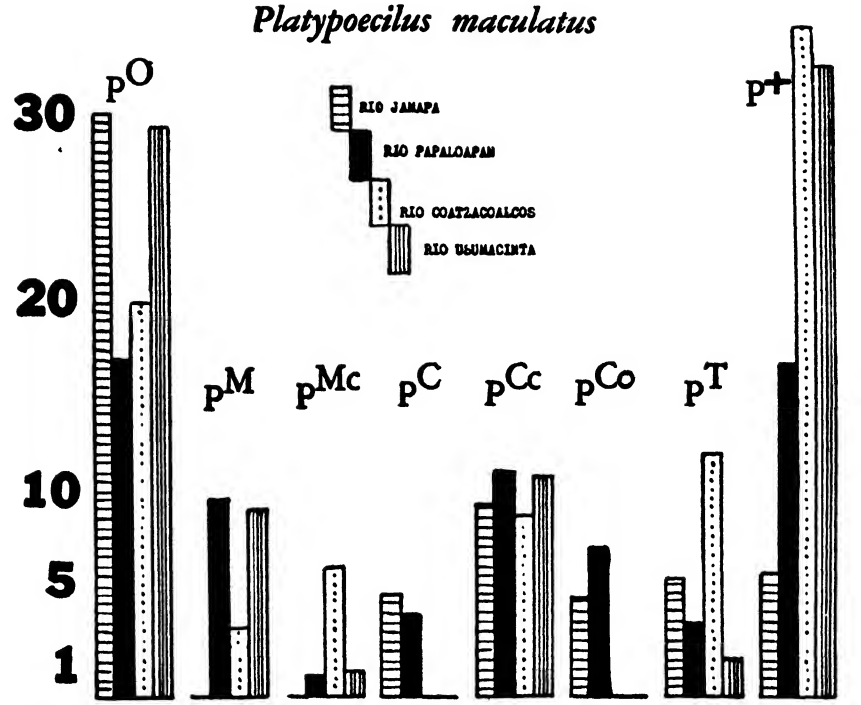


FIG. 5

The Frequencies of the Seven Genes and the Universal Recessive for the Four River Populations of *Platypoecilus maculatus* Based on Percentage Values Obtained from Table VI.

The Rio Jamapa platyfish lack the genes P^M and P^{Mc}. The Rio Papaloapan platyfish have all the seven genes. The Rio Coatzacoalcos platyfish and those of Rio Usumacinta lack genes P^C and P^{Co}. The latter two populations may be distinguished on the basis of the relative frequencies of their other genes.

On the basis of these analyses (particularly those in Table VI) a diagnostic "key" to four distinct populations may be constructed:

1. *Key to Natural River Populations of* Platypoecilus maculatus

A. Population with genes *O*, *M*, *Mc*, *C*, *Cc*, *Co* and *T* present ..... Rio Papaloapan

AA. Population with some of above genes wanting ..... B

B. With genes *C* and *Co;* but no *M* or *Mc* ..... Rio Jamapa

BB. With genes *M* and *Mc;* but no *C* or *Co* C

C. With frequency of genes *T* more than 10% and *Mc* more than 5% ..... Rio Coatzacoalcos

CC. With frequency of genes *T* less than 5% and *Mc* less than 5% ..... Rio Usumacinta

TABLE II

Tail Pattern Frequencies in Platypoecilus maculatus *for all Stations in the Rio Papaloapan*

	'67		'02		'32–7		'39–40		'39–43a		'39–43b		'39–43c		'39–43d		'39–38		'39–41		'39–35	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
+	2	2	4	4	9	6	20	17	65	106	8	9	22	10	66	65	119	76	19	9	1	4
O	...	...	6	6	9	10	12	8	67	85	11	8	28	20	59	44	147	89	14	10	5	1
M	...	...	6	2	4	3	30	16	54	55	8	7	17	7	35	39	41	29	10	5	...	...
Mc	...	...	1	1	2	2	...	...	6	5	...	...	4	1	7	5	3	...	...	3	...	...
C	...	2	5	...	6	...	10	3	22	21	1	4	8	6	11	10	23	13	7	3	2	1
Cc	2	...	6	6	9	6	38	24	57	48	10	9	20	10	39	32	59	35	9	7	2	...
Co	...	...	2	...	3	4	10	6	38	30	10	2	13	5	26	21	58	38	5	1	2	2
T	2	...	4	1	5	1	2	...	21	9	2	3	6	2	13	18	29	13	3	4	...	...
OM	...	...	...	1	...	...	4	2	22	10	5	5	8	...	17	10	26	14	2	3	2	1
OMc	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...	...	...	...	1
OC	...	...	...	1	1	2	3	1	9	10	1	...	3	2	7	5	9	8	...	1	...	...
OCc	1	2	3	3	2	1	3	8	21	17	3	7	11	5	19	14	15	18	5	1	1	3
OCo	...	...	3	...	3	1	4	5	18	13	1	4	5	2	13	14	20	12	3	4	...	...
OT	...	...	...	...	4	2	...	...	6	6	...	2	2	...	8	9	4	6	3	2	...	...
MC	...	...	...	...	...	...	1	...	5	5	...	...	...	...	7	5	...	...	...	...	...	...
MCc	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	2	...	1	...	...
MCo	...	...	...	...	1	...	...	5	13	9	4	3	...	1	10	11	8	4	4	...	...	...
MT	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	...	...
McCc	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	2	...	...	...	1	...
CCo	...	...	1	...	1	...	1	1	3	4	...	...	1	...	7	2	1	6	...	2	...	...
CT	...	...	...	...	...	...	...	...	1	1	...	...	1	1	2	3	2	...	...	...	...	...
CcCo	...	...	...	1	2	1	2	6	13	8	...	4	2	2	9	3	3	6	1	...	...	...
CcT	...	...	...	...	...	...	...	2	3	2	2	...	3	...	...	2	6	1	3	...	...	...
CoT	...	...	...	...	1	...	...	...	6	5	...	2	2	...	3	2	1	...	...	1	1	...
Total	7	6	42	26	62	39	140	104	450	449	66	69	157	74	358	314	583	371	88	57	17	13
Grand Total	13		68		101		244		899		135		231		672		954		145		30	

'67 refers to Sumicrast's 1867 collection (now at the Smithsonian Institute). '02 refers to Meek's 1902 collection (now at Chicago's Natural History Museum).
'32–7 refers to the Gordon-Whetzel-Ross collection of 1932. The '39 series refers to the Gordon-Atz-Gordon collection of 1939.
(The two last collections are at the Museum of Zoology, University of Michigan.)

TABLE III

Tail Pattern Frequencies in Platypoecilus maculatus *for all Stations in the Rio Coatzacoalcos*

	Jesus Carranza		El Jule		Almagres	
	♀	♂	♀	♂	♀	♂
+	4	1	4	3	18	11
O	1	.	2	.	12	9
M	.	.	1	.	1	2
Mc	.	.	3	1	3	1
Cc	.	.	.	.	10	1
T	.	.	1	.	10	4
OMc	.	.	1	1	..	1
OCc	.	.	1	.	..	2
OT	.	.	.	.	1	1
CcT	.	.	1	.	2	1
Totals	5	1	14	5	57	33
Grand Total	6		19		90	

These collections were made in 1939 by Señor S. Coranado and are at the Museum of Zoology of the University of Michigan.

A "key" of this type is workable if the samples are taken over several points in a river system to avoid the chance of an atypical river population at an isolated locality; and of course, the sample should be large enough, about 200. The "key" has been helpful in determining, or rather narrowing down, the locality from which the early stocks of the common platyfish were originally collected and shipped alive to European aquarists in 1909–1911.

The early history of the platyfish has been traced by Gordon (1931) who obtained most of his data from the articles and notices published in two German journals *"Blätter für Aquarien und Terrarienkunde"* and the *"Wochenschrift für Aquarien und Terrarienkunde."* The significant points are these: the platyfish common in aquaria in the early days had four tail patterns *O*, *Mc*, *Cc* and *T* as here defined. It was from these aquarium-reared stocks that Gordon and Fraser (1931) worked out the autosomal multiple allelic series for the four dominant genes for the caudal patterns. Bellamy (this paper) also working with domesticated strains had platies with *O*, *Mc* and *T*. There were no representatives of *M*, *C* or *Co* in the early importations of living fishes for the aquarium. Since all the patterns are found in the Rio Papaloapan it is not likely that the early aquarium fish collectors obtained their platyfish from this river. Nor is it likely that they caught them in the Rio Jamapa, which is quite close to the big seaport

TABLE IV

Tail Pattern Frequencies in Platypoecilus maculatus *for all Stations in the Rio Usumacinta, Lake Petén Area and at Uaxactún*

	Río de la Pasión Headwaters				Río de la Pasión Arroyo Subin		Laguna de Yalec		R. San Pedro de Mártir		Laguna de Zotz		Laguna de Eckikil		Laguna de Petenxil		Arroyo Ponteil		Laguna de Petén				Uaxactún	
Station No.	1	2	3	4	5	6	7	8	9	10	11		12	14	15		16	17	18	19	20		21	
Collecting No.	115a	121	118	113b	102	135c	88	90	91	87b	67		36d	42	94		19	8	11	30	37a		1931	
Sex	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀	♂	♀ ♂	♀ ♂	♀	♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂		♀	♂
+	. .	1 .	16 7	1 .	. .	. .	. .	. .	. .	1 .	87	50	. .	4 .	5 2		. 1	4 1	1 2	. 1	. .		.	1
O	1 .	2 1	18 11	1 6	1 .	. 1	. .	1 .	. 1	6 .	50	47	1 .	6 2	1 2		3 .	2 2	3 .	. .	. .		1	1
M	. .	4 .	10 4	2 .	. .	. .	. .	. .	. .	. 2	18	4	. .	1 3	3 3		. .	2 .	. .	. .	. .		.	.
Mc	. .	. .	4 1	. .	. .	. .	. .	. .	. .	. .	..	..	. .	. .	. .		. .	. .	. .	. .	. .		.	.
Cc	. .	1 .	9 4	2 1	. .	1 .	. .	. .	. .	1 1	26	11	. .	. 1	1 3		1 .	. .	. .	. .	. .		.	.
T	. .	. .	3 3	2 .	. .	1 .	. .	. .	. .	1 .	..	..	. .	2 .	. 1		. .	. .	. .	. .	. .		.	.
OM	. .	1 .	2 2	1 .	1 .	. .	. .	. .	1 .	. .	1	4	. .	. .	1 .		. .	. .	. .	. .	. .		.	.
OMc	. .	. .	.. ..	. .	. .	. .	. .	. .	. .	. .	..	..	. .	. .	. .		. .	2 .	. .	. .	. .		.	.
OCc	. .	. .	5 1	. .	. .	. .	. .	. .	1 .	1 .	7	5	. .	. .	. .		. .	. .	1 .	. .	. .		.	.
OT	. .	. .	3 2	. .	. .	. .	1 .	. .	. .	. .	..	..	. .	. 1	2 4		. .	. .	. .	. .	1 .		.	.
MCc	. .	. .	.. ..	. .	. .	. .	. .	. .	. .	1 .	2	1	. .	. .	. .		. .	. .	. .	. .	. .		.	.
CcT	. .	. .	.. 1	. .	. .	. .	. .	. .	. .	. .	..	..	. .	. .	. 1		. .	. .	. .	. .	. .		.	.
OCg	. .	. .	.. ..	. .	. .	. .	. .	. .	. .	. .	..	..	. .	. .	1 .		. .	1 .	. .	. .	. .		.	.
Totals	1 .	9 1	70 36	9 7	2 .	2 1	1 .	1 .	2 1	11 3	191	122	1 .	13 7	14 16		4 1	11 3	5 2	. 1	1 .		1	2
Grand Total	1	10	106	16	2	3	1	1	3	14	313		1	20	30		5	14	7	1	1		3	

These collections were made by Drs. C. L. Hubbs, H. van der Schalie and J. Van Tyne and are at the Museum of Zoology of the University of Michigan.

TABLE V

Tail Pattern Frequencies in Platypoecilus *in the Rio Jamapa and a Regrouping of the Pattern Frequencies from the Other Three River Systems*

	Río Jamapa		Río Papaloapan		Río Coatzacoalcos		Río Usumacinta		Totals	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
+	32	25	335	308	26	15	120	65	513	413
O	169	108	358	281	15	9	97	74	639	472
M	...	...	205	163	2	2	40	16	247	181
Mc	...	...	23	17	6	2	4	1	33	20
C	30	17	95	63	..	..	...	...	125	80
Cc	47	42	251	177	10	1	42	21	350	241
Co	25	19	167	109	..	..	...	...	192	128
T	34	20	87	51	11	4	9	4	141	79
OM	...	...	86	46	..	..	8	6	94	52
OMc	...	...	4	1	1	2	2	...	7	3
OC	38	24	33	30	..	..	...	...	71	54
OCc	46	23	84	79	1	2	15	6	146	110
OCo	45	27	70	55	..	..	...	...	115	82
OT	33	25	27	27	1	1	7	7	68	60
MC	...	...	13	10	..	..	...	...	13	10
MCc	...	...	3	3	..	..	3	1	6	4
MCo	...	...	40	33	..	..	...	...	40	33
MT	...	...	1	1	..	..	...	...	1	1
McCo	...	...	4	....	..	..	...	...	4	...
Cco	9	2	15	15	..	..	...	...	24	17
CT	6	3	6	5	..	..	...	...	12	8
CcCo	3	1	32	31	..	..	...	...	35	32
CcT	...	...	17	7	3	1	...	2	20	10
CoT	3	4	14	10	..	..	...	...	17	14
OCg	...	...	...	...	..	..	1	1	1	1
Totals	520	340	1970	1522	76	39	348	204	2914	2105
Grand Total	860		3492		115		552		5019	

The Río Jamapa collection was made by Gordon in 1939; it is deposited in the Museum of Zoology of the University of Michigan.

of Veracruz, because this river population has the patterns *C* and *Co*, patterns missing from the early aquarium stocks. This narrows the likely collecting area down to the Rio Coatzacoalcos and the Rio Usumacinta. We now know that platyfish may be taken near the mouth of the Rio Coatzacoalcos from the collections of Señor Coronado, but the only record we have of platyfish from the Rio Usumacinta are those from the upper

TABLE VI

The Analysis of the Single Tail Patterns in the Four River Populations of Platypoecilus maculatus

Platypoecilus maculatus	No.	P^O		P^M		P^{Mc}		P^C		P^{Cc}		P^{Co}		P^T		P^+	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Rio Jamapa	860	277	32.2	...	...	...	...	47	5.5	89	10.3	44	5.1	54	6.3	57	6.6
Rio Papaloapan	3492	639	18.2	368	10.5	40	1.1	158	4.5	428	12.5	276	7.9	138	4.0	643	18.4
Rio Coatzacoalcos	115	24	20.9	4	3.5	8	6.9	...	...	11	9.5	...	...	15	13.0	41	35.7
Rio Usumacinta	552	171	31.0	56	10.1	5	0.9	...	...	63	11.4	...	...	13	2.4	185	33.5

The Rio Papaloapan population has all the seven tail patterns.

The Rio Jamapa population does not have P^M or P^{Mc}.

The Rio Coatzacoalcos and the Rio Usumacinta populations do not have P^C or P^{Co}; but these populations differ greatly from each other in their frequencies for P^M, P^{Mc} and P^T.

reaches of the river not likely to be visited by sailors with a few hours or days leave to catch fish for aquarium fish dealers. Thus it is likely our domesticated aquarium reared platyfish were caught in the Rio Coatzacoalcos near the port of Puerto México (Coatzacoalcos).

2. *Survey of the Local Populations of* P. maculatus *in the Rio Papaloapan*

Adequate numbers of *P. maculatus* were difficult to collect owing to the short periods of time spent in the field. However, at four stations, the necessary conditions for making large collections possible were present: the time, late February — early March was right, it being late in the dry season; and the localities were suitable, being small. The isolated pools contained concentrated populations of fishes which formerly occupied larger bodies of water earlier in the season. A summary of four populations in the Rio Papaloapan is given in Table VII.

Three of the stations, 39–43, 39–38 and 39–41 (shown in Fig. 3 as 43, 38 and 41), were only half a kilometer apart. It was likely that they had been joined previously, at the height of the wet season, and had been isolated for about one month before the collections were made. Soon after our collections were made the water in pools had evaporated completely. Photographs of the gradual disappearance of pool number 39–43 may be seen in Gordon (1940).

Some of the data on the collection points are as follows:

"Field No. 39–43: The collection was made 3½ kilometers south of Papaloapan, Oaxaca, in a pool which had been part of a large lagoon between railroad kilometer posts 149 and 150 (Transisthmus Line—Veracruz to Tehuantepec). The water was clear but choked with Chara-like aquatic plants. The pool measured 30 by 15 feet and was about 1 foot deep; the bottom was muddy; the shore-lines were jungles of low growing shrubs and vines; the temperature of the water was 25° C. at the northern end and 30° C. at the southern part. The first collection at this station was made by Evelyn and Myron Gordon on March 4 and further collections on March 6, 7 and 10, 1939.

"Field No. 39–38: The collection was made 4 kilometers south of Papaloapan, Oaxaca, in a pool which was part of a lagoon at the railroad kilometer post number 149. The water was clear with large patches of *Utricularia;* the bottom was muddy at some points and sandy at others; the shore-lines were jungles. The pool measured 25 by 15 feet, was about 2 feet deep, and the temperature of the water was 25° C. The collection was made by Gordon and Atz on February 20, 1939.

"Field No. 39–41: The collection was made 4½ kilometers south of Papaloapan, Oaxaca, between railroad kilometer posts 149 and 150. The

TABLE VII A

The Geographically Isolated Local Populations of Platypoecilus maculatus *in the Rio Papaloapan*

Platypoecilus maculatus	No.	P^O No.	%	P^M No.	%	P^{Mc} No.	%	P^C No.	%	P^{Cc} No.	%	P^{Co} No.	%	P^T No.	%	P^+ No.	%
'39–40	244	20	8.2	46	18.9	...	...	13	5.3	62	25.4	16	6.6	2	.8	37	15.1
'39–43	1937	332	16.6	222	11.5	28	1.4	83	4.3	225	11.6	145	7.5	74	3.8	351	18.1
'39–38	954	236	24.7	70	7.3	3	.3	36	3.8	94	9.9	96	10.1	42	4.4	195	20.4
'39–41	145	24	16.6	15	10.3	3	2.1	10	6.9	16	11.0	6	4.1	7	4.8	28	19.3

For localities see Fig. 3.

TABLE VII B

The Geographically Isolated Local Populations of Platypoecilus maculatus *in the Rio Usumacinta and the Lakes of Petén, Guatemala*

Platypoecilus maculatus	No.	P^O No.	%	P^M No.	%	P^{Mc} No.	%	P^C No.	%	P^{Cc} No.	%	P^{Co} No.	%	P^T No.	%	P^+ No.	%
Rio de la Pasión	133	40	30.1	20	15.0	5	3.8	...	...	17	12.8	...	...	8	6.0	25	18.8
Laguna de Zotz	313	97	31.0	22	7.0	...	...	...	...	37	11.8	...	...	...	...	137	43.8
Laguna de Petenxil–Petén	77	21	27.3	12	15.6	...	...	...	...	6	7.8	...	...	3	3.9	21	27.3

The Rio de la Pasion group consists of collections 1 to 4, of Table IV.
The Laguna de Zotz collection is collection number 12 of Table IV.
The Laguna de Petenxil and Petén represent collections 14 to 19 of Table IV.
For localities of these three groups see Fig. 4.

water was clear, contained large clumps of Utricularia and Nitella, and its temperature was 25° C. The pool measured about 25 by 10 feet, was about 2 feet deep and its banks were lined with jungle growth. The collection was made by Evelyn and Myron Gordon and Atz on February 27, 1939.

"Field No. 39–40: The collection was made in a lagoon and spring beside the Rio Papaloapan, 3 kilometers north of San Bartolo, Oaxaca. The water of the lagoon was cloudy but that of the spring quite clear; the spring run section was about 10 feet long, 3 feet wide and choked with aquatic plants. Palms grew within the immediate vicinity of the lagoon but the land all around was cultivated extensively by the Standard Fruit Company for the production of bananas. The lagoon measured about 100 by 20 feet and was about 3 feet deep; the water in the lagoon was stagnant and its waters in various areas had different temperatures: at the spring end, 25° C., at the center, 28° C., and towards the far end, 35° C. Most of the platyfish were taken in the spring run in the coldest water, while all other species were taken in the warmer waters. The collections were made by Gordon and Atz on February 25, 1939."

The pool at 39–40 (40 in Fig. 3) was a more permanent habitat since it was formed by a small spring. It probably survived the 1939 season. On Fig. 3, station 40 appears near the Rio Papaloapan but actually it is about 15 feet higher. Thus, if the platyfish population at this point were to be wiped out by an extreme drought, the renewal of its platyfish population must come from the main river. This implies that the Rio Papaloapan at this point must rise at least an equivalent number of feet. From reports of those who have spent a rainy season in the vicinity it seems that the river may rise to that elevation.

Four species of platyfish, *P. couchianus*, *P. xiphidium*, *P. variatus* and *P. maculatus*, have this point in common: all of them may be found in relatively large numbers in regions of springs. Often these springs are situated along the banks of the larger rivers. They are in the direct path of the raging flood waters during practically the whole of the rainy season. Their platyfish populations, as discrete units, must be reëstablished after every rainy period. It would be important to analyze these populations year after year.

The three populations at 43, 38 and 41 are fairly uniform. But the population at 40 differs in a number of details, being quite low in the value of P^O and P^T but high in P^M and P^{Cc}.

3. *Survey of the Local Populations of* P. maculatus *in the Rio Usumacinta*

By combining small collections according to larger geographical regions, three population units may be identified in the Rio Usumacinta: (a) an upper headwater unit of the Rio de la Pasión in Alta Vera Paz;

TABLE VIII A

Distribution of the Platypoecilus maculatus *Tail Pattern Frequencies in Time*

Platypoecilus maculatus	No.	P^O No.	%	P^M No.	%	P^{Mc} No.	%	P^C No.	%	P^{Cc} No.	%	P^{Co} No.	%	P^T No.	%	P^+ No.	%
Rio Papaloapan																	
1867	13	3	23.1	...	...	...	...	2	15.4	2	15.4	...	...	2	15.4	4	30.8
1902	68	12	17.6	8	11.8	2	2.9	5	7.4	12	17.6	2	2.9	5	7.4	8	11.8
1932	101	19	18.8	7	6.9	4	3.9	6	5.9	15	14.9	7	6.9	6	5.9	15	14.9
1939	3310	608	18.4	353	10.6	34	1.0	145	4.4	399	12.0	267	8.0	125	3.7	616	18.6

The comparative study of collections from the same general area taken over a period of 70 years.

TABLE VIII B

Distribution of the Platypoecilus maculatus *Tail Pattern Frequencies in Time*

Platypoecilus maculatus	No.	P^O No.	%	P^M No.	%	P^{Mc} No	%	P^C No.	%	P^{Cc} No.	%	P^{Co} No.	%	P^T No.	%	P^+ No.	%
Rio Papaloapan																	
March 4, 1939	899	152	16.9	109	12.1	11	1.2	43	4.8	105	11.6	68	7.5	30	3.3	171	19.0
6, 1939	135	19	14.1	15	11.1	...	...	5	3.7	19	14.1	12	8.9	5	3.7	17	12.6
7, 1939	231	48	20.7	24	10.4	5	2.2	14	6.1	30	13.0	18	7.8	8	3.5	32	13.8
10, 1939	672	103	15.3	74	11.0	12	1.8	21	3.1	71	10.6	47	7.0	31	4.6	131	19.4
Totals	1937	322	16.6	222	11.4	28	1.4	83	4.3	225	11.6	145	7.5	74	3.8	351	18.1

The comparative study of collections from the same pool taken over a period of 7 days at Station '39–43.

(b) the Lagunas de Petén and Petenxil have a distinctive population compared with that of (c) Laguna de Zotz.

In a preliminary paper Gordon (1943) erroneously stated that the platyfish of the Rio Usumacinta (that is, the Guatemalian group) did not have the genes P^{Mc} and P^{T}. That statement was based upon the analysis of the population of the Laguna de Zotz alone. Since it was the largest single unit and the most compact group, it was believed that it represented the whole population. Study of Table VII B shows, however, that the missing genes P^{Mc} and P^{T} are found in the smaller Rio de la Pasión group.

P^{T} is present but P^{Mc} is missing in the Laguna de Petén-Petenxil population. In this latter group, or to be specific, in Laguna Petenxil and in the Arroyo Ponteil, two specimens were found to have a distinctive tail pattern which is listed in Table IV as *O Cg*. The *O* is the usual one-spot but the *Cg* represents the *Guatemala crescent*, a pattern not found elsewhere in all the populations of *P. maculatus*. It closely approached the single crescent pattern *C* but it is broader over all and particularly so at the ends of the crescent. It resembles closely the pattern described by Kerrigan (1934) and by Gordon (1937) in the domesticated swordtail. It may be this was the particular platyfish pattern (*Cg*) which was introduced in the early platyfish-swordtail hybrid stocks to produce the swordtail variety known as *rachovi*. Gordon (1946a) has recently described how several wild platyfish patterns may be transferred to aquarium bred swordtails by the process of introgressive hybridization.

VI. Gene Frequency Distribution in Time

The Sumicrast collection of 13 specimens from the Rio Papaloapan, although small, is extremely important since it was made so long ago, 1867. It may be compared with collections from the same river taken in 1902, 1932 and 1939. The outstanding feature of the earliest collection is that its members have representatives of four out of the possible seven dominant alleles for the caudal patterns, *O*, *C*, *Cc* and *T*. The absence of *M*, *Mc* and *Co* may possibly be characteristic of the locality —— Cosamaloapan, Veracruz —— but this is doubtful. It is more likely that the absence of the latter three alleles may be attributed to chance due to the smallness of the sample.

The Meek 1902 collection of 68 specimens from El Hule (now known as Papaloapan), Oaxaca, may be compared more favorably with our 1932 and 1939 collections since all were taken within a radius of 10 miles of each other and the number of individuals in the populations is more adequate for this kind of study. The similarities, as indicated in Table VIIIA and Fig. 6 in the genetic composition of the platyfish populations, are apparent despite a time lapse of 30 and 37 years. The most remarkable similarities

in gene frequency are found in the distributions of the *O*, *Mc* and *Cc* genes. Those that had a high frequency incidence in 1902, specifically the genes *O* and *Cc*, had a high incidence in 1932 and 1939. At the same time, *Mc* had a low frequency incidence in 1902 and it was correspondingly low in 1932 and 1939. The value for *M* was intermediate 11.8% in 1902; it fell to 6.9% in 1932, but rose again almost to the 1902 value in 1939, 10.5%.

The frequencies of genes *C* and *T* apparently show a diminishing progression. For example, *C* fell from the high of 7.4% in 1902 to 4.2% in 1932 and to the low of 3.7% in 1939. On the other hand, the frequencies of the gene *Co* on the basis of available data show an apparent progressive increase, 2.9%, 6.9%, 8.0% for 1902, 1932, and 1939, respectively.

These relatively long time lapse studies in the genetic structure of natural populations involving the period between 1867 and 1939 may be compared with another, a short time lapse study involving a period of a week, March 4–March 10, 1939. Specifically, collections within the same

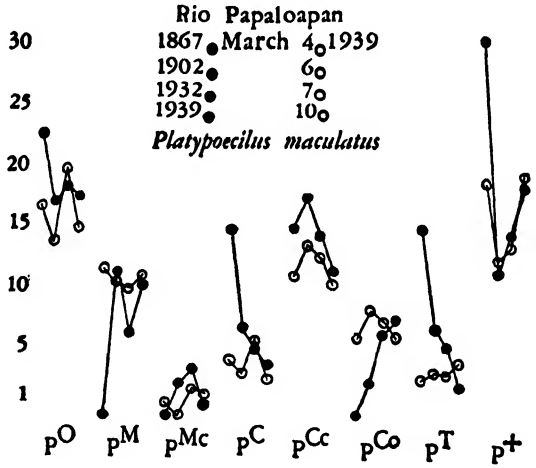


FIG. 6

Distribution of the Pattern Genes of *Platypoecilus maculatus* in Time

Reading from left to right in each gene group, the solid circles represent, in per cent, the frequency of a particular gene in 1867, 1902, 1932 and 1939. In the same manner, the open circles indicate the frequencies in per cent of the corresponding genes in collections made from the same locality on March 4, 6, 7 and 10, 1939. The data upon which this figure is based may be found in Table VIII. (The 1867 collection was quite small, containing only 13 specimens; the others are represented by 68 or more individuals.)

Note that the fluctuations in the 7-day time-lapse series are little less extensive than, and even sometimes parallel to, those in the 70-year series; an indication that the changes in the 70-year series are probably largely due to chance variations in the samples.

pond were made on March 4, 6, 7 and 10. The results of analysis of the platyfish populations for those days appear in Table VIIIB and Fig. 6. There is a general agreement in values between the long and the short time lapse series. It seems to make little difference, on the basis of these studies, whether samples are collected 70 years apart or 7 days apart, the genetic structure of a natural population in this species appears to remain fairly constant. If this constancy is supported by a more detailed analysis, then this may be an example of the automatic maintenance of hereditary variability in natural populations at a constant level, once such a level has been gained, in accordance with the principle represented by Hardy's formula. Hardy (1908) worked out his equilibrium formula for populations heterogeneous for a single pair of alleles. From the data presented here, it may be that his formula may be extended to include a large series of alleles. It is, of course, also possible that the present example of constant genetic structure might be the result of selective differentials, constant in time in a given locality.

TABLE IX

Distribution and Variation in the Number of Dorsal Fin Rays in Platypoecilus maculatus *— an Effective Taxonomic Character*

P. maculatus	7	8	9	10	11	Total	Average	δ
Rio Jamapa	.	1	27	71	1	100	9.72±.05	.48
Rio Papaloapan								
1867 Cosamaloapan	.	.	4	7	2	13	9.85±.19	.69
1902 Obispo	.	.	16	46	1	63	9.76±.06	.46
1932–1939 Papaloapan	.	.	34	74	4	112	9.73±.05	.52
Rio Coatzacoalcos	.	1	88	21	.	110	9.18±.04	.43
Rio Usumacinta								
Rio de la Pasión	.	.	13	58	.	71	9.82±.05	.39
Rio San Pedro de Mártir	.	.	17	6	.	23	9.26±.09	.45
Laguna de Zotz	.	.	37	160	3	200	9.83±.03	.41
Laguna de Petén	1	0	15	42	0	58	9.69±.07	.57

These data are from Revision of the Xiphophorin Fishes (HUBBS AND GORDON, *Ms.*).

There seems to be no appreciable difference in the number of dorsal fin rays in the populations of the Rio Jamapa and the Rio Papaloapan, see Table IX. Nor are there differences with respect to this character between the collections made in 1867, 1902 and in 1932–1939.

The platyfish population from the Rio Coatzacoalcos stands out by the relatively low value of its dorsal fin ray count, 9.18 and so does the population from the Rio San Pedro de Mártir, 9.26. These may reflect difference which may be attributed to geographical isolation, although the latter population is quite small and may be attributed entirely to chance. If it should be shown that the Rio Coatzacoalcos platyfish differ consistently in this character from those of the Rio Usumacinta there is then available a further criterion for the distinctness of these geographically isolated groups of the same species.

VII. Sexual Isolation in the Platyfish and Swordtail

Dobzhansky (1941) has pointed out that differences in courtship behavior, or sexual isolation, in different species may be completely effective, barring hybridization in nature, yet under laboratory conditions these bars may frequently be surmounted. This is true in the *Platypoecilus maculatus Xiphophorus hellerii* relationship. Reference to Table X will show that in ten collections both platyfish and swordtails were taken from the same body of water.

In over 10,000 specimens collected and studied carefully (approximately 8,000 platyfish and 2,000 swordtails) not a single hybrid was found — yet, under laboratory conditions, particularly when no choice of mating partners is offered, hybridization between these fish species is the rule rather than the exception. As Gordon (1931) has indicated, the platyfish were first imported in 1907 and the swordtails in 1909 to serve as aquarium species. Hybrids in aquaria were reported in 1910. While the swordtail is the larger species and its gonopodium has a secondary hook at its tip — hybrid matings between the species are successful in direct and reciprocal crosses. The hybrids are fertile for the most part and this may be related to the fact that each has 48 chromosomes, according to Friedman and Gordon (1934).

The behavior patterns of platyfish and swordtails differ. Gerschler (1914) concluded that the "temperment" of the swordtail is dominant over that of the platyfish from his experiments in hybridization. In some of the second generation he obtained individuals that looked like platyfish (having no sword-like extension to their tail), but had the behavior of the swordtail — an instance of segregation of characters. Breider (1939) has confirmed this statement.

Preliminary tests in the Laboratory of Animal Behavior at the American Museum of Natural History, under Dr. Frank A. Beach's direction, reveal that the behavior patterns in the two species are distinct. When, after six adult virgin platyfish and an equal number of virgin swordtails were placed in a 75 gallon aquarium (which is enormous as far as average aquarium conditions are concerned), a male swordtail was added, he definitely sought out females of his own species. In another preliminary series of tests in smaller aquaria, no hybridization took place when there was a free choice.

Ecological conditions in nature may strengthen the sexual isolation factors in the prevention of hybridization. The swordtail is primarily a headwater species — for instance, in the hills of the Hacienda San Bartolo, station 39–42, only swordtails were found. A description of this station is as follows:

"Field No. 39–42: The collection was made in the Arroyo Zacatispan

at its headwaters in the hills of San Bartolo, 10 kilometers south of Papaloapan, Oaxaca. The mountain stream was clear, contained no aquatic vegetation, and its waters were fairly cool, 20° C. The brook was 5 feet wide, its current was slight, and it was covered by a dense jungle growth of shrubs and creeping vines."

In other river systems, in the Rio Antiqua headwaters at Jalapa and Rio Bejucos, only swordtails were found, and similar conditions were found in the headwaters of the Rio Jamapa at Córdova, and Rio Blanco at Orizaba. Near the mouths of the rivers, or in low-lying areas, often the platyfish are found to the total exclusion of the swordtails. Indeed, when all available collections of these two species are listed, Table X, there are far more collections that represent one or the other species than those that represent both.

1. *Summary of Distribution Data*

Number of localities where *X. hellerii* only are found	15
Number of localities where *P. maculatus* only are found	5
Number of localities where there are more *P. m.* than *X. h.*	8
Number of localities where *P. m.* and *X. h.* are about equal	2

From these facts we may conclude that the swordtail is geographically isolated from the platyfish for the most part. Where both occur together in the same river, the swordtail has a typical ecological niche that differs from that of the platyfish. To the geographical, sexual and ecological factors in isolating mechanisms another may be added which is unique, that of viviparity. In platyfish and swordtails, sperms in the form of spermatophores are inserted during copulation through the genital pore of the female by means of the male's gonopodium. The gonopodium and its suspensorium (see Gordon and Benzer 1945) consist of the modified anal fin, and a complex of modified hemal and interhemal spines. Many authors claim that the tip of the gonopodium is inserted, during the instantaneous copulation act, into the genital pore of the female, but owing to the fact that most gonopodia are not closed tubes but more like open troughs, it is difficult to accept this simple explanation of the mechanics of sperm transfer. The spermatophores, of course, are minute bodies, the gonopodium is about 10 mm. long. In the presumed copulatory act the gonopodium is moved from a posterior pointed position in an arc of about 160° to an anterior directed position and it is then thrust towards the female and instantaneous contact is made. If, prior to the act, the spermatophores must be moved to the gonopodium tip, they must first be moved from the male's genital pore along an open groove of the gonopodium and held for a period within the groove without spilling out. Since the spermatophores

must be moved *under water*, this explanation seems inadequate to account for the facts.

After copulation, more sperms are introduced than are needed to fertilize a complement of eggs. The largest number of fertilized eggs in a complement reported for the platyfish is 156, and about 200 for the swordtail. The usual number is considerably less than half of these numbers. Sperms not participating in the immediate fertilization process do not die, but remain viable and capable of functioning at a future time for as long as six months, during which time they are carried by the female in the folds of the oviduct. At approximately four week intervals, females release their young. About seven days after the first brood is liberated the second complement of mature ova are fertilized by sperms carried by the female (Hopper 1943). After an initial copulatory period segregated females may produce a brood at monthly intervals for six months.

Thus when swordtails, which live for the most part in headwater areas of streams, are swept downstream and enter a platyfish population, it is likely that the mature females of both species are already mated, gravid and carry sperms of their own male species. The female swordtails probably have sufficient swordtail sperms to last them throughout the dry season.

Many factors are in force to prevent the interchange of genes between the swordtail and platyfish under natural conditions. The number and variety of morphological differences between *Xiphophorus hellerii* and *Platypoecilus maculatus* are impressive. For example, significant differences may be found, in their average body size, their body index referring to the relation of their depth to their length and other proportions, the number of dorsal fin rays, the number of vertebrae, the terminal structures of the gonopodia, the number of ribs, the shape of the skeletal elements of their gonopodial suspensorium, the number of scales in the lateral line and their secondary sexual characters, particularly the development of the sword-like extension of their tails.

There is a vast difference in the two fishes in the number of inherited, natural color patterns: for instance, *P. maculatus* has seven tail patterns (with five more possible), five macromelanophore patterns and three red patterns. The swordtail has one rare spotted variety the genetics of which has not been studied. The frequency of this one distinctive variety in nature is less than 1%. The swordtails differ in body proportions in a quantitative manner from one locality to another, and four subspecies have been recognized on these bases, but the differences are overlapping. The many inherited patterns described in *X. hellerii* by Kosswig (biblio. in 1939) and Breider (biblio. in 1939) are based on studies of domesticated individuals and represent, in most instances, no more than specific *P. maculatus* genes in *X. hellerii* — like hybrids. Gordon (1946a) has demon-

strated the process of introgression by which the platyfish comet gene P^{Co} was transferred to the swordtail, and this author described a number of common and colorful aquarium-bred swordtails which owe their distinctive patterns to a similar process.

They possess differences of a physiological nature, too, for example, in their response to flicker, in the length of the maturation period, in the number of young carried, in the earliness of germ cell differentiation, and in tumor responses (Gordon and Smith 1938). Finally, they differ in a

TABLE X

The Ranges in México, Guatemala and British Honduras of Platypoecilus maculatus *and* Xiphophorus hellerii

		X. hellerii	*P. maculatus*
1	Rio Chachalacas, at Encero	+	0
2	at Plan del Rio	+	0
3	Rio Antigua, at Jalapa	+	0
4	at Rio Bejucos	+	0
5	Rio Jamapa, at Rio Chico (Córdova)	+	0
6	at Plaza de Agua (El Tejar)	0	+
7	Rio Papaloapan, at Rio Blanco (Orizaba)	+	0
8	at Otopa	+	0
9	at Motzorongo	+	0
	at Papaloapan:		
10	39–40	−	+
11	39–43	−	+
12	39–38	−	+
13	39–41	0	+
14	39–45	−	+
15	39–42	−	+
16	Rio Tonto	+	+
17	San Bartolo	+	0
18	at Obispo	−	+
19	at Cosamaloapan	0	+
20	at Achotal	+	0
21	Rio Coatzacoalcos, at three localities	−	+
22	Rio Usumacinta, at Rio de la Pasión	+	+
23	at Rio San Pedro de Mártir	0	+
24	Arroyo Xotal	+	0
25	Laguna de Petén	0	+
26	Laguna de Zotz	−	+
27	Rio Hondo, Uaxactún	+	0
28	Belize River, at Rio Privación	+	0
29	at Rio Frio	+	0
30	Rio Motagua	+	0

+ = Present; 0 = Absent; − = Present but in the minority.
Data from Hubbs and Gordon, The Revision of the Xiphophorin Fishes.

number of psychological responses, according to Gerschler (1914) and Breider (1939). (A complete bibliography on these details will be published in a Revision of the Xiphophorin Fishes in a forthcoming paper by Hubbs and Gordon.)

The differences between the species are insufficient to prevent hybridization with subsequent production of some fertile offspring — when the species are confined in small aquaria. This may be because they are given no free choice of their mating partners, or because more probably, as Beach (1942) showed, there are sufficient individual variations for sexual excitement within the members of species to account for the breakdown of the usual mating behaviors in animals.

The important isolating factors that are effective at the present time, in preventing an interchange of genes in these two species in nature are not morphological, physiological nor geographical, but psychological factors aided by ecological conditions. The swordtail is a headwater species while the platyfish is a lowland form (Gordon 1939). The part that geographical isolation factors played in the past in separating these species are still effective in many places, as may be seen in the presence of swordtails in many rivers not occupied at all by platyfish (Table X).

In another cyprinodont group the ecological isolating factor breaks down, for the coastal *Mollienisia latipinna*, according to Hubbs and Hubbs (1932), hybridizes with the upland form *M. sphenops*. Just how frequently this occurs cannot be told because the hybrids, which are females only, are fertile and are capable of reproducing their own peculiar physical type by a process of gynogenesis. Since the hybrids were taken in the Rio San Fernando (R. Conchos) with *M. sphenops*, it is presumed the hybrid females mate with *M. sphenops* males.

VIII. Polymorphism

Mayr (1942) had previously used some gene frequencies data (1867–1939) to illustrate his point that "neutral polymorphism is due to the action of alleles approximately neutral as regards survival value." He cites several examples in addition to one concerning *P. maculatus* — Streseman's birds, Gerould's butterflies, Kinsey's gall wasp, Dobzhansky's beetles and Diver's snails — in support of his view that neutral polymorphism is more widespread than Ford (1940) would admit. According to Ford (1940), "genetic variability is divisible into four types: (1) disadvantageous varieties eliminated by selection and maintained at a low level by recurrent mutation of the genes controlling; (2) variations due to the effects of genes approximately neutral as regards survival value; (3) those dependent upon genes maintained by a balance of selective agencies; and (4) advantageous varieties controlled by genes spreading through the population and dis-

placing their allelomorphs." At a later point he adds that the third and fourth types constitute polymorphism. Here two or more well-marked forms, capable of appearing among the offspring of a single female, occur with frequencies high enough to exclude the maintenance of the rarest of them by recurrent mutation.

Ford (1940) lists as instances of balanced polymorphism the well-known cases of Nabours' ground locusts, Diver's snails and Winge's fish. Gordon and Fraser (1931) pointed out that the color pattern variability in *P. maculatus* had many similar features of polymorphism. Polymorphism is associated with close linkage and with the existence of a relatively common "universal recessive."

Further evidence of "neutral" polymorphism may be found in the natural populations of *Lebistes reticulatus* of northern South America. The genetics of this species has, of course, been known owing to the work of Winge, and its polymorphism has been discussed by various authors, notably Fisher and Haldane. Large series of the species were collected in Venezuela in their native habitat by Franklin F. Bond and are on deposit in the Museum of Zoology at the University of Michigan. A casual inspection of the large collection reveals a wealth of wild patterns. Under the section, "Mutation as a Basis for Racial and Specific Differences," Dobzhansky (1941) lists additional instances of polymorphism, some of which may be of the "neutral" type. Of course the important feature in all of these examples is the proof that the various components of a series are genetic.

The maintenance of balanced polymorphism in *P. maculatus* may be influenced by the short life-span of the local populations of this species. Gordon (1939 and 1940) pointed out that large numbers of platyfish may be found in impermanent ponds. During the rainy season, roughly from May to October, heavy rains cause the large rivers to rise greatly. The Rio Papaloapan overflows its banks in the vicinity of Papaloapan nearly every year, according to members of the Standard Fruit Company. The overflow waters fill inland depressions, many of them being former river beds of the meandering stream. For a time the depression pools are interconnected by waters sweeping across the land. When the rain and flood waters subside, November to April, a series of disconnected pools are formed which become smaller and smaller as the dry season progresses. By intensive seining in these restricted bodies of water at intervals during the last stages of their existence, some idea was gained of the factors that determined the survival of the isolated population. In one pond, as station 39–43, collections were made on March 4, 6, 7 and 10, 1939. The list of species taken and their relative frequency are given in Table XI. Some of the species are definitely predatory fishes, such as Belonesox and large Cichlosoma and Astyanax;

others were food competitors, such as smaller Astyanax, Hemigrammus, Gambusia, Xiphophorus, Mollienisia, Rivulus and Pseudoxiphophorus; perhaps a number of large Rhamdia catfish should be included here. The average size of most members of the population in our collections decreased as the dry season advanced. The outstanding factors in eliminating the larger fishes were fish-eating birds: kingfishers, egrets and other herons. Our seining practices could not have accounted for the disappearance of

TABLE XI

A List of all Species of Fishes Taken with Platypoecilus maculatus *and with* Xiphophorus hellerii

	Rios									
	Jamapa	Papaloapan								
	39–45	32–7	39–40	32–6	39–42	39–43	39–38	39–41	39–35	39–32
Characinidae										
Astyanax fasciatus aeneus	...	6	32	5	17	278	45	11	13	14
Hyphessobrycon compressus	...	...	...	..	..	56	2	...	..	..
Pimelodidae										
Rhamdia guatemalensis oaxacae	1	...	1	9	2	25	8	...	7	1
Cyprinodontidae										
Rivulus robustus	...	...	3	..	..	5	5	...	..	..
Rivulus tenuis	...	...	...	..	..	1	6	...	3	1
Poeciliidae										
Gambusia nicaraguensis sexradiatus	...	3	20	1	..	1100	97	54	55	..
Belonesox belizanus premaxillaris	13	13	...	..	..	46	8	10	20	2
Pseudoxiphophorus b. bimaculatus	...	2	12	3	22	5	69	...	15	5
Poecilistes pleurospilus lutzi	...	...	2	..	..	....	....	...	..	..
Platypoecilus maculatus	828	101	567	..	..	5148	1473	413	48	5
Xiphophorus hellerii strigatus	...	60	7	3	33	41	60	...	19	4
Mollienesia sphenops	59	4	74	..	3	21	4	1	18	1
Centropomidae										
Centropomis undecimalis	...	...	1	..	..	....	....	...	..	..
Cichlidae										
Cichlosoma fenestratum	...	...	...	..	..	....	....	...	..	1
Cichlosoma octofasciatum	23	...	10	..	1	....	2	1	1	2
Cichlosoma aureum	8	...	6	..	..	....	....	...	12	6
Cichlosoma salvini	...	1	1	..	..	127	....	...	..	3
Eleotridae										
Dormitator maculatus	74	...	...	..	..	....	....	2	..	..
Gobiomorus dormitor	...	1	1	..	..	....	....	1	..	5
Eleotris pisconis	1	...	...	..	..	....	....	...	..	..
Synbranchidae										
Synbranchus marmoratus	...	...	...	..	..	1	....	...	..	1

For position of some of the stations see Fig. 3. (32–6 and 39–42 are the same locality.) Data from Hubbs and Gordon, The Revision of the Xiphophorin Fishes.

the large predatory species, for the pond on March 4 was rather extensive and it was impossible to fish out any part of it thoroughly. On the last day of our collections, the predatory and non-predatory fishes were represented chiefly by their young. In pond 39–43 there was a total loss of the fish population owing to the complete loss of water. The main rivers and their larger, permanent tributaries are the platyfish reservoirs.

IX. Levels of Speciation

The four independent river populations of *P. maculatus* provide a good example of speciation at the lowermost level of genetic differentiation and the term "speciation" is used here as Simpson (1944) uses it to include the process of subspecies formation or "raciation" as well as that of species formation. The degree of differentiation shown in this species has not reached a point requiring taxonomic designations but, as Simpson points out, the event may prove that they are the beginning of changes that may become permanent and important in evolution.

A step higher in the level of differentiation is illustrated by the neighboring species *P. variatus* which, when studied by purely taxonomic procedures, breaks up into three subspecies. They are found to the north in the rivers Tecolutla and Cazones, and in the great Rio Panuco drainage system. Gordon (1943) in a preliminary statement indicated that each of these three subspecies has a distinctive pattern complex which coincides with, and is parallel to, the conventional taxonomic criteria. A report on this species will follow.

A still higher step in the level of differentiation in platyfish populations may be found in the two species just mentioned: *P. maculatus* and *P. variatus*. Mayr (1942) and Dobzhansky (1941) have suggested that these taxonomic units may better be regarded as subspecies chiefly on the basis of geographical replacement. The subject is being studied from many viewpoints. The conventional distinguishing taxonomic characters between these species are plentiful, and their number increase as additional anatomic studies are made. For instance, Gordon and Benzer (1945) showed their gonopodial suspensorial systems vary in many details, and preliminary psychological studies show striking differences in courtship behavior.

A third species, *P. xiphidium*, is restricted to the Rio Soto la Marina, north of the Pánuco-Tamesí river complex. This species has a number of distinctive patterns which are genetic. Finally, a fourth species, *P. couchianus*, a uniformly marked species, is found in a restricted portion of the Rio Santa Catarina which is a relatively minor tributary of the Rio Grande. This is the northernmost outpost of the platyfishes' range. The distribution of the species is presented by Gordon and Smith (1938). The unique uniformity of *P. couchianus* is coupled with an extremely small range and the

absence of predation. Gordon (1935) found over 99% of the total number of individuals collected in a spring pool about 25 feet in diameter. The species had been considered rare ever since it was discovered in 1859, but we caught over 500 in a short time in 1930 in a single pool at Santa Catarina and could have gotten many hundreds more. In its restricted area, *P. couchianus* has no apparent predators for no predatory fish or birds were found with them; and fish-eating birds would have extreme difficulty in spearing these tiny fish in their thick jungle habitat of Chara-like plants. On the other hand, *P. maculatus*, the most variable and most widespread species of the group, has been taken with Belonesox and other fish predators and fish-eating birds have been seen wading in their pools. These details seem to support Mayr's (1942) view in questioning Worthington's (1940) generalization that predation impedes the multiplication of species. Apparently just the opposite effect is seen in the platyfishes — at least as far as multiplicity of genotypes is concerned.

From the recent experimental work of Sumner (summary, 1945) on color background conditioned Gambusia and the conclusions that the conditioned fishes survive predation better than the non-conditioned ones, Dobzhansky (1941) has given credence to the statement that this type of protection has developed under the influence of natural selection. Occasionally fishes are found in nature in Mexican ponds so cloudy with suspended matter that it was impossible to see through one inch of their waters. Under these conditions it was noticed that all the fish species in the pool, prey and predators, had become equally pale by the loss of melanin granules in their pigment cells. The whole population reacted uniformly so that the delicate balance between prey and predator, in terms of visibility, or lack of it, remained essentially as before. The key to the problem is, as Sumner states, that the capacity for adjustment to a changed visual environment varies widely in different individuals of the same species. It is this variable color reaction capacity which has been subject to natural selection. Other factors are involved in the problem of conspicuousness *vs.* protective coloration. In pools 39–38 and 39–43 on the initial collections, predatory fishes like Belonesox, large Cichlosoma and Astyanax were taken commonly with the fishes upon which they preyed: Gambusia, Platypoecilus, Hyphessobrycon, *etc.* Later collections in the same ponds yielded only the forage fishes and the young of the larger species. The agents which were responsible for removing the large and effective predator fishes were the kingfishers, herons, egrets and other fish-eating birds. They apparently picked out the larger specimens simply because they were more conspicuous to them. Thus the predator fishes were themselves preyed upon. Other details concerning the adaptability of fishes to their environment and the relation of these details to speciation

have been treated by Hubbs (1940). Some of the genetic implications in this study have been discussed elsewhere (Gordon 1946a).

X. Summary

1. The genes for seven tail patterns in *Platypoecilus maculatus* make up a series of dominant, multiple alleles. The patterns have been used as indices in the analysis of the genetic composition of natural fish populations of the Rios Jamapa, Papaloapan, Coatzacoalcos and Usumacinta of Mexico and Guatemala. The genes are P^{O}, P^{M}, P^{Mc}, P^{C}, P^{Cc}, P^{Co}, P^{T} and P^{+} the recessive.

2. The entire series of seven patterns is found only in the platyfish populations of the Rio Papaloapan. The Rio Jamapa platyfish lack the genes P^{M} and P^{Mc}. The platyfish of the Rios Coatzacoalcos and the Usumacinta lack P^{C} and P^{Co} but these populations may be distinguished on the basis of the frequencies of their other genes.

3. A number of smaller, local, isolated populations within the Rio Papaloapan system and within the Rio Usumacinta system have distinctive gene frequencies.

4. By comparing the gene frequencies of platyfish populations taken in the vicinity of Papaloapan, Oaxaca at various time spans — 1867, 1902, 1932 and 1939 —it was found that most of the genes have remained at fairly constant frequencies. Some apparently show a slight progressive increase while others show a slight decrease.

5. By comparing the gene frequencies of populations taken from the same area at different periods within a week — March 4, 6, 7 and 10, 1939 — the same general constancy was found.

6. Population samples taken 70 years apart and those taken 7 days apart are apparently similar.

7. *Platypoecilus maculatus* is sympatric with *Xiphophorus hellerii* at ten collection areas out of thirty where one or the other, or both, were found. No hybrids have been discovered in nature, yet h ybridization between these species is common under aquarium conditions.

8. *P. maculatus* differs from *X. hellerii* in many morphological, physiological and psychological traits. Geographical isolation factors are still effective in isolating platyfish and swordtail populations at many places at the present time. Where the species inhabit the same river, the platyfish are found near the lowlands while the swordtails are found in the headwater areas — indicating an ecological difference between them. When the species are sympatric, living in the same pond, psychological solating mechanisms prevent the interchange of genes.

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Immunogenetics[1]

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CONTENTS

I. INTRODUCTORY REMARKS

The term "immunogenetics" was proposed by the author some years ago to designate studies in which the technics of both genetics and immunology were employed jointly. A somewhat analogous word, "immuno-

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chemistry," has been in good repute for many years in another field; its adoption encouraged the proposal of this combination of words. The term indicates the study of genetic characters as yet only detectable by immunological reactions.

At the present writing, all findings in immunochemistry substantiate the belief of most immunologists, as ably expressed by Wells (84), that antigens recognizable by immunological technics are definite chemical substances. That is, in all cases in which it has been possible to analyze chemically an immunological difference between antigens, such differences have been shown to involve the chemical structures of the substances being compared and not simply their physical states. Furthermore, following the discovery by Landsteiner (43) that chemical substances of simpler form than proteins could be antigenic *if attached to proteins* — these substances being called haptens — it was found by Heidelberger and Avery (22, 23) that the specificity of the different types of the pneumococcus (*Diplococcus pneumoniae*) depended upon carbohydrates in the capsule of the organism, not on proteins. In other words, biological specificity need not be attributed only to proteins; simpler substances also may produce biological specificity, particularly if they are attached to proteins. (The reader is referred to a recent monograph by Landsteiner (45) on the general subject of the specificity of serological reactions. In it are given references to numerous pertinent articles, review papers and other books dealing with immunology and immunochemistry.)

Indeed, much of the present information concerning proteins is due to the findings of immunologists. As Landsteiner (44) has stated, "Because of the difficulties in working with substances of high molecular weight, one is as yet far from the goal of chemically characterizing the single proteins and determining the constitution of these substances, which rank as the most important components of living matter. Hence, it was not the use of the ordinary chemical methods, but the application of serological reagents, which led to an important general discovery in protein chemistry, namely, that the proteins in various animals and plants are different and are specific for each species. The multiformity is increased by the fact that also various organs contain particular proteins." And it is probable that for some time to come many classes of chemical substances in biological material will be detected first by immunological technics, with the expectation that chemical analyses may follow.

II. Antigens of Human Cells and Related Phenomena

1. A *and* B *Antigens*

A knowledge of the protein specificities of different species led Landsteiner (41, 42) to the search for specificities within a species. This resulted

in the recognition of the well-known cellular characters **A, B** and **O** of humans, commonly called "blood groups." The explanation now generally accepted of the inheritance of these cellular characters is that the causative genes form a triple allelic series, with both **A** and **B** being dominant to their absence, or **O**. This was first postulated by Bernstein (1) as a result of purely statistical considerations. (The reader is referred to the excellent book by Wiener (86) for a discussion of the various theories of heredity of the blood groups, as well as for references to and discussions of articles on the blood of humans and other animals.) It was and is of considerable practical importance that the correct explanation of the inheritance of these antigens be known, because of their use in medico-legal cases, particularly those in which parentage is in dispute.

As all students of biology are aware, the serum of an individual belonging to group **O** contains isoantibodies (isoagglutinins) α and β against the antigens **A** and **B**, respectively, so that the serum of such a person will agglutinate cells containing **A, B** or **AB**. Likewise, the serum of an individual of group **A** contains the β isoagglutinin, that of group **B** has the α, while that of group **AB** has neither. A knowledge of the interactions between the serum and cells of these groups has been of inestimable value in preventing accidents in blood transfusions.

Beyond stating that there is a reciprocal relationship between the presence of the antigen and the absence of the specific antibody, very little can be said of these normally occurring isoantibodies. As was stated above, both isoagglutinins (α and β) are present in the serums of individuals lacking the dominant antigenic characters, while neither isoantibody is present in those carrying both **A** and **B**. It has been postulated (see Wiener (86) for references) that both isoagglutinins α and β are normally present in all humans, but that α is absorbed from the serum of individuals possessing antigen **A**, that β is absorbed when **B** is present, and that both are absorbed from the serum of **AB** individuals. This explanation may eventually be found correct, yet to the writer it seems that there is great need for further experiments on various aspects of the isoantibodies themselves. This will admittedly be more difficult than studies of the cellular antigens.

2. M, N *and* P *Antigens*

Somewhat later, Landsteiner and Levine (46, 47) identified additional cellular antigens in humans, now known as **M** and **N**. These behave in inheritance as a pair of contrasting characters, so that all individuals possess either **M, N** or **MN** (the heterozygote). At the same time, these authors reported the detection of another property, **P**, in human cells. There is no question of the existence of this antigen, but satisfactory

reagents for its detection have been and still are difficult to obtain regularly. These cellular characters are genetically independent of **A** and **B**.

3. **Rh** *Antigen*

In a search for further antigens in human cells, Landsteiner and Wiener (52) found that anti-rhesus serum from guinea pigs and rabbits would differentiate some human bloods. The cellular component first identified thus is now called **Rh** (contraction of rhesus) and, as Wiener (90) has stated, its discovery has solved two major medical mysteries. Despite care in matching blood groups prior to transfusions, there occasionally were hemolytic reactions following transfusions. Wiener and Peters (93) pointed out that the **Rh** component was undoubtedly the causative factor in many such reactions, and this proposal has been substantiated. With the accumulation of more evidence it is believed that about 90% of similar hemolytic reactions can be attributed to the **Rh** antigen. An individual who lacks the **Rh** character and who has been sensitized to it by a previous transfusion or pregnancy is potentially susceptible to a hemolytic reaction if transfused with blood containing **Rh**. Thus another test for compatibility of bloods is added to those already known, thereby making safer the transfusion of blood in humans.

a. Erythroblastosis Fetalis. The other puzzle to the medical profession was that of certain women who had infants with hemolytic disease (erythroblastosis fetalis) and in some cases a record of unexplained stillbirths. Levine and co-workers (57a, 57b) provided a reasonable explanation for the majority of these cases. That is, if the mother lacks the **Rh** agglutinogen (is **Rh**-negative, or **Rh**−) and the father possesses it (is **Rh**-positive or **Rh**+), she may develop antibodies against an **Rh** fetus. These antibodies, in turn, may pass through the placenta and hemolyze the blood of the fetus, the result being one or another of the manifestations of the disease. Cases are also known of hemolytic disease in infants when the mother is **Rh**+ (57). The antigen chiefly involved will be referred to later in this paper.

There are still many unanswered questions about this biological phenomenon. For example, only about one in fifty of the **Rh**− females involved in the critical matings (**Rh**+ males×**Rh**− females) are known to become sensitized (90); the others seemingly are but little, if at all, affected. It is generally accepted that the human placenta is permeable to antibodies, at least in the later stages of pregnancy. Hence, a satisfactory explanation is yet to be offered as to why antibodies to **Rh** should primarily be involved in hemolytic disease, at least more often than those to other antigens of human cells. On the other hand, Levine (53, 55) proposes and cites the opinion of other workers that there may be an interaction between

mother and fetus of the A and B antigens and their antibodies. This whole field certainly warrants much further investigation.

b. Antibodies and Rabbit Embryos. In this connection, experiments done on the passage of induced antibodies through the placental wall in rabbits are of interest. Levine and Landsteiner (58) reported the existence of two agglutinogens in the corpuscles of the rabbit. These were called H_1 and H_2 by Castle and Keeler (7), who proposed that the genetic explanation for the occurrence of these two antigens, singly or together, and the absence of both (group O), was that of a triple allelic series of genes. It was further demonstrated by Keeler and Castle (37) that females lacking both H_1 and H_2 (therefore belonging to group O), but in whose serums antibodies to both antigens had been induced, could produce young with either antigen by mating to bucks homozygous for one or the other component. It is well known that, in the rabbit, there is maternal transmission of antibodies through the placenta to the young. The striking point of these experiments is that, from such females carrying both antibodies, the serum of young with agglutinogen H_1 in their cells had agglutinins to H_2 but no trace of that to H_1. And the serum of those with antigen H_2 carried the agglutinin to H_1 but not that against H_2. In other words, the developing fetuses were protected in some manner from an antibody antagonistic to their cells. It has also been demonstrated in doves (36) that each of a pair of parabionts developed antibodies against the cells of the co-twin, and these antibodies were seemingly present only in one member of the pair.

Thus it appears possible that a different situation exists in the rabbit and human, respectively, for protection of the fetuses against at least some of the isoantibodies of the mother. Unfortunately, only a small number of female rabbits were tested in the above experiments. Results with much larger numbers would be welcomed, so that it might be learned whether or not the festuses of any immunized females could be affected by the antibodies of the mother. Comparable information in other species should also be obtained on this important and fundamental subject.

4. *Subgroups of Cellular Characters*

The division of the cellular character A into subgroups A_1 and A_2 is well established and has been critically reviewed by Wiener (86) and others. Wiener also cites the various proposals for subgroups of other antigens of human cells. Just how many of these proposals are based on results obtained with immune serums (reagents) of different specificities and how many represent actual subgroups, is a matter for further experimental work to decide. It seems clear that the criteria of both immunology and genetics should be met before a subgroup can be completely accepted.

Currently the division of the **Rh** antigen into subgroups, or subtypes, is of great interest. Wiener (87) has postulated six allelic genes to account for the varieties of types of **Rh** agglutinogens. Eight subtypes of **Rh** blood are proposed under this scheme, these having a wide range of frequencies. These subgroups are recognized by their differential reaction toward three kinds of anti-**Rh** agglutinins, each presumably having different specificities. The absence of any reaction with these **Rh** antiserums indicates **Rh**− blood, although Levine *et al.* (57) and later Race and Taylor (72) have described antiserums that reacted with all **Rh**− bloods as well as with those of one or more **Rh** subgroups. The antigen demonstrable by these immune serums was called "**Hr**" (reversal of **Rh**) by Levine and co-workers and "St" by Race and Taylor. It should be stated that the **Hr** antigen seems to be most often involved in cases of hemolytic disease in infants in which the mother is **Rh**; it is also a factor to be considered in transfusions. A change in the original nomenclature has been advocated by Wiener (88), and this includes suggestions made by Race *et al.* (73). It is now proposed (91) that there are eight allelic genes for the **Rh** and **Hr** agglutinogens, although the reactions of all the possible subgroups have not yet been observed.

The various aspects of the **Rh** and **Hr** antigens in medicine represent a rapidly growing field of research. The possibility that there may be an association between the **Rh** factor and feeble-mindedness has incited considerable interest. A paper by Snyder *et al.* (79) refers to their own and other observations. For a much more comprehensive treatise than has been attempted in this paper, the interested reader is referred to review articles, among others, to those by Boyd (4) and Wiener (89). However — to make a general comment on this work — the writer would like to see the technic of agglutinin-absorption more widely used in the differentiation of the various **Rh** types. Also the suggestion by Levine (56) is heartily seconded, that the nomenclature of the various subtypes should at some future date be considered by an international committee of geneticists and serologists. In fact, the nomenclature of all cellular antigens might well be considered by such a committee.

5. *Antigens in Tissues and Body Fluids*

Much work has been done to determine in what other cells of the body the antigens of the red blood cells may be found. This has been largely done with the **A** and **B** components. If, as Haldane (20) has proposed, "the gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connexion with the chromosome," one might anticipate that an antigen would be present in most, if not all, cells containing the causative gene. Actually, as summarized by

Boyd (3), the **A** and **B** antigens have been demonstrated in nearly all tissues, body fluids and secretions (as the saliva).

On the other hand, several investigators have reported their inability to demonstrate the presence of **M** and **N** substances in the body tissues and fluids other than in the blood cells. Comparatively few such tests have been made with the **Rh** antigen, with negative findings (54). However, Kosjakov and Tribulev (38) claimed that, by the use of different technics, **M** and **N** could be shown to be present in tissues other than blood cells. And more recently Boorman and Dodd (2) have substantiated this claim with respect to the presence of **M** and **N**, and report that **Rh** is also demonstrable in some tissues and in the saliva.

To the writer, the presence or absence of the **A** and **B** antigens in the saliva represents an interesting example of the dependence of a gene upon its substrate for the specificity of its final product. Schiff and Sasaki (76, 77) proposed that the presence of **A** and **B** in such secretions as the saliva was due to the presence of a "secretor" gene, (*S*), their absence was conditioned by a "non-secretor" allele, (*s*). Further, whether **A, B** or **AB** was found in the secretions depended upon which was present in the blood cells and tissues. That is, the gene *S* presumably produced an enzyme which would act in a particular organ to effect the presence of **A, B** or **AB** in the secretions if either antigen or both were in the organ. It can also be demonstrated, but with more difficulty, that the *S* gene is active in **O** individuals. It is interesting that the antigens are primarily alcohol-soluble in the erythrocytes and water-soluble in the secretions. There is evidence, furthermore, that the water-soluble substance of the saliva is actually formed in the salivary glands and is not simply a disintegration product of the alcohol-soluble substance. Many ramifications of this subject are discussed, references are given to pertinent papers and the results of much experimental work are reported by Hartmann (21).

There are other known antigenic substances for which a genetic system has not yet been shown. The best known of these is the Forssman antigen, which is antigenically related to the **A** of humans. This is a collective term covering substances which will produce hemolysins to sheep red blood cells when injected into rabbits. It is widely, but not necessarily randomly, distributed in animals, and less widely in some bacteria and higher plants. Usually it is found in the organs and not in the blood cells of the animal species possessing it. For example, it has been demonstrated in the organs but not in the blood cells of the guinea pig. It is found in both blood cells and organs of chickens, however, and in sheep and goats it is found only in the blood cells. Its occurrence in animals and plants is summarized by Boyd (3), who also gives examples of other antigens in various species that are serologically related.

6. *Cellular Antigens of Humans in Related Species*

At the outset, it may be stated that all data so far assembled allow the conclusion that the genes giving rise to antigens produce their end-results irrespective of the genetic complex in which they are placed. For example, the genes of humans and higher apes producing A of the blood cells appear to be homologous, for the A substances in these species are indistinguishable and presumably identical. And yet it is doubtful that anyone would claim that the genic complexes of any of these species are the same. If the above conclusion be accepted, an additional statement can be made —— namely, that the antigens of various species can readily be compared by immunological technics to determine their probable identity or similarity. These technics involve the absorption of agglutinins from the immune serum. A comprehensive explanation of this useful technic, its limitations and advantages, has been given by Krumwiede *et al.* (40). It is generally believed that every cell contains many antigens and that a serum immune to these cells has a counterpart of, or antibody specific to, each antigen of the cells. The interactions between them and the results following antibody-absorption may be illustrated in a hypothetical case, as follows.

Agglutinins in Antiserum (anti-ABC)	Absorption by Cells Containing	Agglutination of Cells Containing Antigens			
		ABC	ABD	BCE	CEF
abc	–	+++	++	++	+
abc	A	++	+	++	+
abc	AB	+	0	+	+
abc	BC	+	+	0	0
abc	ABC	0	0	0	0

As stated above, the basic assumption underlying the interpretation of the results of agglutination obtained following agglutinin-absorption is that each agglutinin reacts only with its specific antigen. Thus *a* interacts only with *A*, *b* with *B* and *c* with *C*. If absorption of an antiserum containing *a*, *b* and *c* is done with cells containing *A*, *B* or *C*, singly or in any combination, any resulting agglutination of cells will be by virtue of the interaction of one or more of the agglutinins (as *a*) with its particular antigen (*A*). For example, if the cells used in absorption contain *A*, agglutinin *a* will be removed from the antiserum, leaving *b* and *c*, and reactions will be possible only with cells carrying either *B* or *C*, or both.

Thus if an antiserum to human cells is made into a "reagent" which contains antibodies only to antigen B, this fluid may be tested on the bloods of other species. The reagent has been found to agglutinate the cells of some individuals of orangs and gibbons (48). Further, when

reactive cells of these species were used in absorptions of the reagent, the agglutinins were removed not only for the absorbing cells but also for human cells containing B. From these results it may be stated that the B of orangs and gibbons is indistinguishable from, and presumably identical with, the B of humans.

A different sort of relationship was revealed when the reagent for human B was tested with cells of lower monkeys (49). The cells of the Lemuridae, or lemurs, and of the Platyrrhina, or New World monkeys, were reactive with the reagent, while those of the Cercopithecidae, or Old World species, were not. But when the reactive cells of these species were used in absorptions, the antibodies to B were only partially exhausted, for reactions still remained for human cells containing B. That is, the cells of lower monkeys which were agglutinated in such tests contained a substance similar to, but not identical with, antigen B. Genetically, if the antigens of two or more species are identical, the causative genes are also probably homologous; if the antigens are similar but not identical, the genes presumably will also be similar, but cannot be considered as homologous.

Recently it has been shown (6, 92) that A-like or B-like antigens could be demonstrated in secretions or organ extracts of some species of higher apes and lower monkeys in which the erythrocytes gave no reactions. That is, supposedly the gene for the antigen was present in the individuals concerned but its effect was not demonstrable in the blood cells. One must, therefore, take into consideration the fact that an antigen being present in one kind of cell does not always mean that it is demonstrable in another. Apart from the substances which are particular to certain organs, as cited by Landsteiner (45) and by Loeb (60), one would anticipate a more or less common antigenic substrate being typical of the cells and tissues of an individual. However, since the interaction of antigen and antibody is largely a phenomenon dependent on the antigens at or near the surface of the cell, it is possible for a character to be present in the cell but demonstrable, if at all, only under exceptional conditions. For example, in human erythrocytes a component (*T*) is reported by Friedenreich (17) to be detectable only after the action of the environment on the corpuscles. It seems reasonable to believe that there are many antigens within cells in addition to those found at the surface of the cell.

III. Species Relationships

Until recently most comparisons of antigenic relationships among species have primarily employed the serums, presumably serum proteins, as the source of antigens. The classical studies of Nuttall (66) represent the first comprehensive work of this kind. These have been followed by numerous other studies on animal serums, cited by Boyden (5). Land-

steiner and Van der Scheer (50) first used the red blood cells in a systematic differentiation of two species and their hybrid —— horse, donkey and mule —— whereas the serums of these species are difficult, if not impossible, to differentiate serologically. Their results, plus the findings (22, 23) that the immunological specificities of the pneumococci were amenable to a chemical explanation, suggested to the writer that there was excellent experimental material in the species hybrids and backcross hybrids in pigeons and doves which Professor L. J. Cole had produced. These have generously been made available for the experiments.

1. *Cellular Antigens in Species Crosses*

a. Pearlneck and Ring Dove. The technical procedures employed in these studies involve either direct use, or slight variations, of the agglutinin-absorption technics discussed previously. The details have been described elsewhere (27, 32). Briefly, a definite differentiation of the cells of any pair of related species of pigeons and doves has been possible only after the antiserum to one has been absorbed by the cells of the other. That is, after an antiserum to Pearlneck (*Streptopelia chinensis*) has been exhausted of part of its content of antibodies by mixing it with an excess of the cells of Ring dove (*St. risoria*), it becomes a reagent or test-fluid which will agglutinate the cells of Pearlneck, but not those of Ring dove (27). The cellular antigens of Pearlneck reactive with this reagent are called the "species-specific" characters of Pearlneck. Similarly, species-specific components of Ring dove as compared with Pearlneck are readily demonstrable. Further, the cells of the hybrids between these two species appear to contain all the antigens shared by the parent species, and most, but not all, of the characters specific to each parent. In addition, the cells of all hybrids —— 29 or more —— produced in this cross over a period of years possessed a "hybrid substance" not found in either parent (24, 27). The immunological reactions on which these differentiations are based are presented in Table I.

Evidence that the species-specific antigens of each parental species segregated in a Mendelian fashion was obtained in backcrossing the species hybrid and selected backcross progeny to representatives of each parental species. At least ten substances which distinguish Pearlneck from Ring dove have been obtained in unit-form, called *d*-1, *d*-2, *d*-3 . . . *d*-12 (26, 35). That is, backcrosses to Ring dove of birds carrying a particular cellular character produced only two kinds of offspring in approximate equality; namely, those with and those without the antigen. The Ring dove is distinguished from Pearlneck by approximately the same number of cellular substances. These have not been obtained as single substances

TABLE I

Antigenic Relationships Between the Blood Cells of Pearlnecks, Ring Doves and Their Hybrids

Immune Serum	Absorbed by Cells of	Titers of Agglutinations with Cells of		
		Pearlneck	Ring Dove	F_1
Pearlneck	.....	8, 9	8, 9	8, 9
Pearlneck	Ring dove	7, 8	0	7, 8
Ring dove	.....	8, 9	8, 9	8, 9
Ring dove	Pearlneck	0	6, 7	6, 7
F_1	.....	7, 8	7, 8	7, 8
F_1	Pearlneck	0	5	5
F_1	Ring dove	6	0	6
F_1	Pearlneck and Ring dove	0	0	3, 4

The digits represent the highest dilution of antiserum at which agglutination was visible microscopically; if the first dilution was 90, 1 = 90, 2 = 180, 3 = 360 . . . 9 = 23,040. "0" = no clumping in the first or absorbing dilution.

From IRWIN, M. R., AND COLE, L. J., *J. exp. Zool.* **73**, 85–108 (1936).

for the females of Pearlneck and of the species hybrids rarely produce viable squabs under our conditions.

The presumed unit antigens of Pearlneck are immunologically distinct, with possibly a few exceptions (26). The tests by which this point may be established involve the absorption of Pearlneck antiserum by Ring dove cells plus those of one or more birds carrying a particular antigen, as *d*-1. Only the agglutinins for the *d*-1 character will be removed from the reagent for Pearlneck cells; all others will remain and each will react with its specific cellular component. This process must be repeated for the different antigens of Pearlneck; the results of such tests have provided the data required to postulate the existence of the different antigens in the backcross hybrids. These are given in Table II. From these results it may be concluded that one or more genes on ten or more chromosomes of Pearlneck produce antigens which make the cells of Pearlneck different from those of Ring dove.

b. Columba guinea×*C. livia and Others.* Employing the same technics of genetics and immunology, it has been demonstrated that there are five or six cellular antigens which distinguish the triangular-spotted pigeon of Africa (*Columba guinea*) from the domesticated form of the common pigeon (*C. livia*). These were called **A, B, C, D, E** and **F** (32); the character **D** was available as a single substance for only a few tests. Also, several backcross hybrids from mating to Ring dove the species hybrid between

TABLE II

Agglutination Interactions of the Species-Specific Pearlneck Components

Cells	Ring Dove	Titers for the Different Cells of Anti-Pearlneck Serum, First Absorbed by Ring Dove Cells, then by Cells of Backcross Birds Containing, Respectively, One of the Following Pearlneck Characters									
	Alone	*d*-1	*d*-2	*d*-3	*d*-4	*d*-5	*d*-6	*d*-7[1]	$\frac{d\text{-}4}{d\text{-}8}$	*d*-9	*d*-11
Ring dove	0	0	0	...	...	...	...	...	....	...	...
Pearlneck	7, 8	...	...	...	...	...	...	...	...	...	...
F_1	7, 9	8	8	8	8	7, 8	7, 8	7, 8	8	8	6, 8
d-1	3, 4	0	3, 4	3, 4	3, 4	2, 3	3, 4	4	2, 4	4	4
d-2	2, 4	2, 4	0	3, 4	2, 3	2, 3	3	3, 4	2, 4	3	4
d-3	1, 2	2	1, 2	0	1, 2	1, 2	2	?	2	1, 2	2
d-4	2, 4	2	2	2	0	2, 3	2, 3	2, 3	0	?	2, 3
d-5	5, 6	5, 6	5, 6	6	5, 6	0	5, 6	6	5, 6	6	6
d-6	2, 4	3	3, 4	2, 3	2, 3	2, 3	0	3	3, 4	3	·2
d-7[1]	2, 4	3	3	2, 3	3	2, 3	3	0	1, 3	3	2, 3
d-4.*d*-8	3, 4	2, 3	3	2	2	2, 3	2, 3	1, 2	0	2	2, 3
d-9	2, 3	2, 3	2, 3	2, 3	?, 2	2, 3	2, 3	2, 3	?, 1	0	2, 3
d-11	7, 8	8	8	7, 8	8	8	7, 8	7, 8	8	8	0

Symbols: same as used in Table I. This table includes the majority of the reactions given in a paper by Irwin (26), plus experimental results obtained in later trials. A ? indicates a doubtful reaction at the first dilution of serum.

[1] These cells appear to contain two substances.

From IRWIN, M. R., *Genetics* **24**, 709–721 (1939).

livia and Ring dove have been available at one time or another over a period of years. A segregation of cellular components specific to *livia* has been observed (27a) in the blood cells of these birds, although it has not been possible to test the cells of each bird for antigenic content with those of all the others. Further, the antigenic characters peculiar to Senegal (*St. senegalensis*) in contrast to Ring dove are now in the process of being obtained in unit form (30, 31).

The results obtained in comparing the blood cells of any two species which hybridize allow the general conclusion to be made that the two species contain antigens in *common* and that each species contains one or more antigens *specific* to itself. Furthermore, the divisibility of these cellular components in backcross generations has shown that the species-specific components are heritable according to current genetic theory. Undoubtedly the same kind of antigenic relationship exists between related species which do not hybridize. In genetic terms this means that each species of a related pair possesses genes which produce the substances shared by each. These genes would be homologous in the two species, although their linkage relationships need not be the same. In addition,

each species possesses genes which differentiate it from the other or contrasted species. Just what the relationship of the genes for the species-specific characters of each of two related species, respectively, are to each other is at present only a matter of speculation.

c. Pearlneck, Ring Dove and Senegal. Returning to the antigenic relationship between Pearlneck and Ring dove, it is a matter of interest to inquire whether the substances peculiar to Pearlneck may be present in part or *in toto* in other species. By immunological tests alone it has been shown (29) that Senegal shares a part or all of the various antigens peculiar to Pearlneck and most of those common to Pearlneck and Ring dove. These tests are shown in Table III. Briefly, Senegal antiserum absorbed by Ring dove cells reacted with each of the specific components of Pearlneck, indicating that Senegal contained antigens at least similar to all those of Pearlneck. And when the cells of both Senegal and Ring dove were used in the exhaustion of Pearlneck antiserum, the resulting reagent no longer reacted definitely with the cells of any unit-substance of Pearlneck except *d*-2, *d*-6 and *d*-11. (Numerous subsequent trials have always given negative reactions with cells carrying *d*-3 and *d*-7; at only one time of testing has the reaction been positive.) That is, presumably all of Pearlneck antigens *d*-1, *d*-3, *d*-4, *d*-5, *d*-7, *d*-4.*d*-8 and *d*-9 are present in Senegal cells, but only a part of *d*-2, *d*-6 and *d*-11 are present. Hence, a segregation of these three characters (*d*-2, *d*-6 and *d*-11) would be expected to occur in the backcross progeny of the species hybrid between Pearlneck and Senegal in matings to Senegal. Genetic confirmation of this expected result was actually obtained (29) for *d*-6 and *d*-11; the Pearlneck antiserum in use at the time of the tests did not distinguish the fraction of *d*-2 peculiar to Pearlneck as compared with Senegal.

From the above results it may be concluded that Pearlneck differs from Ring dove in ten or more cellular antigens, but from Senegal in only a part of three of these same antigens, the others being shared by Pearlneck and Senegal. A gene or genes on three chromosomes of Pearlneck would thus account for the known antigenic difference of the cells of Pearlneck from those of Senegal. A comparison of the unit-antigens of Pearlneck and Senegal, respectively, in contrast to Ring dove would, therefore, be of considerable significance. From such comparisons a clearer picture might be expected to emerge of the relationship of substances in Senegal to *d*-2, *d*-6 and *d*-11 in Pearlneck.

d. Comparisons of Antigens in Species. Another example of how one species may share with others a part or all of the antigens which distinguish it from a particular species has been reported (25) for the cellular characters which differentiate *guinea* from *livia*. It was found that Pearlneck and Ring dove of the genus Streptopelia possessed substances in

TABLE III

Agglutination Interactions, with Various Absorbed Antisera, of the Cells of Pearlneck, Ring Dove and Senegal, and of Cells Carrying One of the Species-Specific Characters of Pearlneck

Anti-serum	Absorbed by Cells of	Cells												
		Species				Unit-Pearlneck Substances								
		Pearl-neck	Ring Dove	Senegal	$F_1 \frac{P.N}{R.D.}$	*d*-1	*d*-2	*d*-3	*d*-4	*d*-5	*d*-6	*d*-7[1]	$\frac{d\text{-}4}{d\text{-}8}$	*d*-9	*d*-11
Pearlneck	Ring dove	8,9	0	7,8	7,8	3,4	3,4	0,2	3,4	5,6	3,4	3,4	3,4	2,4	7,8
Senegal	Ring dove	7	0	8,9	7,8	3,4	3,4	3,4	3,4	5,6	2,3	2,3	2,3	1,3	3,4
Pearlneck	Ring dove and Senegal	7,8	0	0	7	0	0,3	0, ±	0	0	2	0, ±	0	0	7,8

Symbols: same as in Table I; ± = weak or doubtful agglutination at the first dilution of antiserum. This table differs from that given by Irwin and Cole (29) to include positive agglutination of the *d*-2 substance in the use of another Pearlneck antiserum absorbed by both Ring dove and Senegal cells.

[1] Two substances.

From IRWIN, M. R., AND COLE, L. J., *Genetics* **25**, 326–336 (1940).

common with *guinea* (genus Columba), which in turn distinguished *guinea* from *livia*. Since these antigens had been obtained in unit-form in back-cross hybrids, they became "testers" by means of which their presence or absence in other species could be detected.

TABLE IV

Sharing of Unit-Characters of Guinea, Not in Common with Livia, with Pearlneck and Ring Dove

Immune Serum	Absorbed by Cells of	Agglutination of Cells of								
		Species				Unit-Substances of Guinea				
		Livia	Guinea	Pearl-neck	Ring Dove	A	B	CD[1]	E	F
Guinea	Livia	0	8	6	4	2	5,7	5,7	5,7	1,2
Pearlneck	Livia	0	6	8,9	6	2,3	0	5	2,4	2,3
Ring dove	Livia	0	4	5,7	8	2,3	0	2	2	2,3
Guinea	Livia and Pearlneck	0	7,8	0	1,2	0	6	3	7	0
Guinea	Livia and Ring dove	0	8	5,6	0	0	6	5	7	0

Symbols: same as given in Table I.
[1] These cells may have contained C and D, and are therefore called the CD complex.
From IRWIN, M. R., *J. Genet.* **35**, 351–373 (1938).

From the reactions given in Table IV it may be deduced that **A** and **F** of guinea are likewise present, presumably *in toto*, in Pearlneck and Ring dove. That is, two of the substances which are *common* to Pearlneck and Ring dove are the **A** and **F** of *guinea*. Both Pearlneck and Ring dove also possess only a part or parts of **CD** and **E**, but not of **B**. Hence the component **B** and parts of **CD** and **E** are peculiar to guinea in contrast to *livia*, Pearlneck and Ring dove.

Thus it is seen that the *d*-2, *d*-6 and *d*-11 antigens of Pearlneck are only partially shared by Senegal, and also that the **CD** complex and **E** of guinea are partially shared by either Pearlneck or Ring dove, or both. As has been previously suggested (25, 29), these relationships may be explained in either of two ways, or by a combination of them. If any one of these components, as *d*-11, were produced by the action of a single gene, it would be concluded that a gene with an effect only similar to *d*-11 was present in Senegal. On the other hand, if two or more genes on a single chromosome of Pearlneck produced *d*-11, then one or more genes in Senegal might have an effect either identical with, or similar to, only a part of *d*-11. It is probable, although not proven, that more than one gene on the respective chromosomes work together in the production of most or all of these species-specific characters, so the second alternative is slightly favored as an explanation of the resemblances observed.

A hint of the antigenic relationships existing between related species is provided by a diagrammatic representation of the cross-reactions given in Table IV. A diagram of the antigens of one species, as *guinea*, is rela-

FIG. 1

Diagram of the Sharing of Cellular Antigens of *guinea* with *livia*. [From Irwin (25)

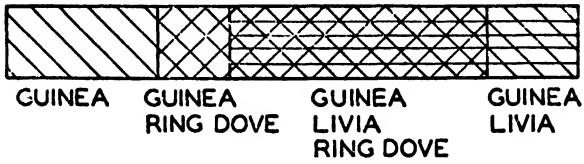

FIG. 2

A Diagram of the Cellular Components of *guinea*, Showing an Approximation of the Proportions Shared with Ring Dove and with *livia*. From Irwin (25)

tively simple if its antigenic composition is compared with that of only one other species, as with *livia* in Figure 1. The complexity is increased two-fold if the antigens of Ring dove are included (Fig. 2). The tests necessary to assign the approximate proportions of the latter figure are given elsewhere (25) together with more complex diagrams for the antigens of each of these four species —— *guinea*, *livia*, Pearlneck and Ring dove —— in relation to those of the other three.

e. Antigens of the Genus Columba. Comparable but more extensive comparisons have been made of the interrelationships of the cellular substances of 11 species of the genus Columba (34). In these comparisons it was shown that at least a part of the antigens peculiar to one species in contrast to another were usually shared with several other species. Further, for certain species in relation to others it was noted that a given species might share (*a*) a complex of cellular components with one species, (*b*) the same complex or the same complex plus additional characters with another species, (*c*) all the components of the second species plus others with a third, *etc.* The antigens of each species interlock in intricate but more or less unique patterns with those of the others, except that a group of antigens appeared to be shared by all the species. However, each species studied in its relationships to the others was definitely an entity. An observation made possible as a result of these studies was that antigenically

these 11 species tended to form two more or less distinct groups, depending upon habitat in the Old or New World, respectively (11). A parallel grouping of these 11 and other species of Columba was proposed by Cumley and Cole (8), using various color patterns as the criterion of classification. These studies point definitely toward a single main line of divergence from a common ancestral stock in each of the two hemispheres. Whether other species of Columba, not included in one or the other of these studies, would conform to these groupings is, of course, an open question.

2. *Antigens in the Serum*

The question arose early in the studies of the segregation of cellular antigens in backcross hybrids as to whether the causative genes might also have effects on the serum proteins. In the light of our present knowledge it appears unlikely that the same antigens would be involved in the cells and serum, but the causative genes might be the same. In order to differentiate the serums of two species, it was necessary to absorb the precipitating antibodies (precipitins) from an immune serum by absorption with the serum of the contrasted species. The technic is much more difficult than that of agglutinin-absorption, and generally less than half the antiserums contain antibodies capable of distinguishing the serum of a species from that of another closely related to it. Also, in the precipitation test the antigen, rather than the antiserum, is diluted serially, so that the same reasoning as to the results can hardly be applied as if the antiserum, not the antigen, is diluted.

However, it was possible to distinguish the serums of any two related species, each from the other, on the basis of the presence of the reaction of a reagent with the serum of one species as compared with an absence of reaction with that of another. Without making any implications as to the chemical structure of the serum antigens —— which presumably are proteins —— the reactive part of the serum antigens of one species as compared with another has been called *species specific.*

a. Species Crosses in Doves. A segregation of serum antigens specific to Pearlneck has been observed (9) in the progeny of successive backcrosses to Ring dove. It is probable that there are at least three such antigens peculiar to Pearlneck as compared to Ring dove, whose distribution in the backcross families simulates that expected if each were caused by one or more genes on a particular chromosome. Further, there seemed to be independent segregation of the antigens of the cells and serum, thus eliminating the possibility that the antigens of the serum might be disintegration products of the cells. A parallel segregation of species-specific components of the serum, and their probable genetic independence of those of the blood cells, has been found in backcross hybrids of three other kinds

of species hybrids — namely, those between Pearlneck and Senegal (12), between Senegal and Ring dove (13), and between pigeon and Ring dove (33). It appears that a minimum of two to five serum antigens distinguishes the serum of one of these species from that of the other, implying, naturally, one or more genes on as many different chromosomes as there were antigens peculiar to a species.

These are the first experimental results which show that genes have an effect on the proteins of a species which distinguish it from another. (The complement-deficient guinea pig (74) has, of course, been for many years a unique example of genic effects on one kind of serum protein. And Landsteiner (45) pointed out that the differences in normal antibodies within a species is presumptive evidence of individual variation in proteins.) Thus the question, raised many years ago by Loeb (59) as to whether the known differences in proteins between species could be explained on a Mendelian basis, can now be answered in the affirmative. One may also state that the taxonomic relationships of animals, and presumably also of plants, as revealed by serological studies, undoubtedly represent gross genetic relationships of the species under study.

b. Individual Differences in Human Serum. Differences in the serum antigens between species are definitely a reality, even between those of closely related species. A pertinent question then is that of whether protein differences are to be expected only *between* species or will they also be found *within* a species? Some peculiar results (unpublished) were obtained by Dr. R. W. Cumley in studying the species hybrids of pigeon and Ring dove, which were easily understandable on the assumption that some pigeons were heterozygous for genes affecting serum antigens. With this evidence of the possibility of individual differences in serum antigens as a background, Cumley and Irwin reported (10) that such differences were demonstrable in the serums of humans. If these are found to be heritable and are actually differences in proteins rather than in substances associated with them, a new class of substances will be available for research not only in humans but in other animals as well.

3. *Correlation of Antigens with Other Characters*

There are no morphological characteristics of the various species which have been associated with the species-specific antigens, either of the cells or serum. Some such correlation still may be detected, although at the moment it appears rather improbable. Also, there is very little hope that cytological studies of the various hybrids would show a correlation of chromosome behavior with the species-specific antigens, at least not until technics are found which will make more precise the examination of material with many small chromosomes. A report by Painter and Cole

(70) is evidence for stating that there are approximately 30 or more pairs of chromosomes in pigeons and doves.

Shrigley (77a) observed that various types of abnormalities of the sperm, occurring infrequently in both Pearlneck and Ring dove, were increased markedly in the species hybrids. Further, the backcross hybrids to Ring dove which antigenically (cells) resembled the Ring dove more than the species hybrid had a lesser proportion of abnormalities than did those which still carried several Pearlneck-specific characters. Seemingly, these abnormalities were a reflection of some sort of disharmony between some of the genes of Pearlneck and those of Ring dove.

4. *Remarks on Genetic Relationships of Species*

The antigens of the blood cells and serum of these different species may now be used together to portray genetic relationships between the species more accurately than would be possible with either alone. For example, Pearlneck differs from Ring dove in that one or more genes on at least ten or more chromosomes produce cellular antigens specific to Pearlneck. (It is assumed that the chromosomes involved are genetically independent.) Also, there appear to be a minimum of three other chromosomes which carry genes with species-specific effects on the serum proteins. Thus, if each of these two species has 30 or more chromosomes, genes on somewhat less than half of this number in Pearlneck have effects on the blood which distinguish it from Ring dove. Reciprocally, there are probably nine or ten cellular antigens — implying the same number of chromosomes — peculiar to Ring dove in contrast to Pearlneck; there is no information as to the number of serum antigens. Also, Senegal probably differs from Ring dove in about the same number of cellular characters (31), and in three or four antigens of the serum, making the over-all picture between Senegal and Ring dove very much the same as that just stated for Pearlneck and Ring dove.

However, there appears to be much less difference between the antigens of Pearlneck and Senegal than between those of either of these species in relation to Ring dove. That is, as stated earlier, Pearlneck differs from Senegal primarily in the effects of genes on three or four chromosomes acting on the blood cells, and of genes on probably two or three other chromosomes acting on the serum. Until recently these three species (Pearlneck, Ring dove and Senegal) were classified as being in three different genera. Peters (71) has grouped them all in the genus Streptopelia. Antigenically, however, Pearlneck and Senegal are more closely related than is either species with Ring dove, indicating various degrees of similarity possible among related species. Antigenic relationships between well-defined species with a lesser number of chromosomes involved in the

differentiation than the minimum of five to seven of the probable 30 or more pairs of Pearlneck and Senegal may yet be found.

Naturally, there may be antigens of either or both cells and serum distinguishing any pair of these species which have not yet been observed. It is probable, however, that the majority of species-specific cellular characters not now recognized will be demonstrated, if at all, at a lower dilution of immune serum (from rabbits) than has been used in our tests. For example, individual differences in Ring doves have been detected, but at a dilution of 1:2 or 1:5 of the absorbed immune serum, whereas, in our hands, a reagent which does not react at 1:60 with any species-specific antigen is hardly usable. In brief, and without attempting to consider all the ramifications which this line of reasoning involves, the antigenic differences between the species which have been analyzed experimentally seem definitely to be produced by genes on less than half the chromosomes of a species. If genes on other chromosomes are involved in these differences between species, it would seem in the light of our present knowledge that (*a*) their effects are less pronounced antigenically than those of the known substances, or (*b*) a lesser number of genes on other chromosomes have such effects. In these different studies, the species do not appear to be differentiated by multiple genes, having individually small effects, on all the chromosomes, as proposed by Muller (65), but rather by genes — possibly multiple — on less than half the chromosomes.

Some observations have been made on the behavior of the chromosomes carrying genes affecting species-specific characters when in the species hybrid. The most pertinent example is that of the distribution of substances specific to Senegal in the first backcross offspring to Ring dove of hybrids between these two species (31). The antigens specific to Senegal — and therefore the probable nine or ten chromosomes with the genes producing these antigens — appeared to segregate at random in the first backcross generation. This suggests more or less normal pairing in the species hybrid of these particular chromosomes with partners in Ring dove. The observed distribution in succeeding backcross generations (data not to be published) and less extensive data in the first and second backcross progeny of the cross between Pearlneck and Ring dove (27, 28) entirely corroborate the above statement.

One other finding of interest is that which concerns the hybrid substance from the Pearlneck-Ring dove cross, cited earlier. (Although this component was found in all hybrids of this cross and a hybrid substance related to the major part of it was noted in the species hybrids between *livia* and Ring dove (27a, 35), hybrids between other species (30, 32) were not characterized by the appearance of an antigen different from that in either parent. Species hybrids in ducks also contain a hybrid substance

(62). Thomsen (81) reported complementary action of genes on antigens of chicken cells, which is the only known evidence to date of such interaction on antigens within a species.) By testing the occurrence of this new substance in backcross hybrids, it appeared to consist of three parts, each immunologically distinct from the others (35). One of these was always associated with the *d*-4 character, one with *d*-11 and a third seemingly in loose linkage with each of several Pearlneck specific antigens — *d*-1, *d*-2, *d*-3 and *d*-12. Assuming that the gene or genes producing this third part of the hybrid substance were linked, respectively, with the one or more genes producing the presumably genetically independent antigens of Pearlneck, the conclusion would be that a gene or genes with duplicate effects in interaction were located on several chromosomes of Pearlneck. An alternative explanation of this association would be that this third fraction was produced by one or more genes on but one chromosome of Pearlneck interacting in the hybrids with one or more genes from Ring dove, and the supposed linkage with each of the several antigenic characters of Pearlneck might then be due to chance alone.

IV. Cellular Antigens in Cattle

1. *Number and Interactions*

The number of chromosomes which carry genes for species-specific antigens, plus probably an additional number which have genes for antigens common to two species, suggests strongly that there may be many more antigenic components in the blood cells of a species than has often been thought to be typical of such cells. Such a concept was held by Todd as stated in a report (82) of work on cattle cells, and particularly in a later report (83) on differentiation of corpuscles within families of chickens. The work in this laboratory by Ferguson (15) and Ferguson *et al.* (16) shows that there are 30 distinct antigenic components in cattle cells. Furthermore, additional antigens in this species have been noted (unpublished data primarily by Clyde Stormont and R. D. Owen) and at present 40 are demonstrable. Most of these can be detected singly, so the number of different combinations possible in the bovine species is 2^{40}, or over a trillion — well beyond human comprehension. If the assumption be admitted that each antigen is a chemical entity, biochemical individuality within a species is certainly more than a definite possibility — it is practically an actuality, at least in cattle.

Although, as stated above, the majority of these substances in cattle cells may be detected independently of the others, genetically many of them seem to be associated. An extreme example is that of the antigens called **B**, **G** and **K** (80). Either **B** or **G** may occur alone or together, whereas

K has never yet been definitely observed except in combination with both **B** and **G**. Furthermore, there are genetically two kinds of animals possessing **B** and **G** together — namely, (*a*) those in whose offspring **B** and **G** separate as expected if the parent were a heterozygote, and (*b*) those whose offspring either contain both **B** and **G** or neither. Seemingly there is linkage of the causative genes of these three substances or they are produced by genes in an allelic series. Thus, under the second explanation, one gene would produce **B**, another **G**, another **BG** together and another **BGK**.

That is, under this explanation there are some genes (for **BG** and for **BGK**) which can do the work, or more, of two alleles (for **B** and **G**, respectively). There are other examples of this peculiar ability of certain genes to do the work of two alleles. The occurrence in the chimpanzee of a substance similar to but not identical with the heterozygote — **MN** — of humans (51, 85) may be interpreted as indicating that the chimpanzee possesses a gene with an effect similar to those of the alleles for **M** and **N** in humans. McGibbon (62, 63) has shown that a pair of contrasting antigens which differentiate the Muscovy duck (*Cairinia moschata*) from Mallard (*Anas Platyrhynchos*) are further divisible, as if the various fractions were caused by either linked or allelic genes. If the antigenic complexes in the Muscovy are produced by a series of alleles, rather than by linked genes, the combination noted in certain species hybrids of parts of the otherwise contrasting antigens is another instance of what probably is a single gene producing the same effect as a pair of alleles. Also, Wiener (87) has postulated the same kind of explanation for the appearance of certain variants of the **Rh** complex of humans.

In general, immunological reactions with cells containing but a single dose of an antigen (the heterozygote) are indistinguishable from those with cells carrying a double dose (the homozygote). For example, in humans, no differentiation is possible between cells containing **AO** or **AA**, **BO** or **BB**. Slight differences in reactivity have been noted, however, in cells with **AB** and **MN**, respectively, as compared with the homozygotes; for references to the experimental work, see Wiener (86). However, quantitative differences have been noted by Olson (67) between chicken cells from the homozygote and heterozygote, respectively, for a particular antigen, in that an appreciably higher titer was typical of the cells from the homozygote. A few antigens in cattle cells give differential quantitative reactions between the heterozygote and homozygote; they are the exception rather than the rule.

2. *Differences Between Two Breeds*

The cellular characters of humans, particularly the O, A and B substances, have been extensively used in a study of their distribution in human races. Critical discussions of this topic, along with references to reports of experimental work and to several review papers are given, among others, by Boyd (4) and Wiener (89). Only exceptionally is there a distinction between races by virtue of the absence of an antigen (therefore the gene) in one race and its presence in another. The genetic concept of races is that differences between them are primarily the result of differences in gene frequency among genes common to the races. Evidence as to the validity of this concept is supplied in a study by Owen *et al.* (69) of differences between two dairy breeds (biologic races) of cattle — Holstein and Guernsey — for which were found only quantitative differences in the frequencies, in one breed or the other, of most of the 30 antigens used in the tests. It is, of course, to be anticipated that the presence, as compared with the absence, of some alleles may constitute a part of the differences between races — just as it makes for individuality — but probably the proportion of such differences will be only a small part of the total.

3. *Identical Blood Types in Fraternal Twins*

An approach which promises to provide new information on the embryological formation of blood cells is indicated in a study by Owen (68) of cellular characters among twins in cattle. Instead of most fraternal twins having unlike blood types, the majority of them had identical blood types. A rational explanation of this finding is that, as a result of vascular anastomosis between twins in cattle, there is an interchange of embryonal cells which are ancestral to the erythrocytes of the animal. These persist in either twin, and it can be demonstrated in certain twins that the blood of each actually has a mixture of two types of corpuscles.

V. General Remarks

Unpublished analyses (by C. J. Stormont and R. D. Owen) of the genetic relationships of these 40 substances of cattle cells now recognized indicate that the causative genes are located on perhaps ten of the chromosomes of cattle. (Krallinger (39) reports 30 pairs of chromosomes in cattle.) In other words, some are caused either by linked genes or by one or more allelic series. There now appear to be two groups of these, one group being comprised of 15 or more antigens including B, G and K mentioned above, the other group having less than ten components.

Thus it is seen that the number of chromosomes recognized at present (probably ten) with genes having antigenic effects to make for individ-

uality *within* a species (cattle) is practically the same as the number in Pearlneck or Senegal (nine or more in each species) which carry genes for antigens which set either apart from Ring dove. Furthermore, the number of known cellular antigens in cattle is much greater than those which separate any two species tested to date which have produced fertile hybrids. Hence the somewhat anomalous situation presents itself in which individuals of the same species (cattle) may differ in more antigens than do several well-defined species. If there were multiple genes with antigenic effects on each of the chromosomes which differentiate one species from another, the number of species-specific antigens might be increased many-fold. Even with that possibility, it seems reasonably clear that the number of antigens need not be the deciding factor in differentiating species. It is fully realized that such a comparison as stated above is valid probably only to a limited extent. What is required for a critical appraisal of the part antigenic differences may play in differentiating two species is a knowledge first of the substances which make for variation *within* each species. With this information, it would be possible to determine for each species which components are held in *common* and which are *specific* to each, respectively. It is not known to what extent the antigens which make for individuality within cattle, for example, separate the bovine from another related species. It is known, however, that there are differences within species of doves in at least a few of the antigens which distinguish one species from another, as reported by Irwin and Cole (30) and other unpublished data. Further comments on this situation would be primarily speculative, and this is reserved for a later time.

It may confidently be expected that some physiological function of the genes with antigenic effects may eventually be discovered. Except for the **Rh** factor in humans, no association of any cellular antigen with a vital process is known at present. It is surely not unreasonable to believe that such genes have effects other than on the cells, or at least are linked to others which do. Only two cases of linkage of genes, one having an antigenic effect, are known at present. A linkage in the rabbit between a gene for a cellular character and one for brachydactyly is reported by Sawin *et al.* (75). Also, evidence for the location on the sex chromosome of a gene producing specific effects in Mallard in contrast to Muscovy has been noted by McGibbon (64). Attention is called to the reports of Gorer (18) and of Lumsden (61) who, respectively working with mice and rats, have demonstrated independently that the success or failure in transplanting tumor tissue depended in part upon antigens demonstrable in the red blood cells and the formation of antibodies to them.

There are various other phases of immunogenetics in which progress may be expected in the future, in addition to an expansion of any activity

described in the foregoing pages. Among these may be included the problem of the relation of genetics to organ specificities, which still remains to be explored fully. A recent report by Snell (78) is an index of future activity in that field. Also, an early question by Guyer (19) as to whether an antibody may influence the genic complex in rabbits has been revived by Emerson (14) in Neurospora. Further researches along this general line are indicated. Also, studies of the antigens of the cells should include the cooperation of chemists in attempts to determine the chemical structure of these cellular components. And finally, studies on antigens of the serum should include the technics of physical chemistry to characterize the individual proteins and to determine what protein or proteins *within* and *between* species are affected by genes. Results of considerable interest in several fields should be obtained if such a program becomes possible.

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The Origin and Evolution of Maize

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CONTENTS

I. METHODS OF INVESTIGATING CULTIVATED PLANT ORIGINS

Alphonse de Candolle (22) in his "Origin of Cultivated Plants" described four methods for investigating the origin of cultivated species.

These are (*a*) botany, with particular reference to geographical distribution, (*b*) archaeology and paleontology, (*c*) history and (*d*) philology. In concluding his discussion of the utility of the various methods considered, De Candolle strongly emphasized the necessity for combining the different methods as no one alone is capable of providing a final solution to the problem and even a combination of all can usually do no more than lead to a strong probability that a given solution is correct.

To de Candolle's four methods we can now add a fifth; the method of cytology and genetics. In some respects this method is the most promising of all, for it is capable of attacking the problem at its roots through investigation of gene changes and chromatin rearrangement. But cytogenetics, like the older methods, cannot alone solve the problem of the origin of a cultivated plant. This method, too, usually leads only to probabilities. The necessity, which de Candolle emphasized, of making use of all available methods in attacking the problem of the origin of cultivated plants has not diminished with the years and is as great today as it was in his period.

De Candolle's conclusions regarding cultivated plants in general apply with particular appropriateness to the specific problem of the origin of maize. For, of all of the cultivated plants which have been subjects for studies relating to problems of origin, none has at once commanded more attention and proved more puzzling and perplexing to its investigators than Indian corn or maize. The problem of its origin has been of interest to a wide variety of students. To the botanist maize is particularly intriguing because of its high degree of specialization, its great diversity of types and its apparently complete lack of wild forms. What could have been the nature of the wild or primitive maize from which the multitude of present-day varieties has developed? Where, when and how was a species, so hardy that it could survive in the wild, converted to a cultivated plant so specialized and so dependent upon man's ministrations that it is no longer capable of an independent existence in the absence of man's protection?

These questions also interest the anthropologist and archaeologist to whom the problem of the origin of maize is of considerable importance. Its solution would throw much-needed light upon the origin of the prehistoric American civilization, the Inca of Peru and the Maya and Aztec of Middle-America, civilizations which, in certain material and intellectual achievements, were more advanced than the contemporary cultures of the Old World. There is little doubt that maize was the basic food plant upon which these pre-Columbian civilizations rested. Indeed, there was scarcely an advanced culture in any part of North or South America which did not depend partially or almost wholly upon maize for food. And when

America was discovered and colonists began to arrive from the Old World maize became, in a sense, the bridge over which European civilization traveled to a foothold and permanent occupation of America (77). Thus the historian, like the botanist and anthropologist, has an interest in maize and its origins.

II. Previous and Present Hypotheses on the Origin of Maize

There have been numerous studies and much speculation upon the origin of maize and three rather distinct hypotheses have had a vogue during the past century and more: 1. that it originated from pod corn which differs from normal maize primarily in the fact that the seeds or caryopses are enclosed in glumes (81); 2. that maize originated from teosinte, its closest relative, by direct selection, by large-scale mutations, or by the hybridization of teosinte with another grass, now unknown (13, 44, 26, 27, 28, 30, 52, 21, 82); and 3. that maize, teosinte, and the more distantly related genus, Tripsacum, have descended along independent lines from a remote common ancestor (75, 83–86).

In recent years, especially since the discovery of the close relationship of maize and teosinte, the first of these hypotheses has been largely abandoned and the remaining two, especially the second which provides a rôle for teosinte, have held the stage. And, since teosinte is known only in Mexico and Guatemala, it has been generally assumed that maize must have had its origin there. Anthropologists, recognizing the great importance of maize to the pre-Columbian American cultures and civilizations, and assuming that maize agriculture must have had its origin in the general region where the progenitor of maize grew wild and where maize was first domesticated, have been inclined to regard Central America as the seat of early American civilizations.

Some years ago my colleague, Dr. R. G. Reeves, and I, convinced that none of these hypotheses was completely adequate, and convinced, too, that the problem could not be solved with the evidence then at hand, initiated a series of cytogenetic studies designed to discover how the genes and chromosomes differ from each other and resemble each other in maize and its relatives. New evidence derived from these studies and considered in the light of previous evidence, not only from genetics, cytology and morphology, but also from archaeology, ethnology and history, led to the development of a tripartite hypothesis: 1. that cultivated maize originated from a wild form of pod corn which was once, and perhaps still is, indigenous to the lowlands of South America; 2. that teosinte, the closest relative of maize, is a recent product of the natural hybridization of Zea and Tripsacum which occurred after cultivated maize had been introduced by man into Central America; 3. that new types of maize originating

directly or indirectly from this cross and exhibiting admixture with Tripsacum comprise the majority of Central and North American varieties (73).

Many additional investigations have been made since this hypothesis was published. It is the purpose of this chapter to consider briefly the earlier evidence on the origin of maize, to review the cytogenetic studies which led to the tripartite hypotheses described above and to summarize the evidence which has since accumulated, much of it until now unpublished, both in support and contradiction of this hypothesis. If special emphasis is given to the cytogenetic aspects of the problem it is, first, because such emphasis seems appropriate in a book which is primarily concerned with advances in genetics and, secondly, because other parts of the problem have already been adequately treated elsewhere. The reader is referred to Weatherwax (86) and Mangelsdorf and Reeves (73) for detailed treatment of other aspects of the problem.

III. The Relatives of Maize

Since a wild form of maize has never been discovered, and since the only suspected fossil maize (29) has now been shown to be an artifact (20), an attack upon the problem of the origin of maize must concentrate to a large extent upon the maize plant itself and its relatives.

Maize is a grass, though in some respects a most unusual one, as the herbalists and early botanists clearly recognized. Within the grass family (Gramineae) maize is assigned to the tribe Maydeae (more recently designated as Tripsaceae) a tribe comprising eight genera, five of which are Oriental and three American. The Oriental genera which include Coix, Schlerachne, Polytoca, Chionachne and Trilobachne, are all native to the region extending from India and Burma through the East Indies into Australia.

The American genera of the Maydeae were formerly regarded as three, Zea, Euchlaena and Tripsacum, commonly known as maize, teosinte and gama grass, respectively.

As a result of their studies of the comparative morphology of maize and teosinte and of hybrids between them, Reeves and Mangelsdorf (80) have recently concluded that teosinte is not entitled to generic rank, and have proposed that the two species of teosinte *Euchlaena mexicana*, Schrad, annual teosinte, and *E. perennis* Hitchc., perennial teosinte, be regarded as species of Zea. Whether or not this proposed taxonomic change will be generally accepted remains to be seen. In the meantime, however, there is no doubt that teosinte is much more closely related to maize than is Tripsacum.

The distribution of teosinte is not widespread. The perennial form has so far been discovered in only one isolated locality in Mexico (31).

Annual teosinte is more common but it, too, has a limited distribution, having been reported from only ten or twelve localities in Mexico and Guatemala. In Mexico, it has been found in several localities, all on the Pacific watershed or in the Central Plateau and all at altitudes of more than 3,000 feet, growing usually as a weed in the maize fields. Jenkins (49) has recently reported teosinte growing more or less as a wild plant in the vicinity of Chilpancingo in the state of Guerrero in Mexico. In parts of Guatemala teosinte behaves more nearly like a truly wild plant. Kempton and Popenoe (57) found a form of teosinte constituting the dominant element in the vegetation over thousands of acres in the vicinity of San Antonio Huixta in the Department of Huehuetenango. McBryde (*cf.* Mangelsdorf and Cameron, 69) found approximately the same form growing in great abundance between Santiago Petatan and Santa Ana Huista. On the other hand the teosinte which Kempton and Popenoe found growing near Jutiapa and Lake Retana in Guatemala is, like most of the teosinte of Mexico, "weedy" in its nature, being largely confined to fence rows and the margins of rice and corn fields. Anderson (8) questions whether even the teosinte at San Antonio Huixta can be considered as a wild species as it is growing on unforested land which presumably was once forested and has since been cleared by man for the planting of crops.

In Mexico and, to a lesser extent, in Guatemala, teosinte is constantly hybridizing with maize and the hybrids are backcrossing to both maize and teosinte. This means that there is a constant introgression of maize germplasm into teosinte and of teosinte germplasm into maize. One consequence of this is that some of the Mexican varieties of teosinte have become almost indistinguishable from maize except for their fruit which retains the characteristics of teosinte. The teosinte of Chalco in the Valley of Mexico exhibits the pilosity of the leaf sheaths and the strong pigmentation of leaf and stalk characteristic of the maize with which it is found growing. Some plants of Chalco teosinte have become so maize-like as to bear seeds with yellow endosperm. There is some question whether a "pure" form of teosinte exists, either in Mexico or Guatemala.

Tripsacum, the third American representative of the Maydeae, comprises several species, all of which are truly wild plants. A preliminary survey of the genus by Cutler and Anderson (36) puts the number of species at seven. Tripsacum appears to have its center of diversity in Central America with a range extending in both directions to North and South America. Its occurrence in South America is more widespread than had previously been supposed. Dr. Hugh C. Cutler, who has traveled and collected extensively in central South America, in recent years has found Tripsacum in a number of localities in Brazil and Paraguay from which it had not previously been reported.

IV. Chromosome Numbers in the Maydeae

The haploid chromosome number of maize and its relatives, including several Andropogoneae, is set forth in Table I (p. 179). So far as chromosome numbers alone are an indication of relationship, maize would appear to be more closely related to the Oriental Maydeae and to the majority of Andropogoneae than it is to the American Maydeae. This fact, among others, has led Anderson (8) to suggest that maize may be an amphidiploid hybrid of a five-chromosome Sorghum and a five-chromosome species of Coix. Chromosome numbers alone, however, can be quite misleading as a

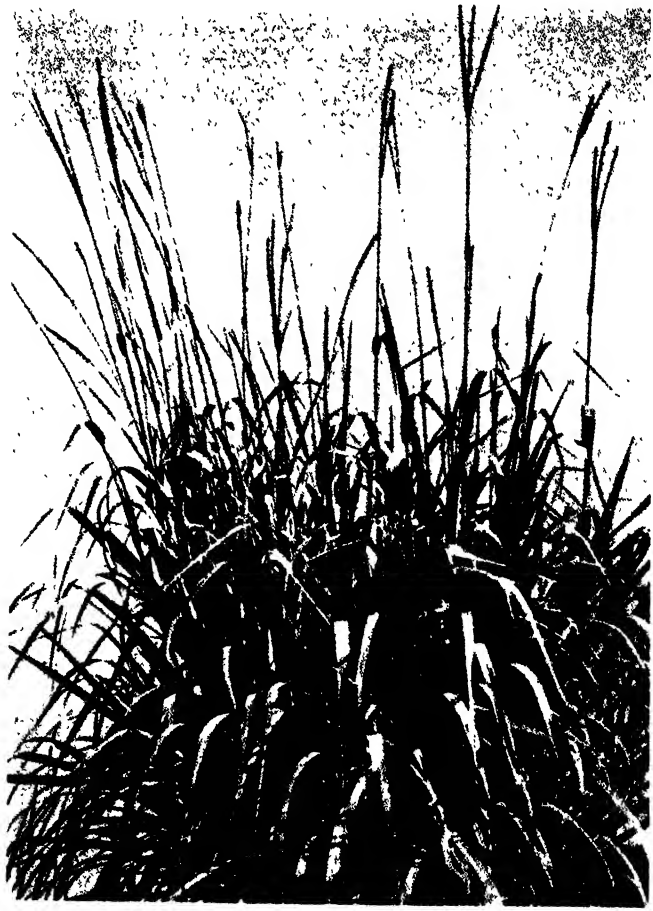

Fig. 1
Plant of *Tripsacum dactyloides* (L.) L.

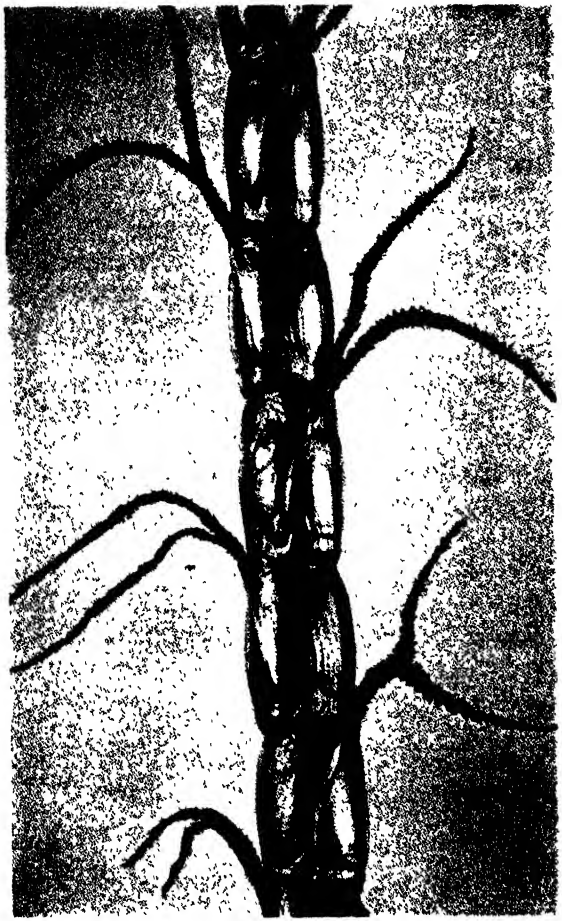

FIG. 2
Pistillate Portion of a Lateral Inflorescence of *Tripsacum dactyloides* (L.) L.
From Mangelsdorf and Reeves (73).

criterion of relationship, as the crossing relationships discussed below will indicate.

V. CROSSING RELATIONSHIPS IN THE MAYDEAE

Failure of two species to hybridize does not necessarily prove a lack of close relationship since there are numerous morphological and physiological barriers, sometimes of a minor nature from the evolutionary standpoint, which can prevent crossing. On the other hand, the fact that two

species can be hybridized speaks of genes which they possess in common. Crossing experiments (*cf.* Mangelsdorf and Reeves, 73, for details) have shown that maize can be crossed with all forms of teosinte and, by the use of special techniques, with several species of Tripsacum. Teosinte and Tripsacum can be hybridized with each other although living plants of this hybrid have not yet been reared. Neither maize, teosinte nor Tripsacum have ever been hybridized with any of the Oriental Maydeae, although by no means all of the possible combinations have been attempted. The cross of maize and Coix, however, has been repeatedly attempted by several investigators and has always failed. The same is true of attempted crosses of maize and Polytoca, and maize and Sorghum, although strangely enough maize has been crossed with sugar cane, *Saccharum offininarun* L., (47, 48) which taxonomically and in chromosome numbers is farther removed from it than Sorghum.

So far as crossing experiments are a criterion of relationship they show that maize is quite closely related to teosinte, is somewhat more distantly related to Tripsacum and is only remotely related to the Oriental Maydeae. Indeed, from the evidence now available, it would appear that maize may be more closely related to some of the Andropogoneae, such as sugar cane, than it is to its relatives among the Oriental Maydeae. And since the principal characteristic which Zea has in common with other members of the Maydeae is monoecism, which has appeared again and again in the plant kingdom and since, in several other characteristics, maize differs decidedly from such plants as Coix (85), it is quite possible that maize is, indeed, genetically more closely related to certain members of the Andropogoneae than to the Oriental Maydeae.

VI. Cytogenetics of Maize-Tripsacum Hybrids

In order to anticipate the conclusions which are later to be drawn from a consideration of maize-teosinte hybrids, it is appropriate to take account first of the studies made upon hybrids of maize and Tripsacum. For it is these studies which have, to a large extent, made intelligible some of the results obtained from maize-teosinte hybrids.

Hybridization of maize and Tripsacum is difficult but can be accomplished by the use of a simple technique which involves shortening the styles or silks of maize (70). The only maize-Tripsacum hybrids which have been studied in detail both cytologically and genetically are those reported in the monograph of Mangelsdorf and Reeves (73) from which the description which follows is largely a condensation.

FIG. 3

Plant of the Hybrid *Zea Mays* L. × *Tripsacum dactyloides* (L.) L. (Right) Compared with its Maize Parent (Left).

From Mangelsdorf and Reeves (73).

1. *The Diploid Hybrid: Zea × Tripsacum*

The majority of the hybrid plants resulting from crossing maize (n = 10) with diploid Tripsacum (n = 18) were quite vigorous (Fig. 3) although a few, for no apparent reason, were decidedly weak and stunted. In their general aspects the hybrid plants resembled Tripsacum much more closely than maize. Nevertheless, their hybrid nature was readily apparent, especially with respect to the perennial habit of growth. Tripsacum is strongly perennial and can be maintained indefinitely by vegeta-

tive propagation. The hybrid of maize×diploid Tripsacum was weakly perennial and, although it lacked rhizomes, was propagated for several years from offshoots. This fact, incidentally, is in conflict with Brieger's (18) later contention that teosinte cannot be intermediate between maize and Tripsacum with respect to its growth habit since there can be no intermediate between the annual and the perennial habit.

The degree of pairing of chromosomes in the first meiotic division of the hybrid was variable but cells in which pairing was completely lacking were found only rarely. When pairing did occur it was feeble except for two or three pairs which had a tendency to unite more closely and regularly. The number of bivalents varied from none to six with three and four occurring most frequently.

Anaphases consisted of all types of behavior from an apparently regular condition to one in which there was no movement of the chromosomes toward the poles. When this occurred a definite nuclear membrane was frequently formed around the entire chromosome complex at, or near, the center of the cell. Sometimes the partition wall which developed, following this failure of nuclear division, was located entirely to one side of the newly formed restitution nucleus with the result that one daughter cell received the unreduced number of chromosomes while the other received none. Apparently it is only those unreduced nuclei which later give rise to functional eggs.

The hybrid was completely pollen-sterile, apparently because the amount of functional pollen was so small that the anthers failed to develop normally and were incapable of dehiscing. The hybrid could be crossed by maize, teosinte (71) or Tripsacum and showed a fertility of 3.4%, 5.8% and 1.3% respectively when pollen from these three species was applied.

2. *The Triploid Hybrid: (Zea×Tripsacum)×Zea*

When the diploid hybrid Zea×Tripsacum, whose only functional gametes were those arising from unreduced nuclei, was crossed by Zea, the result was a population of triploid hybrid plants. These were quite uniform in appearance and much more maize-like than the plants of the diploid hybrid. In their general vegetative characteristics they were similar to plants of Florida teosinte but the ears showed less resemblance.

These triploid hybrid plants possessed 38 chromosomes comprising two genoms of 10 each from Zea and one genom of 18 from Tripsacum. At meiosis there were usually 10 bivalents and 18 univalents although one or more trivalents were occasionally found. These were undoubtedly the result of a feeble union of a Tripsacum chromosome with a pair of Zea chromosomes.

This hybrid, like the preceding one, was completely pollen sterile but was fertile to the extent of 21.3% when pollinated by maize.

3. *Progeny of the Triploid Hybrid*

Among the progeny which resulted when the triploid hybrid was pollinated by maize, all plants possessed 20 Zea chromosomes and many of them one or more Tripsacum chromosomes in addition. The distribution of the plants with respect to the number of Tripsacum chromosomes (as estimated by the amount of empty pollen) was as follows:

No. Tripsacum Chromosomes	No. Plants
0	25
1	76
2	22
3 or more	55

The population is obviously not the result of a random distribution of the 18 Tripsacum chromosomes for, even allowing for gametic and zygotic elimination, the number of $2n$ and $2n+1$ plants is much too large. Apparently, in many cases, all of the Tripsacum chromosomes, except those which have paired with maize chromosomes, are eliminated.

In this generation morphological characteristics of the plants were strongly correlated with the estimated number of Tripsacum chromosomes present. Several plants approached teosinte quite closely in their general vegetative habits. Plants completely lacking Tripsacum chromosomes were usually typical maize plants exhibiting no evidence of Tripsacum contamination. A few $2n$ plants, however, showed definite Tripsacum influence indicating that an interchange of Zea and Tripsacum chromatin had occurred. This possibility was definitely verified by a study described below of certain $2n+1$ plants.

4. *Segregates with a 2n+1 Complex*

Extra Tripsacum chromosomes were rapidly eliminated from the hybrid population in later generations because they were not transmitted through the pollen and, only with some loss, through the eggs. It was possible, however, to make a detailed study of one $2n+1$ stock in which the extra Tripsacum chromosome bore an allele of the *su* gene for sugary endosperm in maize. In this stock the presence of the Tripsacum chromosome was usually recognizable by the starchiness of the seed. Starchy seeds when planted usually produced $2n+1$ plants; sugary seeds from the same ears usually produced normal $2n$ maize plants. Exceptions to the rule demonstrated that there is sometimes an exchange of chromatin

between the Tripsacum chromosome and one or more of the maize chromosomes.

Cytological studies of this stock showed the extra Tripsacum chromosome pairing most frequently with chromosome 1 of maize, sometimes with the short arm, less commonly with the long arm. It also sometimes exhibited partial synapsis with regions on chromosomes 4 and 9.

Apparently the chromosomes of Tripsacum, or at least some of them, are homologous with those of maize to a degree that results in partial synapsis. There is not, however, the chromomere-by-chromomere homology which is found in a pair of completely homologous chromosomes.

5. *Significance of the Results*

The facts that Zea and Tripsacum can be hybridized, that there is some pairing of chromosomes in the hybrid and that there is not only opportunity for interchange of chromatin but also evidence that it sometimes occurs, led to a consideration of the possible consequences of the natural hybridization in nature of these two genera. This in turn led to the realization (73) that teosinte may well be the product of such a hybridization.

The most obvious evidence in support of this suggestion is the fact that teosinte is intermediate between, or identical with, its two putative parents in virtually all of its characteristics. It does not possess, apparently, a single characteristic which it could not have received from either Zea or Tripsacum.

Even is such characteristics as resistance and susceptibility to the attacks of insects such as the flea-hopper, *Perigrinis maidis*, teosinte is intermediate between Zea and Tripsacum. Is it possible that this insect has recognized, long before man, the true nature of teosinte?

VII. Cytogenetics of Maize-Teosinte Hybrids

1. *Historical Review*

The hypothesis that teosinte has played an important rôle in the origin of maize may be said to have had its beginnings with the work of Ascherson (13) who showed convincingly for the first time the close relationship between maize and teosinte. The hypothesis became plausible when Harshberger (43) considered *Zea canina* the ancestor of maize and learned later (44) that this form could be produced by hybridizing maize and teosinte. The hypothesis became well established as the result of a number of genetic and cytological studies which will be briefly reviewed in the following paragraphs.

The chromosomes of teosinte and of hybrids of maize and teosinte

were first investigated by Kuwada (58) who found the chromosome number of Florida teosinte to be the same, 10, as the basic number in maize. He found that in hybrids of maize and Florida teosinte there are ten bivalents, one or more of which sometimes exhibit weak pairing. He also observed that the chromosomes in one of the larger pairs were not equal in length. Longley (61) found ten bivalent chromosomes and completely regular meiotic behavior in hybrids of maize and Chalco teosinte. An investigation of hybrids of maize and the annual teosintes, Florida, Durango and Chalco, by Beadle (15) indicated regular meiotic behavior in hybrids involving Durango and Chalco teosinte and irregularities in the hybrid in which Florida teosinte was one of the parents. The hybrid of Florida and Durango teosintes with maize were further studied by Arnason (12) who, using known translocations to test homologies, found two unequal pairs in the maize-Florida hybrid and a suggestion of reduced chiasma number in chromosomes 8 and 9 of the maize-Durango hybrid.

Longley (63) in a study of the comparative morphology and homology of the chromosomes of maize, teosinte and Tripsacum found important differences in the three species in number and position of the chromosome knobs. Tripsacum was found to have numerous knobs all of which are terminal in position. The annual teosintes from southern Guatemala possess large terminal knobs; those from northern Guatemala have smaller knobs, which however, are numerous and predominantly terminal, while the teosintes from Mexico have internal knobs, scarcely distinguishable from those of some varieties of maize. Several recent papers of Longley on chromosome knobs will be considered later.

O'Mara (76) studied the chromosomes of hybrids of maize with Florida, Moyuta and Nojoya teosintes as well as a hybrid between Florida teosinte and Nojoya teosinte. In the maize-Florida and maize-Moyuta hybrids he found two and four univalents as well as inversion or duplication bridges and fragments. A hybrid of Florida and Nojoya teosintes showed all of the phenomena characteristic of the maize-Florida hybrid. In the maize-Nojoya hybrid, however, no univalents or bridges and fragments were found, and pairing of maize and teosinte chromosomes was regular and complete.

Genetic studies of maize-teosinte hybrids are for the most part in close agreement with cytological observations in showing a close relationship between the two species.

Collins and Kempton (33) recorded an approach to a simple Mendelian ratio in the inheritance of paired *vs.* single spikelets in the F_2 of a maize-teosinte hybrid. In other characters, however, clear-cut ratios were not observed and there was a tendency for all characters to be correlated with each other. Teosinte-like segregates and maize-like segregates appeared

more frequently than would be expected if the differences between maize and teosinte involve a large number of genes inherited independently.

Kempton (54) studied the inheritance of three recessive maize characters; crinkly, ramose and brachytic in maize-teosinte hybrids. His data show, although he did not emphasize the fact, that the gene for brachytic, and perhaps the gene for crinkly as well, exhibit genetic association with the characteristics which distinguish maize from teosinte.

Emerson and Beadle (40) studied genetic crossing-over in maize-teosinte hybrids and demonstrated that, in nineteen teosinte chromosome

FIG. 4
Plant of Florida Teosinte.
From Mangelsdorf and Reeves (73).

regions tested (nine in Durango, six in Chalco and four in Florida), all except one showed approximately the same crossing-over as that of maize. In the exceptional region, the *C-wx* region on the ninth chromosome, no crossing-over occurred in the maize-Durango and maize-Florida hybrids. Beadle (15, 16) made a special cytological and genetic study of the ninth chromosome in maize-teosinte hybrids using a translocation with chromosome 8 to facilitate the cytological investigations. He found a region of approximately 45 cross-over units in length in which pairing on the short arm of the ninth chromosome between maize and teosinte chromosomes is not complete and in which there is no crossing-over. O'Mara (76) later showed that crossing-over between *C* and *wx* is approximately normal in maize-Nojoya hybrids where pairing of the ninth chromosome is complete.

Hybrids between maize and perennial teosinte have been studied by Longley (61, 62), Emerson (38), Emerson and Beadle, (39, 40) and Collins and Longley (34), and the knobs of perennial teosinte have been described by Longley (63). Because it has the perennial habit of growth, a character generally considered to be more primitive than the annual habit, this form of teosinte has sometimes been regarded as the most primitive of the teosintes. Cytological and genetic studies, however, show it to be an autotetraploid quite similar to the annual Mexican teosintes in the morphology of its chromosomes and in their homology to those of maize. Until a diploid form of perennial teosinte is discovered it is difficult to see how this form can have any bearing upon the problem of the origin of maize.

Mangelsdorf and Reeves (72, 73) studied the linkage relations of the genes which differentiate maize and Florida teosinte, using Mendelian characters of maize to mark the chromosomes. They found little evidence of linkage between the genes which differentiate maize and teosinte and the marker genes on chromosomes, 2 and 6. There was slight evidence of linkage, however, with the marker gene on chromosome 9 and strong evidence of linkage with two marker genes on chromosome 4. They concluded that the genes which differentiate maize and teosinte are not distributed at random over all of the ten chromosomes but are concentrated in particular regions of several of them.

These results, considered in the light of the behavior of maize-Tripsacum hybrids described earlier, led to the conclusion that teosinte differs from maize primarily by a small number of segments of chromatin, assumed to have been received originally from Tripsacum. This hypothesis was tested by studying the segregates of hybrids in the backcross (maize × teosinte) × maize. Here it was found possible to divide the population into sixteen approximately equal classes, which indicated that four major hereditary units were involved. It was postulated that these units represent not single genes but segments of chromatin received originally from

Tripsacum as the result of crossing-over or translocation between maize and Tripsacum chromosomes in natural hybrids of the two genera. Thus it was concluded (73) that teosinte is nothing more than a hybrid of maize and Tripsacum.

In support of this conclusion, it was shown (73) that teosinte resembles one or the other of its putative parents or is intermediate between them in almost all of its characteristics, a condition which would scarcely be expected either if teosinte is the progenitor of maize or if the three species, maize, teosinte and Tripsacum had descended along independent lines from a remote common ancestor.

One of the characters in which teosinte is intermediate between maize and Tripsacum is the number and position of chromosome knobs. It was suggested (73) that chromosome knobs in both teosinte and maize were derived originally from Tripsacum and that "pure" maize uncontaminated by Tripsacum might be expected to exhibit knobless chromosomes. Such maize was sought and found in the Andean region of Peru (73).

2. *Conflicting and Corroborating Evidence*

It is often the case, when a problem involves two or more conflicting hypotheses, that evidence which appears to support one is soon counterbalanced by new evidence which can be interpreted to support the other. This has been true of the maize-teosinte problem. While Mangelsdorf and Reeves (73) were demonstrating that teosinte may well be no more than a recent hybrid of maize and Tripsacum, Beadle (17) and Langham (59) were bringing forward new evidence which appeared to render much more plausible than it had previously been, the hypothesis that maize may be a direct descendant of teosinte. Until Beadle showed that teosinte seeds when popped, burst from their hard, bony shells, it had been difficult to see why primitive man should have had any interest in teosinte as a food plant or made any attempt to domesticate it. Until Langham showed that several of the important characteristics which distinguish teosinte from maize — response to short days, two-ranked spikes and single pistillate spikelets — are apparently inherited as simple Mendelian characters, it had been difficult to vizualize the transformation under domestication of a plant such as teosinte to a plant such as maize.

At the same time Longley (64–67) studied knob positions in maize and teosinte and concluded that there are gradients affecting knob positions and suggested that the difference in knob positions in the two species might well be the result of a relatively small number of mutations affecting these gradients. As a result of his studies on knob positions Longley concluded that Tripsacum is the most primitive of the American Maydeae, standing near the base of the tree, teosinte from southern Guatemala is

next in the series and represents the trunk of the tree, teosinte from northern Guatemala is a major branch while maize and the Mexican teosintes represent the finer branches.

In the light of these new items of evidence, Weatherwax (87) concluded that the objections to the teosinte hypothesis were less formidable than they had been a decade previously.

Brieger (18) reported that his observations on maize-teosinte hybrids are not in agreement with either those of Mangelsdorf and Reeves or those of Langham, although he presented no actual data to show to what extent they differ. He did, however, conclude that pod-corn rather than teosinte is the progenitor of maize.

The hypothesis of Mangelsdorf and Reeves (73), that new types of maize, originating directly or indirectly from the hybridization of maize and Tripsacum and exhibiting admixture with Tripsacum, comprise the majority of Central and North American varieties has received considerable additional support from the studies of Mangelsdorf and Cameron (69) on the maize of western Guatemala. These studies showed that a small area in the Department of Huehuetenango, an area in which Tripsacum grows in profusion and teosinte occurs as the dominant species on thousands of acres, embraces a greater diversity of maize than occurs in the entire United States. Here are found not only the most Tripsacoid varieties in this hemisphere but also remnants of the "pure" maize presumably once involved in the hybridization with Tripsacum. Here is the probable center of diversity of the Tripsacum-contaminated varieties and in this area, significantly, chromosome knob number is strongly correlated with the morphology of the plant.

Reeves (78), in another study showed that varieties with high knob numbers are the predominating type in Mexico and in the countries of northern and eastern South America: Colombia, Venezuela, Dutch Guiana, Brazil and Uruguay. Mangelsdorf and Cameron (unpublished) found that this is also true of Panama, Nicaragua, Costa Rica and Cuba. Reeves (78) has also demonstrated a statistically significant relationship between knob number and distance in either direction from Central America. This agrees with the previous conclusion (73) that maize was distributed over parts of North, Central and South America before teosinte came into being in Central America and maize became subject to modification of its chromosomes by contamination with Tripsacum. Graner and Addison (41) report that the Brazilian Tripsacum, *T. australe*, is lacking in chromosome knobs. If this proves to be generally true, then the fact that teosinte is intermediate between maize and other species of Tripsacum in the number and position of its knobs may lose much of its significance and the facts reported above may demand a new interpretation.

The assumption that there are two more or less distinct types of maize (73) has recently received strong support from studies by Mangelsdorf (68) on the origin and nature of the maize ear. These show that one type of ear, characteristic of the maize of the Guarany Indians of Paraguay, is a lax spike upon which paired spikelets are arranged in whorls at the nodes of a simple rachis. The second type of ear, an extreme form of which can be produced by hybridization with teosinte, is characterized by a rigid spiral phyllotaxy.

3. *New Evidence from Maize-Teosinte Hybrids*

It has become apparent that the question of the origin of maize hinges to a very large degree upon the nature of teosinte. Teosinte is obviously closely related to maize. If it is a good species which once existed in a

FIG. 5
Pistillate Spikes or "Ears" of Florida Teosinte.
From Mangelsdorf and Reeves (73).

truly wild state and from which maize may have originated as a result of a relatively few large scale mutations, then it must continue to be regarded as a possible and indeed, the most probable, progenitor of maize. If, on the other hand, teosinte is, as Mangelsdorf and Reeves concluded, a hybrid of maize and Tripsacum and if as such, or if on other grounds, it can be dismissed as a maize progenitor, then, and only then, is the way opened for a critical evaluation of alternative hypotheses.

Perhaps the only completely acceptable proof of the hybrid nature of teosinte would be the actual synthesis of teosinte through the hybridization of maize and Tripsacum. The possibility of accomplishing this is remote. In species hybrids, as in games of chance, the odds against the fortuitous repetition of a particular and complex combination are almost overwhelming (74). There is, consequently, little likelihood that the hybrid nature of teosinte, if indeed it is a recent hybrid, will ever be completely

TABLE I

Chromosome Numbers in Maize and Related Grasses

Tribe, Genus and Species	Chrom. No		Reference to Recent Literature
	n	2n	
A. American *Maydeae*			
Zea Mays L.	10	20	Mangelsdorf and Reeves (73)
Euchlaena mexicana Schrad.	10	20	Mangelsdorf and Reeves (73)
Euchlaena perennis Hitchc.	20	40	Mangelsdorf and Reeves (73)
Tripsacum dactyloides (L) L.	18	36	Anderson (4)
Tripsacum dactyloides (L) L.	36	72	Anderson (4)
Tripsacum floridanum Porter ex Vasey	18		Longley (63)
Tripsacum laxum Nash		72	Mangelsdorf and Reeves (73)
Tripsacum latifolium Hitchc.	36	72	Mangelsdorf and Reeves (73)
Tripsacum pilosum Scribn. and Merr.		72	Mangelsdorf and Reeves (73)
B. Oriental *Maydeae*			
Coix lachryma-jobi L.		20	Mangelsdorf and Reeves (73)
Coix aquatica Roxb.		10	Mangelsdorf and Reeves (73)
Schleracne punctata Brown		20	Mangelsdorf and Reeves (73)
Polytoca barbata Stapf†		20	Mangelsdorf and Reeves (73)
Polytoca macrophylla Benth.		40	Cameron (23), Hunter (46)
C. *Andropogoneae*			
Manisuris cylindrica (Michx.) Kuntze	9	18	Mangelsdorf and Reeves (73)
Rottboellia glandulosa Trin.		54	Mangelsdorf and Reeves (73)
Sorghum vulgare Pers.	10	20	Karper and Chisholm (50)
Sorghum halepense Pers.	20	40	Karper and Chisholm (50)
Sorghum versicolor Anderss.	5	10	Karper and Chisholm (50)
Saccharum officinarum L.	40–68	80–136	Janaki-Ammal (48)
Saccharum spontaneum L.	30–56	60–96	Janaki-Ammal (48)

† Now *Chionachne Koenigii* (Sprengel) Thwaites, Henrard (45).

and finally proved. The most that can be done is to establish certain facts beyond a reasonable doubt.

Since the publication of our monograph in 1939 I have made extensive studies of a large number of hybrids of maize and teosinte for the purpose of obtaining additional data in answer to four questions.

1. On which chromosomes are located the postulated segments of Tripsacum chromatin which differentiate teosinte from maize?

2. Do different varieties of teosinte have the same Tripsacum segments?

3. Can these Tripsacum segments be transferred individually to maize by repeated backcrossing and, if so, what are their effects?

4. Are the important differences between maize and teosinte due to a relatively few, simply inherited genes as Langham has suggested and, if so, on which chromosomes are these genes located?

The complete answers to all of these questions are not yet available. Nevertheless, considerable progress has been made and the picture becomes clearer with each year of study.

The data of Mangelsdorf and Reeves (73) suggested that Florida teosinte differs from maize by four major and perhaps several minor segments of Tripsacum chromatin and that two of these are located at opposite ends of the fourth chromosome. Later experiments do not completely corroborate this conclusion. Tests involving marker genes on each of the ten chromosomes show that one, but apparently only one, of the segments is located on the fourth chromosome where it shows linkage with the genes, *Su*, Gl_3 and *tu* on that chromosome. A second segment shows strong linkage with the allele of the gene *P* for pericarp color on the first chromosome and a slight indication of linkage with Bm_2 at the opposite end of the same chromosome. A third segment shows a clear-cut linkage with *Wx* on the ninth chromosome. A fourth segment is apparently linked with *G* on the tenth chromosome although the data are still insufficient to establish this linkage beyond doubt. In addition there are strong indications that one of the Tripsacum segments, perhaps one of the minor ones, is located on the third chromosome where it shows a relatively strong linkage with Lg_2 and a weak one with *A*.

The remaining chromosomes show little or no evidence of linkage with teosinte characteristics as determined by these tests.

The new data are thus quite convincing in supporting our previous conclusion (73) that the genes which differentiate maize and teosinte are not distributed at random over all of the ten chromosomes but are concentrated on only part of them.

In a second group of experiments Florida, Durango and Nobogame teosintes were crossed with a uniform inbred strain of maize. The F_1

hybrid was then back-crossed to a second uniform inbred strain of maize to produce a population in which the hereditary variation was wholly the result of segregation of maize and teosinte germplasm. This experiment shows that while Florida and Durango teosinte apparently differ from maize primarily by four hereditary units, presumably segments of Tripsacum chromatin, Nobagame teosinte, which is more maize-like than either Florida or Durango teosinte, seems to contain only three of these segments.

This fact is extremely important, for it indicates that these segments contain approximately the same kind of germplasm and that the chief difference between the more maize-like and less maize-like varieties of teosinte lies in the amounts of this kind of germplasm which they contain.

A third group of experiments has involved the transfer, by repeated back-crossing to an inbred strain of maize of the individual Tripsacum segments of Florida, Durango, "New" and Nobogame teosintes. When the different stocks had become relatively isogenic (after three back-crosses following the first cross) they were selfed to produce stocks homozygous for the Tripsacum segments involved. These experiments show clearly that the

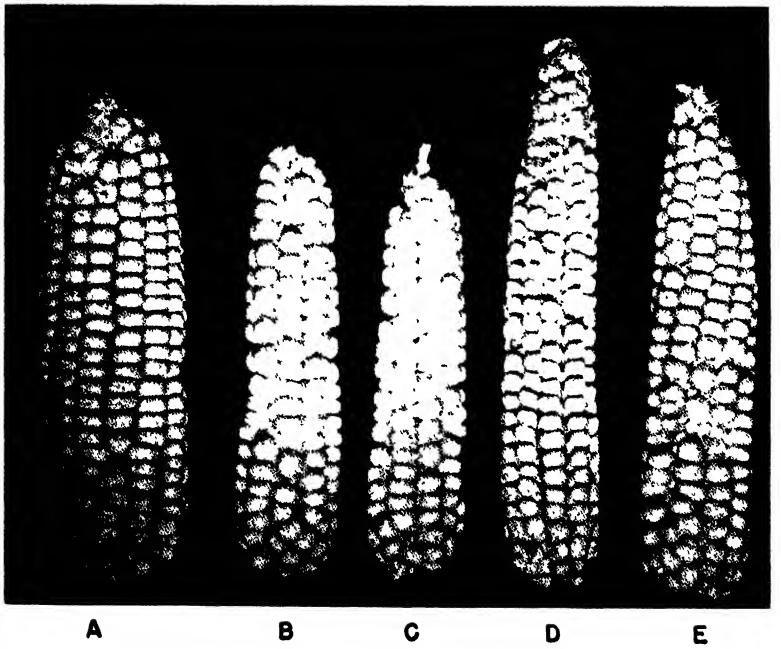

FIG. 6

Four Distinct Types of Ears are Produced When the Chromatin Segments of Florida Teosinte are Transferred to a Uniform Inbred Strain of Maize (A) by Repeated Back-Crossing.

principal differences between maize and teosinte behave in inheritance almost as though they were single Mendelian factors. These units do not behave exactly as single genes, however, for they are capable of breaking up on occasion into smaller units. The units have similar, but not completely identical, effects. All of them, regardless of the variety of teosinte from which they are derived, increase the prominence and horniness of the glumes. All of them have a tendency to reduce the number of rows of grain and the size of the seed (Fig. 6). But they are not completely identical in their effects, even in these characteristics, and they differ considerably in others. And, what is more important, the four types which can be distinguished in Florida teosinte have close counterparts in Durango teosinte. Furthermore, the most Tripsacoid type derived from Florida teosinte (Figs. 6D, 7B) has almost identical counterparts in Durango, Nobogame and "New" teosintes (Fig. 7C, D, E). Each of the four teosintes studied yields a type in which the seeds are small, slightly pointed and

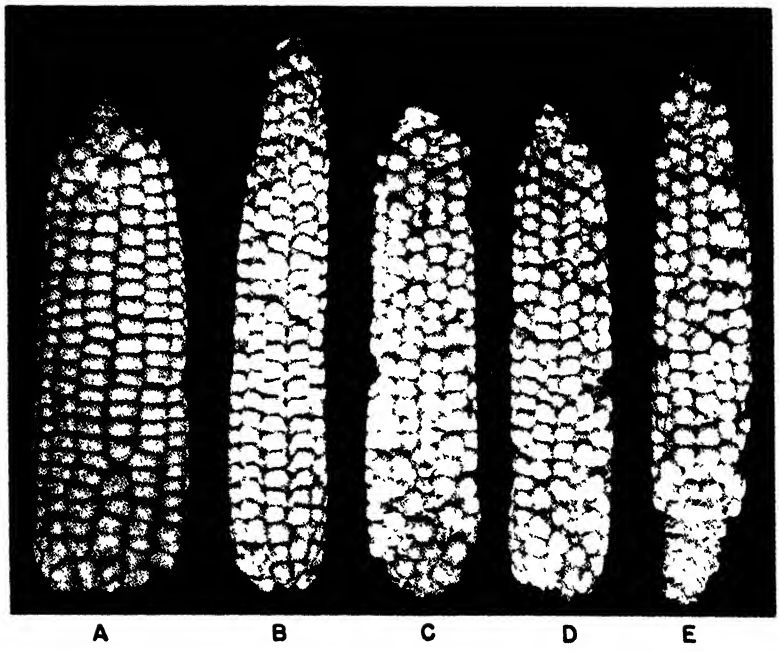

FIG. 7

From Left to Right. A. Inbred strain of maize. B. One of the four types derived by repeated back-crossing of a maize-Florida teosinte hybrid to this inbred strain. C.–E. Corresponding types from Durango, Nobogame and "New" teosintes derived in the same way.

grayish in color, the glumes prominent and strongly indurated, the ear slender and as long as, or longer than, the comparable ear of maize (Fig. 7A).

Linkage studies of these extracted individual teosinte units has only begun but two of them from Florida teosinte have been definitely located on the chromosomes. Stocks homozygous for the Tripsacum segment were crossed with a maize stock having marker genes on nine of its ten chromosomes. The F_1 hybrid was back-crossed to a nine-factor recessive.

In the case of one of the segments the segregation was clear-cut; 64 plants exhibited the effects of the segment: 66 did not. Furthermore, there was clear-cut linkage with the gene *A* on the third chromosome with approximately 25% of crossing-over. This segment is obviously usually inherited intact and is near the center of chromosome 3.

The second segment to be tested exhibited a more complex behavior. It was obviously linked with *Su* on the fourth chromosome and linkage was complete in the sense that individuals with the *Su* gene always showed some effects of the segment from teosinte. It was also apparent, however, that this segment was not always inherited intact as segregates completely lacking its effect occurred only to the extent of about 30% of the total. The simplest explanation, and one which is now being tested by F_3 progenies, is (*a*) that the segment occupies a position near the center of the fourth chromosome which includes the *Su* locus; (*b*) that this segment is not always inherited intact but is sometimes broken as the result of crossing-over; (*c*) that the position of the *Su* gene within the segment is such that it is seldom, if ever, completely separated from the entire segment.

The final group of experiments involves crosses of Durango and Nobogame teosintes with a multiple-factor tester stock to determine first, whether the simple Mendelian inheritance which Langham (59) reported in a maize-Durango cross is characteristic of all teosinte crosses and, if so, to locate the chromosomes on which the factors occur.

Langham reported that in crosses of maize and Durango teosinte the three maize characters (weak response to length of day, paired female spikelets and many-ranked ears) are all dominant to the contrasting teosinte characters and that all segregate as simple Mendelian characters in the hybrids. He interpreted the results to support the theory of the origin of maize from teosinte by a relatively few large scale mutations.

My results do not agree with those of Langham, as a glance at the data in Table II will reveal at once. In no case does the segregation fit a simple Mendelian ratio closely although it approaches such a ratio in the case of many-ranked pistillate spikes *vs.* two-ranked pistillate spikelets. Furthermore the two crosses differ significantly in their segregation, teosinte-like plants with respect to each of the different characters usually, but not invariably, occurring with greater frequency in the Durango cross

TABLE II

Segregation of Characters in Maize-Teosinte Crosses

Characters Segregating	No. of Individuals	
	Maize-Durango F_2	Maize-Nobogame F_2
Many-ranked central spike	166	291
Two-ranked central spike	13	35
Total	179	326
Many-ranked pistillate spike	112	213
Two-ranked pistillate spike	65	86
Total	177	299
Pistillate spikelets mainly paired	96	192
Pistillate spikelets mainly single	81	107
Total	177	299
Weak response to short day*	160	301
Strong response to short day†	21	17
Total	181	318

* Flowered before September 15. † Flowered after September 15.

than in the Nobogame cross. Limited data from a third cross, a maize-Florida cross, indicate that here the appearance of teosinte-like plants is even more frequent than in either the maize-Durango or maize-Nobogame cross. In other words the inheritance of these characters varies with the variety of teosinte involved in the cross. It varies also with the variety of maize, for in a cross of Durango teosinte with a variety of maize obtained from the Guarany Indians of Paraguay the spikelets are predominantly single in F_1, whereas in crosses of Durango and North American maize they are predominantly paired.

It is not to be inferred from these results that Langham's data from maize-Durango crosses are not reliable. Since it can be shown that the inheritance of these characters varies with the variety of teosinte as well as the variety of maize used in the cross, almost any kind of segregation can occur in F_2. It was an unfortunate coincidence that the segregation in Langham's crosses approached a 3:1 ratio so closely and led him to conclude that simple Mendelian inheritance is involved, for this is obviously not the case, at least when other varieties of teosinte or maize are used.

Additional evidence, if more is needed, that the characters which distinguish teosinte from maize are not simple Mendelian recessives, is provided by the linkage tests set forth in Table III. These include, in addition to the characters which Langham studied, a "glume score" which attempts to measure, in a wholly empirical fashion, the degree of development of the horny glume, and "disarticulation score," also an empirical value, which attempts to measure the differences between maize and teosinte in the readiness with which the rachis segments separate from each other. Both of these characters are important ones in differentiating maize and teosinte. It should be added that, in studying the two-ranked *vs.* the many-ranked condition, data from both the ear and the central spike of the tassel were used and, though treated separately in Table II, are combined in Table III.

TABLE III

Linkage Relations of Teosinte Characters with Each Other and with Marker Genes on Nine Chromosomes of Maize

		Nobogame Teosinte × Maize F_2													
	Characters	Bm_2	Lg_2	A_1	Su_1	Y	Gl_1	J_1	Wx	G_1	T.R.	S.D.	P.S.	G.S.	D.S.
Durango Teosinte × Maize F_2	Bm_2 — Chromosome 1		−	−	−	−	−	−	−	−	+	−	−	−	I
	Lg_2 — Chromosome 2	−		−	−	−	−	−	−	−	+	−	−	−	−
	A_1 — Chromosome 3	−	−		−	−	−	−	−	−	I	−	−	I	I
	Su_1 — Chromosome 4	−	−	−		−	−	−	−	−	−	−	+	+	+
	Y — Chromosome 6	−	−	−	−		−	−	−	−	+	−	−	+	−
	Gl_1 — Chromosome 7	−	−	−	−	−		−	−	−	I	−	−	+	I
	J_1 — Chromosome 8	−	−	−	+	−	−		−	−	+	−	I	+	+
	Wx — Chromosome 9	−	−	I	−	−	S	−		−	+	−	−	−	−
	G_1 — Chromosome 10	−	−	−	−	−	−	−	−		−	I	−	I	−
	T.R. — Two-Ranked Spikes	−	+	+	+	−	−	+	+	−		+	+	+	+
	S.D. — Response to Short Day	−	−	−	−	−	−	I	−	+	+		+	+	I
	P.S. — Paired Spikelets	S	−	−	+	−	S	+	S	−	+	+		+	+
	G. S. — Glume Score	−	−	+	+	−	−	+	+	+	+	+	+		+
	D.S. — Disarticulation Score	−	−	+	+	+	S	I	−	−	+	I	+	+	

+ = Linkage. − = Independent inheritance.
I = Indication of linkage. S = Significant deviation not due to linkage.

The data show that there are genes governing the two-ranked condition (of either the ear or central spike or both) on chromosomes 2, 3, 4, 8 and 9 of Durango teosinte and on chromosomes 1, 2, 6, 8 and 9 of Nobogame teosinte.

Genes responsible for teosinte's strong response to length of day definitely occur on chromosome 10 of Durango and possibly on chromosome 10 of Nobogame as well as chromosome 8 of Durango.

Genes involved in the differences between paired and single spikelets in maize and teosinte are found on chromosomes 4 and 8 in Durango teosinte and on chromosome 4 and possibly 8 in Nobogame teosinte.

There are apparently numerous genes acting upon the development of the glume, for glume score shows linkage with the marker genes on chromosomes 3, 4, 8, 9 and 10 in the maize-Durango cross and chromosomes 4, 6, 7, 8 and possibly 3 and 10 in the maize-Nobogame cross.

Approximately the same situation obtains with respect to disarticulation which is correlated with the marker genes on chromosomes 3, 4, 6 and possibly 8 in the maize-Durango cross and with genes on chromosomes 4, 8 and possibly 1, 3 and 7 in the maize-Durango cross.

It should be noted that here, as in the previous experiments of a somewhat different nature conducted with Florida teosinte, chromosome No. 4 is outstanding in both the maize-Durango and the maize-Nobogame cross as a bearer of genes which distinguish maize from teosinte. Probably there are some genes of this nature on all of the ten chromosomes but, if so, they are not distributed at random among them.

Finally, it is important to note in Table III that the various teosinte characters are strongly correlated with each other, a fact which Collins and Kempton (33), treating the problem in a much different way, noted many years ago. Each of the five characteristics by which the plants in these populations have been classified shows linkage, or an indication of linkage, with each of the four others, and this is true in both the maize-Durango and the maize-Nobogame cross. In this connection it is interesting to note that in the numerous linkage tests which Langham (59) made, the only linkage which he discovered was between two teosinte characters: two-ranked ears and single spikelets.

What do these results mean in terms of the genetic nature of the differences between maize and teosinte? It is reasonably clear, I think, that the important characters which distinguish maize and teosinte are each conditioned by a number of different genes or groups of genes. It is also clear that the genes or group of genes which are responsible for one character are also involved in each of the others. Finally, it is apparent, as it has been from other studies already considered, that some chromosomes carry more than their share of the genes which are responsible for

the differentiation of the two species. These genes have repeatedly shown strong linkage with various marker genes on the fourth chromosome. And considering the present data with those mentioned earlier, as well as those previously reported (54, 73, 59), there is some evidence that chromosomes 1, 3, 8, 9 and 10 carry an appreciable number of these genes while chromosomes 2, 5, 6 and 7, although probably not completely lacking in such genes, at least have less then their proportionate share.

VIII. What is Teosinte?

There is now a considerable body of evidence to support the conclusion that the number of inherited units involved in the differences between maize and teosinte is relatively small in spite of the fact that each character involves genes on several chromosomes. In no other way can one account for the relatively high frequency with which teosinte-like segregates and maize-like segregates have been encountered in all of the maize-teosinte crosses for which data are available. It is also evident that each of these units produces effects upon several of the important characteristics which distinguish maize from teosinte. In no other way can one account for the fact that teosinte characters are strongly correlated with each other in hybrids. Furthermore, when these units are "extracted" from teosinte and bred into isogenic stocks by repeated back-crossing to an inbred strain of maize, it is evident that they have similar, if not identical, effects.

What is the nature of these units which differentiate teosinte and maize? Mangelsdorf and Reeves (73) concluded that they are segments of Tripsacum chromatin which have become incorporated into maize chromosomes as a result of the natural hybridization of maize and Tripsacum presumably followed by repeated back-crossing to maize. Extensive recent experiments have furnished no evidence in conflict with such a conclusion. Certainly the units are more than single genes and, although their exact length still remains to be determined, it is evident that some of them, at least, are capable of breaking apart into smaller units. Certainly too, the effects which they produce are the effects which would be expected from blocks of Tripsacum genes.

This is not mere speculation; these are actual facts based upon extensive experimentation. Are there other possible interpretations for the facts? It might be argued, with some reason, that the true nature of teosinte still remains to be discovered since the varieties of teosinte known today are already strongly contaminated with maize. If this were true, then the hereditary units which now differentiate maize and teosinte may represent nothing more than regions of the chromosomes which, located near the centromeres where crossing-over is often decidedly restricted, have remained uncontaminated by maize. Where does this line of reasoning lead? To say

that present-day varieties of teosinte are contaminated with maize is merely another way of saying that they are hybrids of maize and "pure" teosinte. What is "pure" teosinte? What are its characteristics and where is it to be found? If we must assume that present-day teosinte is a hybrid of maize and a wild grass capable of transmitting to the hybrid those characteristics which now distinguish it from maize, is it not more logical to assign that rôle to Tripsacum, which is living and real, rather than to "pure" teosinte which is hypothetical and presumably extinct.

All of the teosintes so far studied have proved to differ from maize not by a few genes with large effects nor by a large number of genes scattered at random over the chromosomes, but by a relatively small number of blocks of genes inherited usually as units. The effects produced by these blocks of genes are exactly those which one would expect blocks of Tripsacum genes to produce. And, since teosinte is like maize or Tripsacum, or intermediate between them, in virtually all of its characteristics, it requires no particular ingenuity to reach the conclusion that teosinte is a hybrid of maize and Tripsacum. The new data presented above strongly support that conclusion. Yet the possibility that there is, or has been, a "pure" form of teosinte must not be overlooked. The only point on which we can be certain is that the varieties of teosinte so far analyzed are not "pure".

IX. Teosinte as the Possible Progenitor of Maize

Opinions on the possibility that teosinte is the progenitor of maize vary from that of Whiting (89), who feels that the teosinte hypothesis has long since been disproved, to that of Langham (59), who visualizes maize arising from teosinte as the result of a few large-scale mutations. The truth probably lies somewhere between these two extremes.

If teosinte is a hybrid, with maize as one of its parents, then it obviously can not have been the progenitor of maize. Suppose, however, for the sake of argument, that teosinte is not a hybrid, but a good species of long standing. If this were true could teosinte have been the progenitor of maize? There are now several reasons for believing that it could not have been.

There is no evidence that teosinte was ever used as a food plant by the American Indians and it is the almost universal opinion among present-day Mexican Indians familiar with the plant that it is valueless as a food plant. The difficulty of removing the seeds from their hard bony shells has usually been regarded as a serious obstacle to the use and domestication of teosinte. Beadle (17) has shown that this difficulty is easily overcome by the application of heat which causes the seeds to explode and to burst from their shells. But there is no evidence that such a method was

ever used by the Indians or that teosinte was ever cultivated by them. Indeed, if it had ever been used it would probably still be used today, even if rarely, for ancient customs have a way of persisting long after better methods and materials are known. The ancient cereal, Amaranthus, for example, is still grown in parts of Mexico in competition with maize, to which it generally is regarded as inferior.

Teosinte is never cultivated for food in Mexico today and, even where it is known, it is regarded as having no food value for man or beast. It is difficult to believe that the Mexican Indians who make use of every part of the corn plant in scores of different ways, and who even gather the cancerous-looking growths of corn smut for food, could be so completely ignorant of the potential food value of teosinte had they or their ancestors ever used the plant for this purpose. The only reasonable interpretation of the unusual indifference to teosinte is that it is a recent arrival which appeared on the scene only after maize, a much better food plant, was already well known.

The fact that teosinte is capable of popping and of bursting from its shell in the process seems somewhat less significant when it is realized that Tripsacum seeds can also be popped. Dr. Reeves and I have found that Tripsacum seeds, which are enclosed in the same kind of hard shell as teosinte seeds, pop as readily as do those of teosinte and are equally palatable. Tripsacum has a much wider distribution than teosinte, occurring in abundance in Central America and extending into the United States as far north as Massachusetts. Yet there is no direct evidence that it was ever used by the Indians as a food plant, although the fact that Tripsacum seeds were discovered by Gilmore (42) among the plant remains in the bluff-dweller caves of the Ozarks, would suggest that some use was sometimes made of them.

But the most serious objection to the hypothesis that teosinte has been the progenitor of maize lies in the characteristics of the two plants themselves. The changes which must have occurred to transform a plant such as teosinte to one such as maize are more numerous and far-reaching than several of the recent students of the problem have supposed. Indeed, the morphological differences between maize and teosinte are greater than those which now exist between the cultivated and the wild forms of any species where both are still known. Some clue to the complexity of the changes necessary to transform teosinte into maize may be gained by a study of the F_2 segregates of a maize-teosinte cross. The transition is illustrated by specimens in Fig. 8.

In a transition from teosinte to maize the single spikelets of characteristic teosinte must become the paired spikelets characteristic of maize and the distichous spike of teosinte must become the polystichous spike

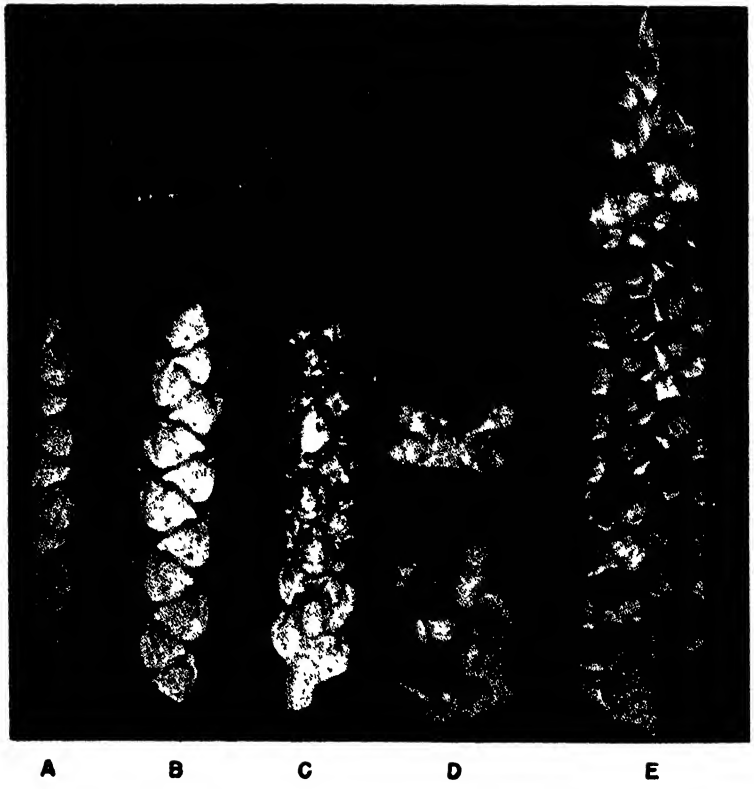

FIG. 8

Transition from Teosinte to Maize Illustrated by the Pistillate Spikes of F_2 Segregates of a Maize-Teosinte Hybrid. The changes from single to paired spikelets, (A-B) from a two-ranked to a many-ranked spike, (A-C) from prominent, horny glumes (A, B, C) to inconspicuous scales (E) and from a fragile, disarticulating rachis (A, B, C, D) to a solid one (E), all involve groups of genes on several different chromosomes.

of maize. These, as has already been mentioned, are not the simple changes which Langham's (59) data indicate — they involve gene changes on at least five different chromosomes. And this is only the beginning. The prominent horny glumes of teosinte must be reduced to inconspicuous scales and this involves gene changes on at least five different chromosomes. The readily-disarticulating rachis of teosinte must be transformed to the rigid cob of maize and genes, or groups of genes, on at least three different chromosomes participate in this process. Finally, the entire pistillate inflorescence must become strongly compacted and, although the linkage relations of the genes for compaction have not been determined, it is

evident from observations made on several maize-teosinte hybrids that this character, too, involves several genes or groups of genes.

The differences between teosinte and maize are complex both morphologically and genetically and it does not seem possible that maize could have been derived from teosinte *during domestication* by any genetic mechanism now known. If maize has originated from teosinte it represents the widest departure of a cultivated plant from its wild ancestor which still comes within man's purview. One must indeed allow a considerable period of time for its accomplishment or one must assume that cataclysmic changes, of a nature still unknown, have been involved. A more reasonable conclusion is that teosinte has played no part in the origin of maize.

Regardless of the validity of the hypotheses which have been, and may be, put forward to replace it, the hypothesis that teosinte is the progenitor of maize is definitely no longer tenable.

X. Pod Corn as the Progenitor of Cultivated Maize

If teosinte is ruled out as a progenitor, what is the origin of maize? Mangelsdorf and Reeves (73), following the botanical opinion of the previous century, postulated that it is derived from a wild form of pod corn once indigenous to the low lands of South America.

1. *Historical Evidence Tested Experimentally*

The hypothesis that maize has been derived from a type of pod corn originated with Saint-Hilaire (81) who, in a letter to the French Academy of Sciences, described a peculiar type of maize which he had received from Brazil. This maize, which he designated as *Zea Mais* var. *tunicata*, was characterized by glumes surrounding the seeds, a condition which Saint-Hilaire regarded as primitive and one which he believed to have been lost during domestication. Saint-Hilaire showed this ear to a young Guarany Indian from Paraguay then living in France, and this young man acknowledged the corn as belonging to his country, stating that it grew there in the humid forest. This obviously is neither proof that pod corn is native to Paraguay nor evidence that cultivated corn originated from a wild form of pod corn. There are, however, several other references to pod corn in South America, one of which in particular, lends a degree of plausibility to the pod corn hypothesis. This is the statement of Azara (14), the Spanish Commissioner to Paraguay from 1771 to 1801, who described four varieties of maize in Paraguay, one of which, "abatý-guaicurú," is certainly pod corn, for he said of it:

" . . . chaque grain est enveloppé à part par de petites feuilles qui ressemblent entièrement aux grandes qui enveloppent l'épi entier."

FIG. 9
The Earliest Printed Illustration of Pod Corn.
From Bonafous, 1836.

Another type of maize which Azara described may also well have been a form of pod corn. His description is as follows:

"Je ne me rappelle pas le nom qu'on donne à la quatrième espèce, dont la tige, beaucoup plus mince, se termine, non par un épi, mais comme le millet, par une espece de discipline à plusieurs cordes, dont chacune est couverté de grains absolument semblables à ceux du maïs, mais plus petits. J'ignore aussi les usages particuliers auxquels on peut l'appliquer. Je sais

seulement qu'en faisant bouillir dans de la graisse ou de l'huile cette espèce de discipline qui contient les grains, ceux-ci crèvent tous sans se séparer, et qu'il en résulte un superbe bouquet, capable d'orner la nuit la tête d'une dame. . . ."

In a previous publication (73) it was suggested that the fourth variety of maize which Azara described may well have been the homozygous form of pod corn which is often earless and which sometimes bears numerous seeds in the terminal inflorescence or tassel. It was further suggested that, if the seeds of such a variety were small and corneous and capable of popping, heating the entire tassel might well result in the peculiar condition which Azara described. It was pointed out, finally, that wild maize may have been of this nature and that its usefulness as a food plant was discovered by primitive man when its inflorescences were accidentally exposed to heat, causing the kernels to explode from their enveloping glumes.

There is some evidence to support such an assumption for the popping of maize is an ancient custom among the American Indians and there are still numerous varieties of pop corn in various parts of South America. Also the word *pisingallo* or variations of it, which is now used in Bolivia and Peru to designate pop corn, has, in two of the early historical references (37, 19), been applied to varieties of maize which were obviously pod corn.

To determine whether the fourth variety of maize which Azara described could be reproduced with a pod corn whose kernels are capable of popping, modern pod corn was crossed with a variety of pop corn and the hybrid repeatedly back-crossed to pop corn. When, after three generations of back-crossing, the kernels had become small and hard, tassels of pop corn which bore seeds in profusion were dipped in a kettle of hot cooking oil. The results were exactly as Azara had described them. The kernels exploded and burst from their enveloping glumes, but remained attached to the tassel to produce the "superbe bouquet" which Azara had so vividly described. A photograph of one of these specimens is reproduced in Fig. 10.

This simple little experiment obviously furnishes no proof that cultivated maize originated from pod-corn. However, since the third variety of maize which Azara described is almost unquestionably the heterozygous form of pod corn it now seems reasonably certain that the fourth variety is a tassel-seeded form of pod corn, probably the homozygous form. In other words there is little doubt that pod corn occurred in Paraguay as recently as a century and a half ago, and it is probable that a homozygous fertile form was known.

2. *Development of True-breeding Pod Corn*

The last fact, if it could be definitely established, would be quite important, for one of the recent objections to the pod corn hypothesis

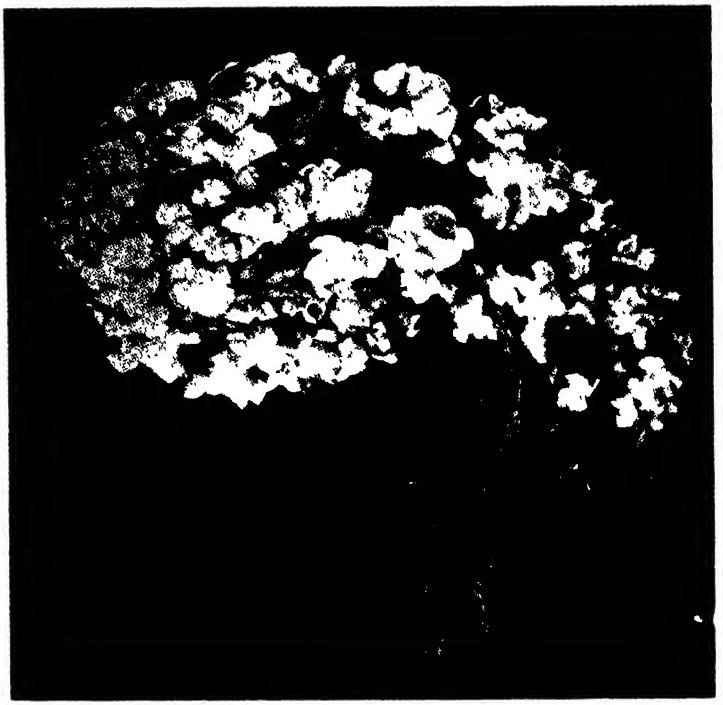

FIG. 10

Pod Corn Was Crossed and Repeatedly Back-Crossed with Pop Corn Until the Seeds Became Small, Hard and Capable of Popping. When the Terminal Inflorescence was Dipped in Hot Oil the Kernels Exploded to Produce the "Superbe Bouquet" which Azara (14) Described from Paraguay in 1809.

has been that homozygous pod corn is usually pollen-sterile and incapable of reproducing itself. It has already been pointed out (73) that this objection is not a particularly formidable one since, if pod corn was the primitive type, then the *Tu* gene of today is a single, wild. relict gene superimposed upon modern varieties which have undoubtedly lost some of the modifying factors which once kept the character under control. In the absence of these restraining modifiers the tunicate character might well result in a monstrous development of the glumes accompanied by partial or complete sterility. If these assumptions are sound, then it should be possible to produce a completely fertile form of pod corn either (*a*) by selecting for restraining modifying factors or (*b*) by substituting the modifier complex of present-day pod corn with a more ancient complex obtained from a primitive variety. Both methods have now been tried.

Modern pod corn has been crossed and repeatedly back-crossed to the Guarany maize from Paraguay which appears to be primitive in a number of characteristics. And although this particular experiment has not yet been completed, and a homozygous form of pod corn has not yet been obtained from it, it is already evident that the Guarany variety contains minus modifiers for the tunicate character. The second experiment involving selection for minus modifiers in modern varieties has been brought to a successful conclusion and a number of fertile, true-breeding stocks of pod corn are now in existence, one of which is illustrated in Fig. 11. The glumes in these stocks have been so reduced by selection for minus modifiers that two doses of *Tu* now produces less effect than was sometimes produced in the original stock by a single dose. And when this homozygous form of tunicate is crossed with some stocks of maize, such as the inbred strain Minnesota No. A158 for example, the development of the glumes is so restrained that it is difficult to see the tunicate character at all except when the seeds are removed. Heterozygous ears of this type are illustrated in Fig. 11.

Brieger (18) has utilized still another method of modifying the tunicate character. He has crossed it with teosinte, which he regards as a good wild species, for the purpose of bringing "wild" modifiers to bear upon the character. From the F_2 of such a cross he obtained forms which he regarded as meeting the requirements for existence in the wild. There is no doubt that such forms occur in hybrids of pod corn and teosinte. Indeed they sometimes occur in the F_1 (*cf.* Fig. 73, Mangelsdorf and Reeves, 73). There is however, considerable doubt that teosinte is a "wild" species. Hence there is no assurance that the "primitive" types obtained from crosses of pod corn and teosinte actually resemble the original maize except in the covering of the caryopsis by glumes.

3. *Evidence of Pod Corn in Prehistoric Times*

Another objection which has been raised to the pod corn theory is that pod corn has been completely unknown in Peru and Bolivia where maize culture reached a high state of development in prehistoric times. It has been suggested (73) that perhaps the tunicate character has disappeared from this region because it is one which would have been selected against by skilled agriculturists — that the absence of pod corn is a measure of the heights to which agriculture has been brought. If this assumption is sound it might be possible to find pod corn illustrated in the prehistoric pottery of the region. A search for prehistoric specimens illustrating pod corn was made in the museums of the United States and a specimen of what appears to be pod corn was found in the Peabody Museum of Yale

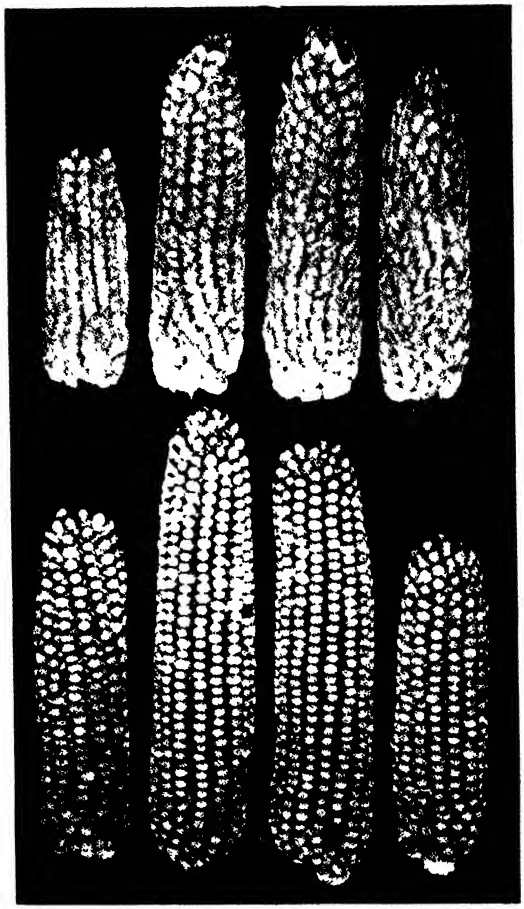

FIG. 11

By Selection for *Minus* Modifiers a True-Breeding Homozygous Strain of Pod Corn was Obtained (Above) in Which the Glumes are not Monstrous. When This Stock is Crossed with Normal Maize the Glumes are so Reduced in the F_1 (Below) as to be Easily Overlooked Although Close Examination Reveals Their Presence.

University (73). Another specimen has since been found in the Museum of the University of Pennsylvania.

Actually, the original objection that pod corn is unknown in Peru and Bolivia has since proved to be unfounded. Cutler, who has made an extensive study of the maize varieties of central South America has found pod corn in Bolivia where, although not common, it is sufficiently well known to be recognized by the natives who call it *paca sara* or "hidden

maize." Cutler (35) has also made the interesting suggestion that pod corn, if it is a relict character, may have been kept in existence and distributed far and wide after selection began to operate against it, by the efforts of the itinerant medicine men who traveled extensively in North and South America and who undoubtedly attributed magical properties to this type of maize.

4. *Evidence for the Pod Corn Hypothesis Summarized*

The above suggestion adds the final touch to a series of facts which now makes the pod corn hypothesis completely plausible. These briefly summarized are:

1. Pod corn was known in Peru in prehistoric times and was illustrated in the prehistoric pottery.

2. Pod corn was repeatedly encountered in South America during the second half of the eighteenth and the first half of the nineteenth century and was designated by the name *pisingallo* which is now applied to pop corn.

3. The peculiar type of maize which Azara described is easily reproduced by crossing modern pod corn with pop corn.

4. The expression of the tunicate character is readily modified either by (*a*) selection or (*b*) the substitution of a modern modifier complex with a primitive one.

5. Completely fertile, true-breeding pod corn has now been developed.

6. A satisfactory explanation for the preservation and spread of pod corn has been suggested.

All of these facts combined still do not prove that pod corn is the progenitor of modern maize. Indeed, final proof of this can come only if wild corn is discovered and found to be homozygous for the tunicate character. In the meantime, if we reject the teosinte hypothesis, on the basis of the evidence previously discussed, then the pod corn hypothesis becomes a plausible alternative and one which, for the moment at least, is confronted with no insuperable objections.

XI. The Probable Nature of Wild Maize

It is always hazardous to speculate far upon the nature of a primitive ancestor which has never been observed. Nevertheless, if maize has originated from a form of wild maize now extinct or still to be discovered, then there are certain reasonable conclusions which can be drawn regarding the nature of the primitive form. It has already been postulated that its seeds were enclosed in glumes and it seems highly probable that this must have been the case even if primitive maize was not pod corn in the sense that it was homozygous for *Tu*. Some of its other characteristics may be

inferred from the nature of varieties of maize in existence today. And at this point we must make a definite distinction between the immediate ancestor of maize, the wild plant with which domestication began, and the remote ancestor of the American Maydeae. Comparative morphology tells us, for example, that the ancestor of maize was probably a perennial bearing perfect flowers — the morphological evidence for this is clear-cut and convincing. But the maize with which domestication began a few thousand years ago may already have been well on the road to its present form so far as these two, and other, characteristics are concerned.

1. *The Seeds*

It is reasonable to suppose that the seeds of wild maize were small and, perhaps, corneous and capable of popping. This supposition is based upon the fact that there are many small-seeded varieties of pop corn in Peru, Bolivia and other parts of South America. Since it is easy to imagine that primitive man practiced selection for larger and softer kernels, but difficult to believe that he would often have consciously sought goals in the opposite direction, one must assume that the small-seeded corneous condition is a primitive one. It is of course conceivable that natural selection under some conditions, even after maize was domesticated, would have brought about a reduction in seed size in the absence of artificial selection in the opposite direction. It is even possible that artificial selection for smaller and harder seeds may occasionally have been practiced by people who used maize exclusively for popping. But both of these conditions, if they occurred at all, must have been rare. The most reasonable explanation for the present-day prevalence of pop-corn varieties in South America is that small, hard seeds capable of popping are characteristic of primitive maize.

2. *Covering of the Ear*

If the seeds of wild maize were covered with glumes there would have been little need for the husks which surround the modern ear of maize. This does not mean that wild corn was completely lacking in husks although it strongly points to such a conclusion. Morphologically the ear of maize is obviously the terminal inflorescence of a lateral branch whose internodes have become drastically contracted. Of this there is no doubt. The question is whether this contraction was characteristic of wild maize or whether it occurred as a result of domestication. The assumption that it was the latter is the more reasonable, since it is difficult to see how husks would have been advantageous in wild maize whose seeds were already covered with glumes, and indeed they might well have been deleterious by interfering with ready dissemination of the seeds. Furthermore, the

primitive Guarany maize from Paraguay, previously mentioned, which contains minus modifiers for the tunicate character also has some genes which affect the development of the husks. In some crosses of this variety, individual plants have appeared in which husks are lacking. Instead, the strongly contracted stalk or "shank" which usually subtends the ear has become elongated so that the individual nodes are readily apparent and the husks, which ordinarily surround the ear, have become virtually normal leaves arising at the nodes of the branch. Thus a condition which has been visualized by Kellerman (51) on the basis of morphological characteristics can now be realistically illustrated by utilizing the genes of a primitive variety.

3. *Nature of the Ear*

The Guarany maize, crossed with pod corn, has also shown the true nature of the ear itself (68). In some instances ears of this cross become so strongly elongated that the arrangement of the spikelets upon the rachis is easily studied. Such specimens reveal that the ear of maize is nothing more than a simple rachis upon which paired spikelets are borne in varying numbers at the nodes. The ear is truly the pistillate counterpart and the morphological homologue of the staminate central spike of the tassel.

There are reasons for suspecting, and some evidence to support the suspicion, that what was is now the ear once resembled the tassel even more than it does at present. It is a common observation, and Werth (88) has assembled data to prove, that the terminal inflorescence of basal branches or tillers is preponderantly staminate or pistillate depending upon the height which the branch attains. If the lateral branches of primitive maize, before they became drastically contracted, attained the same height as the main stalk, then it is likely that they had approximately the same kind of an inflorescence as the latter, not only with respect to proportions of staminate and pistillate spikelets but also with respect to branching of the inflorescence. Whether or not the ear, the terminal inflorescence of a lateral branch, was itself originally branched probably depended on the vegetative vigor of the plant as a whole. If the terminal inflorescence of the primary stalk was branched, then it is quite likely that the inflorescences of the secondary stalks were also branched. The fact that ears with basal branches are commonly encountered in crosses with the Paraguayan Guarany maize would indicate that branching of the lateral inflorescence is a primitive character.

4. *Annual or Perennial?*

Whether wild maize was an annual or perennial is a question upon which few data can be brought to bear. Weatherwax (86) visualized the

ancestor of maize as a profusely branched perennial, and the assumption may well be correct if he were describing the remote ancestor of the American Maydeae. But that the wild maize which primitive man first began to utilize was a perennial is much less certain.

We must, as has already been suggested, distinguish clearly between the remote and immediate ancestors of cultivated plants. And this is especially true in the New World where man, according to the best estimates of anthropologists, appeared on the scene quite recently in terms either of his own history or the evolution of plants. There must have been many species of annuals already in existence when man arrived in America. If so, he certainly would have turned to the annuals for his food, or to plants such as the cassava or sweet potato which, though perennial, yield an annual crop when cultivated or repeatedly gathered. De Candolle (22) expressed the opinion that, at the beginnings of civilization, plants which yielded an immediate return were most highly prized. And Ames (1), although assuming that cultivated annuals have been derived from perennials under domestication, has described a number of characteristics of annuals which render them especially valuable in meeting the needs of agricultural people. One of the most important of these is their enormous potential geographical range. The most tender of annuals are capable of being grown in regions of long and severe winters which they survive as dormant seeds. If the evolution from the perennial to the annual growth habit had already occurred in many species of plants when man appeared, then it is more than probable that annuals would have been included among the plants first domesticated. And it is not unlikely that one of these annuals was the wild form of *Zea Mays*.

There is one bit of indirect, more or less intangible, evidence which has a slight bearing on the problem. The Guarany variety of Paraguay which has already been mentioned in connection with several other characteristics, is an early-maturing variety, in spite of the fact that it comes from the tropical lowlands where late-maturing varieties would be expected to predominate. If this is a primitive variety, as it seems to be, its earliness of maturity suggests strongly that its progenitor was an annual and, perhaps, a short-lived annual.

XII. The Evolution of Maize under Domestication

We may visualize wild maize, the immediate ancestor of cultivated maize, as a short-lived annual, bearing small, hard seeds enclosed in glumes in mixed staminate and pistillate branched inflorescences terminating the primary stem as well as the lateral branches. If wild maize was such a plant, then some of the evolutionary paths which it has followed during domestication in attaining its present form are quite clear. The seeds have

obviously lost their protective glumes and this might easily have been the consequence of a single mutation from *Tu* to *tu*. This mutation may well have been the first step in a long series of changes which followed more or less inevitably. Once the covering was lost from the individual caryopses it became a decided advantage to have the entire ear protected. Hence, both artificial selection and natural selection, acting in a man-made environment, would have favored those variants in which the internodes of the branches had become shortened to such an extent that the terminal inflorescence was partly covered by the leaf sheaths. Under these conditions the basal branches of the inflorescence may well have been a handicap and were eventually eliminated leaving only the central spike remaining. This structure, originally a mixed inflorescence, became predominantly pistillate as its position on the plant became lower through the shortening of the internodes until, with the additional pressure of artificial selection, it eventually became wholly pistillate.

Once the lateral inflorescence was partly enclosed by the enveloping leaf sheaths or shucks, further progress in the same direction could have been considerably accelerated by compaction of the inflorescence itself. Hence, it is not surprising that one of the outstanding characteristics of the modern maize ear is its high degree of compaction (68).

Accompanying these changes there probably was, in many varieties, an increase in the size of the seed and ear. Although many of the smaller-seeded varieties have persisted, there is no doubt that there has been an evolutionary trend, perhaps still operating, toward larger seeds and ears. Evidence for this conclusion is found in a comparison of the large-seeded modern varieties, such as the Cuzco type from Peru, with prehistoric ears and with illustrations of ancient ears on the prehistoric pottery, all of which have smaller seeds than the larger-seeded varieties of today. Furthermore, one can find among the present-day varieties of Peru and Bolivia, small-seeded counterparts of some of the large-seeded varieties. Actually, much of the bewildering diversity of maize in Peru, Bolivia and Ecuador is explicable by the fact that each of a relatively limited number of distinct types occurs in a relatively large number of different sizes.

This is the apparent pattern of evolution of maize before it hybridized with Tripsacum, and the diversity which maize had attained up to this point is probably a good measure of the time which had been involved in its evolution. But once maize hybridized with Tripsacum to produce the ten-chromosome plant, teosinte, new variability must also have come into being with almost explosive force in a relatively short period of time. The evolutionary trends which maize followed must now have involved the absorption of the morphological assets of Tripsacum and the suppression of its morphological liabilities. Since the genes from Tripsacum were

not transmitted singly and independently but by blocks, as segments of chromatin, mere segregation and recombination could not solve the problem. What probably happened was that a whole new modifier complex came into existence. Favorable characters from Tripsacum were accentuated by *plus* modifiers — unfavorable ones were suppressed by *minus* modifiers. There is evidence for this in a comparison of crosses of teosinte with North American maize varieties (presumably Tripsacum-contaminated) and with Guarany maize of Paraguay (presumably almost a "pure" maize). The Tripsacoid maize is strongly dominant to teosinte in some of the important characters, such as paired spikelets and polystichous spike which make maize what it is. The "pure" maize is recessive in these characteristics. Other evidence of a wholly different nature points to the same conclusion. When the hereditary units (presumably segments of Tripsacum chromatin) which distinguish teosinte from maize are transferred individually, by repeated back-crossing to an inbred strain of maize, the number of rows may be reduced, the size of seed decreases, the cob becomes tougher but the spikelets always remain paired. North American varieties of maize are strongly "buffered" against the effects of genes from teosinte which would drastically affect the general morphology of the ear. It is of interest to note that these data from maize-teosinte crosses are in agreement with the conclusions reached by Anderson and Erickson (11) on theoretical grounds.

The evolution of maize since its hybridization with Tripsacum may, therefore, be looked upon primarily as the evolution of systems of modifying factors. There has been an enormous increase in diversity as the result of this hybridization. And from the standpoint of resistance to insects, diseases and unfavorable environmental factors, there has been much improvement. But the fundamental morphological characteristics of the plant as determined by the evolutionary paths which it followed before the hybridization occurred have not been drastically altered.

XIII. The Place and Time of Origin

1. *Probable Native Habitat of Wild Maize*

If cultivated maize arose from pod corn, it probably had its beginnings in South America, for there is no doubt that pod corn was known in several parts of America even in recent times. Furthermore, if teosinte is a hybrid of maize and Tripsacum, it may be inferred that the advent of maize in Central America is comparatively recent for, if maize had always been present in a region where Tripsacum is common, the hybridization might have occurred again and again. Finally, there is little doubt that Bolivia and Peru represent the primary center of maize diversity. There are good

reasons for suspecting, however, that maize as a wild plant had its habitat, not in the Andes where varietal diversity has reached a peak, but in the adjoining lowlands.

Until recently a systematic search for wild maize in South America had never been undertaken. Now at least a reconnaissance has been made. Dr. Hugh C. Cutler has explored parts of southwestern Brazil, Paraguay and eastern Bolivia. He has not discovered wild maize. He has, however, found a widespread distribution of a group of varieties similar to the maize of the Guarany Indians of Paraguay. This variety, if not primitive, at least exhibits primitive characteristics. It possesses many of the known "normal" dominant genes of maize such as brown aleurone and the inhibitor for other aleurone colors. It has a low chromosome knob number and the few knobs which it possesses are small. The rachis or cob is slender and flexible. And it has other, internal characteristics already mentioned such as a strong *minus* modifier complex with respect to the tunicate character and a weak modifier complex with respect to teosinte characters, both of which, in a sense, must be regarded as primitive.

Among cultivated varieties Dr. Cutler has found the maximum diversity on the eastern slopes of the Andes in Bolivia from Sacaba and Cliza on the south to Chulumani and Coroico on the north. The fact that large tributaries of the Amazon, the Rio Beni and the Rio Mamoré, though widely separated in the lowlands, both have their headwaters in this region, may be not without significance.

There is, of course, a possibility that wild corn, if it ever existed in South America, is now extinct. The introduction of cattle and other grazing animals into South America would undoubtedly have led to the decimation of wild maize in many of the places where it occurred. Dr. Cutler reports that large areas of the Matto Grosso region in Brazil are so overgrazed that, if wild maize had ever grown there, it would no longer be in existence. On the other hand there are still vast areas in South America which have received not even a cursory botanical exploration. There is at least a reasonable possibility that wild maize still exists in South America and that it may eventually be found. At least, there is at present no evidence which is seriously in conflict with the hypothesis that cultivated maize arose from a wild maize indigenous to the lowlands of central South America.

2. *Alternative Possibilities*

Anderson (8), although expressing the opinion that it is much too soon to give serious consideration to the problem of the origin of maize, has listed the following additional possibilities (2, 3, 8) with respect to the method and place of origin of maize:

1. That teosinte is a hybrid of maize and Tripsacum but that the original home of maize was in Central America.

2. That teosinte is not a hybrid of maize and Tripsacum but did cross extensively with a South American maize in producing the cultivated maize of North America.

3. That maize is a South American plant which twice became contaminated with Tripsacum, once at a very early date, in South America, a second time, at a much later date in Central America at which time teosinte originated.

4. That maize originated in Asia, possibly as an amphidiploid hybrid of Coix and Sorghum.

The first three of these items do not warrant extensive additional discussion for they shed no new light on the problem and they have at present little, if any, evidence to support them. The last suggestion, that maize may have had its origin in Asia, demands more detailed treatment, for it revives a question which in recent years has been generally regarded as having been satisfactorily answered.

Many of the earlier botanists were of the opinion that maize had an Old World origin. There has, however, never been any convincing evidence to support such an opinion and, on the contrary, the evidence for an American origin has been almost overwhelming. Later, when it became known that maize was already being extensively cultivated in parts of Asia within less than a century after the discovery of America, it was thought by some students of the problem to have had a pre-Columbian distribution in Asia though its American origin was not denied.

The entire problem of a possible Asiatic origin or pre-Columbian Asiatic distribution of maize received a comprehensive and scholarly treatment at the hands of Laufer (60) many years ago. He concluded that the appearance of maize in China in the sixteenth century is an indication of the rapidity with which it spread, once it was introduced to the Old World, rather than evidence that it had been long established there.

No new evidence has been brought forward to contradict Laufer's well-reasoned and strongly-documented arguments. Several of the items which Anderson (8) mentions as lending some plausibility to the hypothesis of an Asiatic origin prove upon examination to be of little real importance. There is, it is true, a unique race of fowls in Chile which lays blue eggs but there is no evidence that the blue-egg character stemmed from a similar race in Asia in prehistoric times. It is apparently the result of a mutation occurring after the fowl had been introduced into America. Pop corn is grown and utilized as food on the island of Timor but there is no indication that it is an ancient food or the principal one. There is no doubt that maize is extensively cultivated by primitive mountainous tribes in northern India, upper

Burma and parts of western China. But this fact loses most of its significance when it is noted that potatoes are also extensively cultivated by some of these tribes and that in others the name of maize is derived from the older name for millet.

Until wild maize is found growing in some part of America or until incontrovertible proof of its previous existence has been established the possibility of an Asiatic origin of maize will remain and cannot be dogmatically dismissed. On the basis of present evidence, however, the odds are very much against it.

3. *The Time of Origin*

There is no way of determining from the evidence at hand the time at which the domestication of maize began. Anthropologists have hoped that studies upon the origin of maize might throw light upon the age of prehistoric American cultures and civilizations. It is doubtful whether this will prove to be the case. The most that can be expected from such studies is indirect evidence with respect to the comparative age of different cultures. The data presented here, for example, show that maize probably had its center of diversity as a cultivated plant in the Andean region of Bolivia and Peru and that the first highly developed maize agriculture probably occurred in South America rather than in Middle America. To the extent that the prehistoric civilizations of America were based upon maize agriculture these facts indicate, though they certainly do not prove, that the civilization of South America is earlier than that of Middle America. They furnish no clue regarding the absolute time at which either came into existence.

Anderson (5, 8), Carter (24) and Carter and Anderson (25) have recently utilized races of maize in studying the cultures of the Southwest and in arriving at important conclusions regarding them. Such studies can undoubtedly be very fruitful if the data are interpreted in the light of all other kinds of data which have a bearing on the problem. They can lead to false conclusions if other pertinent facts are ignored or erroneously interpreted.

One more conclusion can be drawn regarding the time of origin of maize. It need not have been as far back in the remote past as some students of the problem have supposed (32). The diversity of maize in South America represents a relatively long period of domestication but, even here, the evolutionary changes involved have not been great and need not have required many thousands of years. The new diversity resulting from hybridization with Tripsacum was probably of an explosive nature and much of it no doubt was created almost literally overnight.

All of the evidence supports the conclusion that the time which

anthropologists allow for the presence of man in America is ample, and more than ample, for all the changes which distinguish maize from its putative wild prototype to have taken place.

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The Genetics of Cattle*

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CONTENTS

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I. Introduction

Information about the genetics of cattle has been acquired largely by the same methods used in studying human genetics; namely, studying whatever data could be found concerning the resemblances between relatives. In cattle it is sometimes possible, after a hypothesis has been devised, to make matings designed expressly to test this hypothesis and to interpret the results with no more elaborate statistical treatment than a χ^2 test. However, considerations of time and expense usually prevent this actually being done. Hence, most of the literature on cattle genetics interprets the results of matings made for some other purpose than as part of planned genetic experiments.

The completeness of the findings and the validity of their interpretation are usually limited by one or more of the following circumstances: (1) The number of offspring which can be obtained from each mating is small. (2) The interval between generations is long — about five years. (3) The information on collateral relatives is often incomplete. (4) Often environmental conditions are not well controlled, either physically or statistically. (5) Critical test matings are rarely conducted, although sometimes diligent search will reveal some already made. (6) Only the more conspicuous characteristics and the more exceptional occurrences of the same characteristic among several close relatives are likely to be reported to the geneticist for his investigation. (7) The number of chromosomes in cattle cells is large.

Partly offsetting these difficulties is the economic importance of cattle which makes them objects of widespread interest and constant scrutiny. Consequently, the literature on cattle genetics is voluminous, although many of the conclusions and interpretations can be considered as no more than plausible. Because of their economic importance cattle offer a fertile field for applying genetic principles, even when such applications will increase by only a few per cent the rate of progress which the breeder is making toward his chosen goal. Extensive and ingenious investigations may be necessary for discovering how to make such applications.

Attempts to explain cattle inheritance in Mendelian terms began almost with the rediscovery of Mendel's laws, the postulation of a domi-

nant gene for polledness being perhaps the first specific attempt to apply these laws to inheritance in farm animals. Many of the early enthusiasts about Mendelism naturally had a tendency to oversimplify the case and to explain numerous and sometimes complex characteristics as the result of only one or a very few pairs of genes. This should be kept in mind when reading the early reports. In the present review we have refrained from critical comments concerning this in the individual studies mentioned, except when internal evidence in the study itself or from other studies indicated with high probability that the oversimplification was extreme.

The purpose of the present paper has been to assemble the pertinent information in as concise a form as possible. We intend it to supplement rather than replace such excellent earlier reviews as those of Gowen (66), Smith and Robison (193), Ibsen (86), Kronacher (99), and Schäper (181). Although the literature is now so extensive that it is impractical to present a complete bibliography, we have made an earnest effort to include recent papers which presented an important amount of evidence on, or at least strongly suggested the mode of transmission for, various characters.

II. Chromosome Number

Masui (135) concluded that there are 33 chromosomes in the spermatogonia of the male. The sex chromosome moves undivided to one pole during the first division. The other chromosomes, which are bivalent, are divided in the first division, one of each moving to each pole. All the chromosomes, including the sex chromosomes, appear to be divided in the second division. Thus, two types of sperm would be produced, each containing sixteen autosomes, and one containing a sex chromosome in addition. Wodsedalek (221) concluded from his work that the respective chromosome numbers for the two sexes are thirty-seven for the male and thirty-eight for the female. Krallinger (98) established that the diploid number is sixty, consisting in the male of fifty-nine rod-shaped chromosomes and one v-shaped element. Makino (131a) confirmed this and reported 48 for the water buffalo. The most commonly reported diploid number for the other Ungulates is also sixty.

Although these reports are in some disagreement, it is certain that the chromosome number is large and that the male is the heterogametic sex. As yet no linkage groups in cattle have been established although either some linkage or much multiple allelism exists among the antigenic characteristics of the blood. Also linkage has been proposed by some investigators to explain certain character correlation.

III. Color Inheritance

1. *General*

Variations in color naturally attract our attention. There is little economic value associated with any particular color aside from the fact that breeders have esthetic preferences for certain colors and color markings. Also, cattle possessing certain coat colors seem to be better fitted to survive under certain climatic conditions. Attempts have been made to discover whether there are linkage relationships between color genes and genes responsible for economic characters; *e.g.*, Prawochensky (163), Esskuchen (56) and Lauprecht (107). The relationships found were so near to zero that they are doubtful. It is likely that genes for color and genes for economic characters are distributed among all or many of the chromosomes. It would be surprising if linkage among them does not exist, for apparently many pairs of genes are involved in the inheritance of economic characters. However, even if such linkage were successfully demonstrated, it is unlikely that the knowledge would be of much value to the breeder. Crossing over would be continually tending to equalize the coupling and the repulsion phases of the double heterozygotes.

2. *Color Genes*

Ibsen (86) has reviewed the literature on color inheritance. He postulates genes occupying nineteen different loci. The genes will be listed with Ibsen's description of the characters and discussion of the various papers dealing with their inheritance.

a. Red, R. This gene is assumed to be present in all breeds in the homozygous state. It seems unnecessary to mention such a gene if it is universally present in the homozygous state, as the effects of a gene can be known only from the differences in the phenotypes which contain it as contrasted with the phenotypes which lack it. In assuming that *R* is homozygous and hence has no allelomorph, Ibsen explains that the reason red does not always show is that many other genes are epistatic to it. *R* is supposedly responsible for the red pigment in the hair and the brownish pigment of the skin of the nose and eyelids if unaffected by other genes.

b. Black, B. This gene is found in the Aberdeen-Angus and Holstein-Friesian breeds. (36) It makes all the pigmented hairs black and also causes the black pigment in the skin, hoofs, tongue, mouth lining, nictitating membranes and "whites" of the eyes if the white spotting gene *s* does not interfere. The allele of black *b* can only be said to be the absence of *B*.

c. Black Spotting, Bs. This is the type of black found in Jerseys, Ayrshires, and apparently in Brown Swiss also. The black is concentrated

in certain areas. Like the *B* gene, it causes black pigmentation of the skin, hoofs, tongue, nose, mouth lining, eyelids and "whites" of the eyes if the animal is not self *S*. The phenotypic effect of this gene is not fully expressed in newborn calves. Males usually develop more black than females. The recessive gene *bs* is the absence of *Bs*.

d. Modifiers of Bs, M and L. *M* causes much black, *L* causes little black. Ibsen proposes these genes to explain the inheritance of black-and-white and red-and-white in Ayrshires. Wentworth (213) explained the inheritance of these colors as due to a single pair of genes which are sex-influenced in their expression. (Wentworth used the term "sex-limited," but modern usage retains this for characters expressed in one sex only.) By studying results of matings of animals of these colors Wentworth found that assumed heterozygotes were black-and-white if they were males and red-and-white if they were females, the gene for black-and-white being dominant in the male and recessive to the red-and-white gene in the female. Ibsen cites Kuhlman (103) who, in 1915, reported on these colors in Ayrshires in Scotland where all heterozygotes are black regardless of sex. These cattle carry *B*, the factor causing all pigmented hairs to be black. Ibsen, in attempting to refute the hypothesis that a single pair of sex-influenced genes is responsible for these colors, says that *Bs* is the gene which is really involved. The sex influence appears to concern the modifiers of *Bs*. Ibsen states that, because of the great variation in the amount of black pigment *Bs* produces in the hairs, there must be more than one pair of modifiers involved. However, he tentatively postulates the single pair *M* and *L*, *M* being dominant in the male and *L* in the female. The case reported by Lush (119) is explained by Ibsen on the basis of the sex-influenced expression of the modifiers of *Bs*. In this case a Jersey cow showing much black was mated to a bull showing little black to produce a female offspring showing practically no black. This female was mated to a bull showing much black, and the resulting offspring was a heifer as black as her granddam.

e. Brindle, Br. Brindle describes a coat color consisting of irregular narrow stripes of black hair on a red background. It is not usually found in pure breeds in America but occurs frequently in cattle of mixed breeding and has been reported in the F_2 generation of the Angus-Jersey cross and in the F_1 generation of the Jersey-Red Danish cross. A few cases are known in purebred Ayrshires.

Cole (35) explains the inheritance of this character as the result of joint action of two genes. Ibsen is in agreement with this explanation and elaborates upon it, citing the results of Parlour (151) from Jersey-Angus crosses and stating that the segregation of colors conforms to a two-factor hypothesis.

f. Intensity and Dilution, I, i; D, d. Dilution, *D*, causes a genetically black animal to be dun. The recessive dilution factor *i* occurs in Jerseys and Guernseys. However, Ibsen states that "there is no question but that there must be a number of genes responsible for the extreme variation in shade of red in the different breeds."

Watson (209) explains the inheritance of dun as due to a dominant gene which will produce dun whether black is present or not. Wright (229) explained dun inheritance as the result of a dominant dilution factor acting on black. Pitt (160) classified Herefords into two shades, "yellows" (light reds) and "clarets" (dark reds). (Actually, however, many other shades exist.) She stated that clarets tend to breed true while yellows mated to clarets produce both yellows and clarets. No yellow-by-yellow matings were available for study. Ibsen points out that if yellow Herefords carry *D* they should produce duns when mated to intense blacks. This does not occur.

g. Self and Recessive White Spotting, S and s. *S* causes an animal carrying it to be entirely pigmented. Such animals are commonly called self or self colored. *S* and *s* are allelomorphic but *S* is supposedly not completely dominant to *s* according to Cole (35). Ibsen believes that part of this apparently incomplete dominance may be due to genes for dominant white spotting carried by supposedly recessive white-spotted animals.

h. Modifiers of s: Lw and lw, Pl and pl. The gene *Lw* causes a small amount of white. It is incompletely dominant to *lw* from evidence presented by Dunn, Webb and Schneider (48). The action of this pair of genes is entirely quantitative, apparently having nothing to do with pattern.

The gene *Pl* causes pigmentation of the legs to a large extent below the knees. Ibsen states that this gene apparently occurs only in the Ayrshire and Shorthorn breeds in this country but he points out that Holsteins with one or more totally black legs do occur, although rare. In the eyes of the Holstein-Friesian Association of America this constitutes a disqualification and such animals are ineligible for registration. Since such animals do occur, it seems that the gene may be present at a very low frequency in the Holstein-Friesian breed also. The fact that animals showing the character are not registered is probably the reason inheritance has not been studied. Pedigree information has been the basis for most studies of color inheritance.

i. Roan, N. Roan is the intimate mingling of white and colored hairs. Red and white roaning is best known from the Shorthorn breed in which over 40% of the animals are roan or roan with white spots. "Blue roans" are well known from crosses of white Shorthorns with black breeds such as the Aberdeen-Angus or Galloway. Also there are purebreeds

with this color. Examples are the Blue Albion in Britain and the "Race Bleue du Nord" in France. The roaning varies in intensity from animal to animal and on different parts of the same animal. Light roan areas have a high proportion of white to colored hair while in dark roan areas the white hairs may be so scarce that, on a casual inspection, the color might be thought red or black. Frequently some white spotting is present also in Shorthorns. If the spots are small and irregularly scattered, there will be much mingling of the red and white hairs along the edges of the spots and it is possible to call such animals roan and white spotted when they are really red and white spotted. It is generally agreed that genetically the white spotting is independent of the roaning. Also the color of the mature coat is sometimes lighter or darker than the calf coat. Smith (190) mentions a bull which was a red roan as a calf but gradually lost his white hairs and became red as he grew older. Light roans do not approach the whites very closely, however, and it is doubtful that any confusion of white with roan animals ever occurs except by purely clerical error.

Barrington and Pearson (10) proposed that roan is the heterozygote between red and white. In Ibsen's symbols reds would be *nn*, roans would be *Nn* and whites would be *NN*. It should be added that the white Shorthorns are by no means albinos but contain pigmented hairs in their ears and often around the muzzle, the hoofheads and even in the switch, besides having pigment in their eyes. The data available still fit this theory more closely than any other yet advanced. Yet several exceptions to it have been reported. The case of Whitehall Sultan, a white Shorthorn bull who sired 15 red calves out of 59 matings to red cows, is a notable example. One of his white sons, Maxwalton Sultan, produced 13 red calves out of 54 matings to red cows (Evvard, Shearer, Lindstrom and Smith, 57). Wentworth (212) reports from herdbook data four red calves and eight roans from 153 white-by-white matings. If there were no errors in recording and no mutations, such cases would of course prove the single factor hypothesis inadequate. Errors in recording do occur, however, and some believe that they, along with occasional errors in describing dark roans as reds or *vice versa*, are adequate explanation for these occasional cases which are reported but cannot be explained on the one-factor explanation. Wilson (217) and Wright (229) have upheld the one-factor theory. Roberts (178) concluded that if red-and-white animals and red animals are genetically alike except for a spotting factor, the data from the Shorthorn herd at the University of Illinois over a 33-year period conform very closely to the single-factor explanation. A roan calf from a mating of red with red-and-white was the only aberrant individual in all that time. The possibility of the red-and-white having really been a

roan-and-white is real enough that this one unexpected result need not be considered as disproving the theory.

j. Dihybrid Theories. Wentworth (212) proposed separate factors for roan and for white. His roan factor *P* is dominant to the non-roan *p* and is epistatic to *R*, the factor for red, which is dominant to *r*, the factor for white. He thought the exceptions, such as roan or red from white-by-white matings and roan from red-by-red matings were rare enough to be explained as errors in registration. However, Wright (229) showed that, when the necessary gene frequencies are considered, the actual data do not show the proportions required from a population consisting of such genotypes as those suggested by Wentworth.

As part of the explanation of the inheritance of colors in their Shorthorn-Galloway crosses, Evvard *et al.* (57) proposed an extension factor, *E*, which causes pigment to cover the entire coat whereas its allele causes pigment to show only in the points, and a completely dominant factor, *N*, which causes the roaning. As Ibsen points out, roans of four different genotypes would occur if this genotypic picture were true. These would be: *EENN*, entirely homozygous; *EENn*, which would produce no whites when inbred; *EeNN*, which would produce no reds when inbred; and *EeNn*, which would produce all three colors, white, red and roan, as roans actually do.

Duck (47) proposed a dominant extension factor, *E*, which acts on the heterozygote, *Rr*, to produce roan. *RR* animals would be red and *rr* animals would be white. *Rree* animals would be red and white. Smith (190) objected to this theory on two main counts. First, it requires three out of four reds to carry the roan extension factor. The actual data do do not show this to be true. Second, the theory makes it necessary for red-and-white spotted cattle to be heterozygous, which differs from the known situation in other breeds.

To explain the apparent exceptions to the one-factor hypothesis such as those observed among the progeny of red cows mated to Whitehall Sultan and his son, Ibsen tentatively postulated a recessive modifier *rm* which changes genotypic roans to red. According to his explanation, white bulls might be genotypically *RmRm*, *Rmrm* or *rmrm*, the *Rm* gene having no phenotypic effect. Ibsen suggests that possibly the *NNrmrm* animals would be roan instead of white, which would make it possible to produce a true-breeding strain of roan animals. However, it would be impossible to distinguish such roans phenotypically from other roans. If this were the genetic situation, it seems that, because of the preference for roans among many breeders, some roans of this genotype would have been discovered, which appears not to have happened. Moreover, when one considers what frequency a gene like *rm* must have in order to do that

for which it is postulated, it appears that postulating *rm* raises more serious discrepancies than it explains.

The monohybrid explanation of roaning remains the one which most nearly explains the reported facts; yet many will be reluctant to believe that mistakes in recording and in description are adequate to explain all exceptions, especially when there are several centering around one animal as occurred in the case of Whitehall Sultan and his son.

k. Recessive White, wn. Ibsen postulated this gene as the one responsible for the inheritance of the "silver gray" color in the Nellore breed of Indian cattle. He proposes this in opposition to the explanation presented by Manresa *et al.* (132) who assumed that the silver gray color was caused by a dominant gene which, when homozygous, is completely epistatic to both black and the dominant dilution factor, and is incompletely epistatic when heterozygous. Rhoad (168) presented a more critical study. He concludes that there are two distinct hair types produced by a simple allelomorphic pair of genes, *Tp* (unicolored or totally pigmented) and *tp* (tipped or bicolored). The evidence also indicates that the genes responsible for hair pigmentation are also responsible for skin pigmentation, but the data are too meager to establish this point satisfactorily.

l. Dominant White, Wp. This factor is carried by the white English Park cattle and by Swedish mountain cattle (Wriedt, 225). Since animals carrying the factor have pigmented ears and may have colored flecks on the body, Ibsen suggests that the white of these cattle is actually a form of dominant white spotting. Ibsen concludes from observations of photographs of these cattle that black, *B*, and self, *S*, are common since the photographs showed non-whites in groups of whites, these non-whites reputedly being from white parentage in every case.

m. Dominant White Spotting Genes.

α. Hereford pattern, S^H, *S, s.* Ibsen favored a multiple allelic series explanation of Hereford color inheritance, three genes, S^H (Hereford pattern), *S* (self) and *s* (recessive white spotting), making up the suggested series. Other possibilities which have been suggested are: a single-factor hypothesis with a spotting factor epistatic to *S* and a hypothesis in which the spotting factor is epistatic to *S* and *s*.

β. Modifiers of S^H, *Lw, lw; Rn, rn; Re, re.* Ibsen discussed Miss Pitt's proposal (160) of three modifiers of S^H, *Lw, lw* (already described), *Rn, rn* (red neck and absence of red neck; *Rn* incompletely dominant to *rn*) and *Re, re* (red hair around the eyes and absence of red hair around the eyes; *Re* considered completely dominant to *re*). The action of these modifiers is not well established. However, it seems quite certain that such modifying genes exist, for, as Ibsen points out, "There are many

variations in the Hereford pattern and the three pairs of modifiers proposed by Miss Pitt would not by any means account for all of them."

Other commonly seen variations in Hereford pattern are "line-backs" and "brockle-faces." Nothing definite is known as to the inheritance of the "line-back" condition, which consists of white hairs along the center of the back extending from the end of the typical white crest marking at varying distances, sometimes to the tail. Some judges and breeders classify as "line-backs" Herefords which have an isolated fleck of white hair anywhere along the top line farther back than the withers. The fact that the character is discriminated against probably explains the paucity of data for study of its inheritance. Ibsen stated that it appears to be due to a recessive gene because two animals not possessing it occasionally are the parents of a "line-back." It seems rather likely that it may be quantitive in nature since there are so many variations of the amount of white on the back.

The "brockle-face" character consists of large blotches of pigmented hair on an otherwise white face. Ibsen stated that all individuals of this kind are seen from careful examination of photographs to have pigmented legs. Following this he made the statement that "there seems to be no question but that the same factor produces both effects." However, the senior author has seen on Colorado ranges many "brockle-faced" Hereford-Shorthorn crossbreds among which a few with white legs have occurred.

Two European breeds, the Groningen in Holland and the Normandy breed in France, apparently carry the S^H gene, according to Ibsen, but genetic evidence for this is lacking. It seems possible that the patterns of the Hereford and of these two breeds might be produced by different genes since the lineage of the breeds has been separate for a long time. The Normandy breed typically has much more white than Herefords, and the pattern of the white is not as definite.

γ. *Dutch belt*, S^D, S, s. This character consists of a white belt, varying in width and regularity of outline, around the body back of the forelegs. Kuiper (104) explained the inheritance of the condition as the result of the actions of two pairs of genes, B, b (belt completely dominant to absence of belt), and S, s (self and not self). His data seem to agree well with this hypothesis, but Ibsen objected to such an explanation because ss individuals with belts apparently never occur. Since some animals heterozygous for self exist, occasional ss offspring should result from matings between pairs of such heterozygotes. To meet such an objection Ibsen postulated a gene, S^D, which produces the belt and also pigmentation of the remainder of the coat and is allelic and dominant to S and s. Possibly ss animals with belts do occur, but their other spotted areas cause the belted area not to

be recognized as such. If so, Kuiper's explanation might still be satisfactory in spite of Ibsen's objection.

δ. Colorsided, S^c, S, s. Colorsided animals, as the term implies, are colored on both sides of the body with the mid-dorsal line, the underline and the extremities white. The character occurs in some European breeds, yaks and zebus. From the data of Wriedt (223) the factor for this character is dominant to *S* and *s* and, hence, as Ibsen points out, is allelic to them. The evidence indicates, however, that the dominance to *S* is incomplete. Some colorsided individuals, from photographs and descriptions, appear to be very similar to some "line-backed" Herefords. Could it be that the colorsided gene is present in the Hereford breed but with modifiers to restrict the extent of the white? If so, segregation among these modifiers might be responsible for some of the "line-backs" among Herefords.

ε. Inguinal White, In. Gowen (64) presented evidence for the existence of a dominant factor, *In*, which is responsible for white in the inguinal region and is inherited independently of self. The character is especially noticeable on some individuals of the Aberdeen-Angus breed, but the gene producing it is present also in the Holstein-Friesian breed. Gowen appears hesitant to accept the factor fully, but Ibsen considers the evidence "fairly convincing."

ζ. Whitening, w. The "fawn" color of Jersey cattle was explained by Ibsen as due to a single recessive gene *w* for which the Jersey breed is apparently homozygous. The evidence for this is not conclusive. This hypothesis seems somewhat analogous to postulating the single gene for red, *R*, for which all breeds are supposedly homozygous. The character might be due to more than one gene pair since there is variation in the expression of the fawning character. Cole (35) stated that in the F_2 generation of a Jersey-Angus cross no typical light Jersey fawns occurred.

η. Pigmented (Black) Skin Spotting, Ps. Ibsen postulated a single dominant gene, *Ps*, as responsible for black skin pigmentation. This agrees with the conclusions of Gowen (64), Pitt (160) and Pearl (154). The results of Gowen and Pitt indicate that a dominant gene produces black muzzle color, while those of Gowen (reported in the same paper) and Pearl indicate that a dominant gene is responsible for tongue color. Funkquist (61) postulated three pairs of factors for muzzle color in the Stjernsund herd in Sweden.

3. *Genetic Composition of Breeds*

Ibsen presented tentative Mendelian formulas for the color constitution of several breeds, as far as then known. The formulas imply complete homozygosis of each breed for many of these genes, although for some genes they specify that heterozygosis may occur. Estimates of the fre-

quency of the alleles in cases in which heterozygosis of the breed is postulated are not given. These Mendelian formulas which Ibsen tentatively proposed for whole breeds are the most extreme step yet made in that direction, so far as concerns color, although those who study the antigenic characters of blood have gone even farther and have included estimates of the frequency of each allele in the few breeds studied. As an example of Ibsen's formulas, that for the Guernsey was:

bb bsbs dd ii nn psps ss LwLw plpl WW WnWn wpwp
Dd? *Psps* *Lwlw*
lwlw

4. *Albinism*

Several cases of albinism in cattle have been reported but most of these have been sketchy and the genetics has been inconclusive. In some of these cases, a "ghost pattern" has been observed in which, with the proper lighting, a spotting pattern shows on some of the apparently white "albinos." Detlefsen (44) has reported a case in which the dilution factor appeared to be dominant instead of recessive as is the usual case with albinism. Two albinotic calves, one male and one female, were dropped in a grade Holstein herd in Minnesota. This appeared to be a case of segregation of homozygotes. However, when the bull calf was mated back to grade cows, all his calves (approximately twenty) were albinotic as though the bull were a homozygous dominant. This report has been viewed rather skeptically because most cases of albinism are known to be recessive and because it is based on the memory and statement of the owner and not upon records made systematically at the time. An extensive recent study by Peterson *et al.* (156) has established the albinotic dilution as due to a simple Mendelian recessive, substantiating the results of Cole *et al.* (38). The "ghost" pattern appears to be due more to structural abnormality of the hair than to pigmentation. Peterson *et al.* suggest that the condition is due to absence of genes conditioning the presence of the enzyme tyrosinase. These authors cite the case reported by Carstens *et al.* (32) who observed 22 albinotic animals in a Brown Swiss herd. They also mention personal communications concerning cases of albinism in the Red Danish and Hereford breeds. In most cases there is partial pigmentation in the iris of the eye and in the hair in adult animals. This pigmentation develops gradually from birth when there is practically no pigmentation. However, the junior author has himself seen some of the mature albinos in the Red Danish case. These showed all signs of true albinism, including pink eyes and apparent distress when in bright light. Wriedt also reported (225) some true albinism with evident photophobia in strong light.

IV. Horn Inheritance

The inheritance of horns is perhaps the earliest reported example of Mendelism in cattle or, for that matter, any of the larger animals. In 1902 Bateson and Saunders (13) reported that the polled character was dominant over horned. This was substantiated by Spillman (195), Boyd (25), Barrington and Pearson (10), Parlour (151) and Lloyd-Jones and Evvard (111). Gowen (64) presented the first evidence to indicate that this simple hypothesis is not universally adequate, a sex influence being involved. Males heterozygous for horns tend to have horns more frequently than heterozygous females. Cole (35) stated that in the Wisconsin crossbreeding experiments scurs occurred more frequently in the F_1 males than in the females and were larger in the males. Smith (191) presented similar data for Rhodesian native cattle and Park cattle. He concluded that animals heterozygous for the horned condition often have horns if they are not too far removed from undomesticated strains.

To explain the occurrence of many horned animals in the F_1 generation of crosses between homozygous polled and horned cattle, White and Ibsen (215) presented a hypothesis involving four pairs of genes. All cattle are assumed homozygous for the horned gene *H*. (This seems to complicate the explanation needlessly, since anything alike in all the animals contributes nothing toward explaining the genetics of observed differences between them. It only serves as a symbol for the whole genetic complex which makes them cattle.) Polled cattle carry a gene, *P*, which is completely dominant to *p* (non-polled). The gene, *Sc* (which makes otherwise polled cattle have scurs), is sex influenced to the extent that it is dominant in males but recessive in females. The fourth gene is *Ha* ("African" horns). It is epistatic to *P* in the same manner as *Sc*, except that it has not yet been determined whether or not *Ha Ha* females carrying *P* are horned. In applying the theory to explain the results of other investigators the authors assume that the genes are independent.

Churchill (33) reported a case, involving large numbers, in which the expression of the horned-polled contrast was clearly affected by sex, somewhat as it is in Merino sheep. No entirely satisfactory genetic explanation was offered but the females which could be heterozygous were polled, whereas the males from the same group of matings always had horns or at least scurs.

The one-factor hypothesis with polledness dominant in both sexes will explain most of the facts reported about the inheritance of the difference between polledness and horns; yet it is clear that sex sometimes makes a difference and that scurs occur more frequently and are larger in males. The hypothesis of White and Ibsen (215) may explain these complications, but further investigation seems necessary before it is considered

as established. Also, there must be many genes affecting the size and shape of horns.

Dove's work (46) on horn transplantation throws light on the physiology of horn growth, the relations between the bony core and the horny covering, and other tissue interrelationships. Dove concludes that "the development of horns in all of its diverse manifestations can be attributed to the presence of a single genetic factor controlling the production of one leading character — the *os cornu* — by which all the remaining parts are produced as an interaction of tissues."

V. Inheritance of Lethal, Sublethal, Deleterious and Undesirable Factors

1. *General*

To avoid the occurrence of affected animals in herds where the responsible genes are known to exist, or to rid those herds of such genes, requires careful planning of the mating systems to be followed. Berge's comparison (15) of methods of testing for the presence of undesired recessives will illustrate the point. Occasionally animals are discovered which carry undesirable genes but also possess excellent genotypes for other characters. After considering the seriousness of the effects produced by the undesirable genes, some breeders may not wish to sacrifice as much excellent inheritance as would be done if all animals known to possess an undesirable gene were culled. In many cases the best plan may be to strike a compromise between immediately "weeding out" the bad gene and gradually eliminating it without great sacrifice of valuable inheritance which has been gained through years of careful selection.

The division of harmful characters into lethal, sublethal, or semilethal and slightly deleterious characters may be convenient but is somewhat arbitrary as each class grades almost imperceptibly into the one next to it. Complete listing of all such characters is not attempted, as the reports range in a practically continuous series from a few in which the genetic situation is postulated definitely and is supported by a moderately large amount of evidence, to a multitude which merely indicate some tendency for a variably expressed character to "run in families." For more nearly complete lists and references, see Lerner (108) who named 25 "lethals and sublethals" with only brief comments about them, Eaton (49) who listed 14 "lethals" with somewhat more of description, Hutt (85) who described 11 "lethals" with a bit more of detail, and Schäper (181) who listed, with brief descriptions, about a dozen lethal characteristics and more than that many others with effects less severe.

2. *Lethal Characters*

We are listing here, as examples, several of the best established and most widely quoted of these. From the nature of the way in which knowledge about these is gained, those listed tend to be more conspicuous and unusual than is typical. As compared with the lethal genes which have been reported, it is only a guess as to whether a gene which raises or lowers vitality by 20% and produces few or no visible effects besides would have had a tenth as much, or half as much, or one-hundredth as much chance of being noticed and reported.

a. Achondroplasia 1. ("Bull dog" condition.) Calves affected with achondroplasia are seldom carried beyond the fourth month of pregnancy. When aborted they have short legs, short thick heads and are often herniated. This condition was described in detail by Crew (41). It has long been known in Dexter cattle. It is produced by a homozygous gene which when heterozygous produces more nearly normal but extremely short-legged calves. Carmichael (30) reports what appears to be the same condition in African cattle, but this could be caused by a different gene. However, Carmichael mentions one monster which was born alive, indicating a situation somewhat different from that which prevails in the Dexters. Crew explains this condition as the result of an abnormal functioning of the pituitary gland during fetal life. The condition has also been observed by Adametz (1) in Tux-Zillertal cattle.

b. Achondroplasia 2. This condition is more extreme than the type described above, characterized by a short head, cleft palate and extremely deformed jaws. Affected calves may be carried full term and born alive but die within a few hours after birth. The effect is due to a simple recessive gene. It was first reported by Mohr and Wriedt (141) in Norwegian Telemark cattle. Later, Mohr (140a) observed what appears to be the same condition in Holsteins. Brandt (27) observed the condition in two offspring of a grade Jersey cow mated to a grade Guernsey bull and a grade Jersey Bull. He believes it to be the same hereditary form as that reported in Telemarks and Holsteins. He mentions also having observed a "bull dog" calf from purebred Ayrshire parents. Surrarrer (201) reports the same condition in two closely related inbred calves. He contends that the inbreeding indicates that the gene causing the condition is recessive.

c. Achondroplasia 3. A distinctly different form of the "bull dog" condition is reported by Gregory, Mead and Regan (70). The authors designate it a sublethal type which is quite variable in phenotypic expression. It is usually lethal but not always. One reputedly affected heifer lived to be fourteen months of age when she was slaughtered. The character is due to a simple autosomal recessive which was brought to light by inbreeding Jersey cattle.

d. Agnathia. Affected individuals have no jaws or imperfectly developed jaws. The data upon which the report is based are few, but they indicate that the condition is caused by a simple recessive gene which is limited to the male sex. No female monsters of this type have been reported. (Ely *et al.* (52)).

e. Amputated. Affected calves have no appendages or, if appendages are present, they are developed only to the elbows and hocks. The upper jaw is atrophied and the lower jaw is almost completely absent. Cleft palate also occurs. The condition is caused by a single recessive gene in Swedish Holsteins. Monsters are usually stillborn or die shortly after birth (Wriedt and Mohr (228)).

f. Ankylosis. This condition is characterized by ossification of the articulation of the lower jaw and shortening of the jaw. Affected animals are stillborn or die soon after birth. The character is produced by a simple recessive gene occurring in Norwegian Lyngdal cattle (Mohr (140a)). A more extreme condition consists of ossification of all joints and is associated with cleft palate. This character was reported by Stang (196) as probably due to a single recessive gene in German cattle.

g. Congenital Dropsy. Affected animals have an accumulation of water in the subcutaneous tissues and in the thoracic and abdominal cavities. Fluid is especially abundant in the head and neck region. Births occur at term or one to two months prematurely. Larsson (106) reports this character as probably caused by a single recessive gene in Swedish Lowland cattle.

h. Epitheliogenesis Imperfecta. Affected calves have defective skin on the lower legs and mucous membranes of the mouth and nostrils, large hairless patches over the body, deformed ears, dewclaws and hoofs. Births occur at term but individuals soon die from septicemia resulting from bacterial invasion. The condition is reported as being due to a single recessive gene in Holsteins and in Jerseys in the U. S. (Hadley and Cole (73), Hadley and Warwick (74), Regan *et al.* (167), Wipprecht and Horlacher (220)). These reports vary somewhat in their descriptions of the abnormalities observed. The conditions may be caused by different genes, although they appear to be similar.

i. Hydrocephalus. Cole and Moore (37) report cases of internal hydrocephalus (a collection of fluid in the cerebral ventricles). The inheritance appears to be by a single recessive gene. The authors suggest that genes for the characters, "jumpy" (general nervousness and incoördination) and "asymmetry" (asymmetrical skull development) might be recessives also, independent of the hydrocephalus lethal. However, this would mean that the sire used would have to carry all three genes, and, as the authors point out, the probability that one animal would be a carrier of three such rare recessives is very low.

j. Hypotrichosis Congenita. (Hairless.) Hair is present only on the muzzle, eyelids, ears, pasterns and end of the tail. The character is produced by a single recessive gene in Swedish Holsteins and in Jerseys in the U. S. (Mohr and Wriedt (142); Eisele (51); Wipprecht and Horlacher (220); and Regan *et al.* (167)). Surrarrer (201) has also reported the condition but does not state in what breed it was observed. In all these papers, except one, the character is reported as a lethal, the affected animals being carried full term but dying soon after birth. Regan *et al.* call it a sublethal, for they report hairless individuals living for a considerable length of time. In some of the reports this character seems to be the same as *epitheliogenesis imperfecta*, although genetic tests of identity were lacking.

k. Impacted Molars. Heizer and Hervey (81) have reported this character in Milking Shorthorns. The mandible is shortened and the premolars are impacted in the jaw, causing the lateral surface to bulge or break. The molar germs are irregularly placed and reduced. Affected calves are born at term but die during the first week after birth. The evidence indicates that the condition is produced by a single recessive gene.

l. Lameness. A condition which allows afflicted calves to be born alive has been observed by Loje (112) (cited by Eaton) in Red Danish cattle. The condition is characterized by lameness in the hind legs and inability to stand alone. The calves die very soon after birth. The character is probably inherited as a single recessive.

m. Mummification. Loje (112) reports a hereditary condition in Red Danish cattle in which fetuses die at the eighth month of gestation. They have a short neck, stiff legs and prominent joints. This condition, which may possibly be allied with muscle contracture, is inherited as a recessive character. Turner also reported (203) cases of mummification but the evidence for heritability is not strong.

n. Muscle Contracture. Mohr (140a) and Hutt (84) report what appears to be the same condition in Norwegian cattle and in Holstein cattle, respectively. The head is bent backwards, the neck stiff, the front and hind legs are drawn together toward the body and the joints are rigid. The character is inherited as a single recessive.

o. Parrot Beak. This condition is somewhat similar to the impacted molar character reported by Heizer and Hervey. It appears to be inherited as a simple recessive (Annett (3)).

p. Short Limbs. This condition is characterized by short limbs and undeveloped hoofs. Affected calves are usually aborted prematurely. The character is reported by Ljutikow (110) (cited by Eaton) as being inherited as a single recessive in Swiss cattle in Russia.

q. Short Spine. Mohr and Wriedt (143) have reported as hereditary an extreme shortening of the spine in Norwegian mountain cattle. Other

parts of the body appear to be normal except for a distortion in relative positions as a result of the shortened spine. If afflicted calves are born alive, they die within a few hours. The character is reported to be inherited as a simple Mendelian recessive.

r. Spasms. Gregory *et al.* (72) report a condition of hereditary lethal spasms in Jerseys. Calves appear normal until spasmodic muscular contractions begin. Affected animals die within a few weeks after birth. The character is produced by a single autosomal recessive.

Concerning lethal characters it should be noted, in summary, that the expression of many is known to be strongly dependent on the hereditary constitution of the animal without, however, our yet being entirely sure of the number of genes involved, their recessiveness, penetrance, expressivity, *etc.* We cannot be certain that phenotypically similar lethal characters occurring in different breeds are produced by the same gene. Only by making appropriate test matings can we establish definitely the identity or nonidentity of genes producing similar characters in different breeds. An outstanding example of this type of testing is the experiment conducted by T. H. Riches as reported by Punnett (165). A Telemark bull was mated to Dexter cows, and the results showed that the two types of achondroplasia occurring in those breeds were caused by entirely different and independent genes.

3. *Sublethal and Semilethal Characters*

a. Semihairlessness. Craft and Blizzard (40) reported that semihairlessness was caused by a simple Mendelian recessive gene in a Hereford herd in Oklahoma. The calves showing the character were deficient in hair, having only a thin coat of very short, fine, curly hair at birth. As the animals became older, they acquired some very coarse wiry hair but were always deficient in total hair. They did not grow well and they appeared to be wilder than normal calves. Cole (34) reported from a purebred Holstein herd a hair deficiency somewhat similar but accompanied by defective incisor teeth. It was clearly hereditary but a detailed genetic analysis was not possible.

b. Hereditary Congenital Flexed Pasterns. A flexed pastern condition is described by Mead *et al.* (140) in the University of California dairy herd. Two types appear to occur. One type is hereditary and is caused by a single autosomal recessive gene, while the other type is nonhereditary. The expression varies considerably. In the extreme form the pastern is completely flexed so that the calf walks on its pastern joints. Some of the calves have only partially flexed pasterns so that they walk on their tiptoes. The hereditary form occurs in the inbred Jersey herd In such a case as this, where a similar condition is produced by nonhereditary

influences, extreme care must be exercised in the study of the hereditary type.

c. Polydactylism. Morrill (144) has recently reported a condition in Herefords in which affected males possess an extra toe on each front foot. Morrill states that though the data are very meager they indicate that the condition is produced by a single gene which is dominant in the male and recessive to the gene for the normal condition in the female. He terms this typical sex-linked inheritance but it may merely have been a case in which the dominance is reversed between the sexes. The conclusions are based on the fact that all the male progeny of one cow and her daughter have been affected. No females possessing the character have as yet been observed. The character is considered sublethal in effect, because affected animals become so sore-footed as they grow that they have great difficulty in walking.

d. Congenital Cataract. Detlefsen and Yapp (45) concluded that a congenital cataract condition in cattle is inherited as a simple Mendelian recessive. They base this conclusion on the high frequency of cataractous offspring in a group of Holstein-Friesian cattle which were closely inbred to one bull in order to attempt fixing that bull's desirable factors for economic characters.

Gregory *et al.* (71) reported a similar cataractous condition in the inbred Jersey herd at the University of California. They, too, conclude that the condition is produced by a single autosomal recessive gene. Since Detlefsen and Yapp do not describe in detail the condition observed by them, it is impossible to know how similar are the conditions occurring in the two breeds.

e. Night Blindness. Craft (39) reported an eye defect he observed in a Milking Shorthorn herd in Oklahoma. The afflicted animals have difficulty seeing in dim light or at night. The condition seems to be due to an abnormal functioning of the rods and cones of the retina. The data are only suggestive and are not sufficient to establish the mode of inheritance of the character. However, there seems to be little doubt that it is hereditary.

4. *Deleterious and Undesirable Characters*

a. Umbilical Hernia. Warren and Atkeson (208) reported having observed 21 herniated Holsteins in three herds. They were descendants of one common ancestor. No animals not descended from this ancestor were herniated. Hernia is extremely rare in cattle generally, as compared with other mammals. The character appears to be sex-limited, being dominant in the male sex. Inheritance in the female is not clear.

b. Polydactylism. Roberts (177) reported a three-toed condition in Holsteins as being caused by a simple dominant gene. The condition,

from Roberts' description, does not appear to be as harmful to afflicted animals as the type reported by Morrill (144) in Herefords.

c. Bowed Pasterns. An apparently hereditary bowed-pastern condition is reported in Jersey cattle by Atkeson *et al.* (5). On the basis of available data the inheritance could be explained equally well as due to a dominant or to a recessive factor. That the condition is not nutritional appears established by the fact that normal animals grow under the same conditions and that one bull sired affected calves in two different herds.

d. Wrytail. This condition is reported by Atkeson and Warren (6) in Jerseys. It appears to be inherited as a simple Mendelian recessive.

e. Dwarfism. A proportionate dwarfism in Jerseys is reported by Mead *et al.* (139). At birth dwarfs cannot be distinguished from normal individuals. They grow more slowly and can be identified at a year of age. The evidence is not sufficient to establish the character as hereditary, but it strongly suggests that the condition may be produced by a single autosomal recessive gene.

VI. Inheritance of Economic Characters

1. *Milk and Butterfat Production*

There seems to be little value in reviewing in detail the early work already reviewed by Gowen (66) and by Smith and Robison (193). We will offer here only a brief account of recent additions to our knowledge of the genetics of milk and fat production.

The very early studies were not statistical analyses, but some of them served to demonstrate that milk production, fat production and fat content are influenced by heredity. Sedarholm (187) furnished some of the earliest evidence that quality of milk is influenced by inheritance. He found that three of five bulls whose progeny he studied, produced a marked improvement in the quality of their daughters' milk over that of their dams. One bull produced only a slight improvement, and the daughters of the other bull, whose dam's milk was very poor in quality, produced milk which was very inferior in quality as compared to the milk produced by their dams. Kirchner (93) showed by a comparison of fat content of the milk of cows of two families which were crossed that this character is influenced by heredity. Van Norman (205), from his study of a herd of grade Guernseys, furnished evidence that, in general, purebred sires can transmit to their offspring higher milk yield and fat content than ordinary unpedigreed sires.

Rietz (175) presented one of the most critical of the early studies. His evidence indicates that butterfat yield is inherited in such a way as to allow some prediction of an individual cow's record from those of its

ancestors. He also concluded that after maturity butterfat inheritance is much more pronounced than at any fixed point in the growth period. This is in line with the old theory of "dynamic inheritance" which was once accepted by many but was later disproved by Putney (166) and Allen (2) and has been disproved many times since they published.

Wilson (218), with the results of a study of data from 1832 Ayrshires, made the first attempt to establish the number of genes involved in the inheritance of milking capacity. He concluded that milking capacity was produced by the action of a single pair of genes. He later revised his theory to include four pairs of genes producing sixteen grades of production.

Wriedt (227), analyzing the Tranekjaer crosses between Red Danish and Jerseys, concluded that this breed difference in fat percentage was based on one factor. However, the distribution of the F_2 and backcross generations with respect to the means of the parents and of the F_1 in Wriedt's data shows that several pairs of genes were really involved. The various possible comparisons of the difference in parental means and the differences in the variance within the breeds, the F_1, the F_2, and the backcrosses yield 7 to 15 pairs as the minimum number of genes, but the fiducial limits on these estimates are wide.

Hills and Boland (83) suggested two factors, one dominant and the other probably sex-linked. Funkquist (62) postulated at least three homomeric factors to explain fat inheritance. Hansen (76) believed that the best explanation is that there are several factors with greater importance of the female than the male. Yapp (234) concluded from his study of the Bowlker herd records (Guernsey and Holstein-Friesian crosses) that at least ten factors are involved in the transmission of milk yield and still others affect fat percentage. Turner (204) concluded that milk and fat yield are influenced by many genes, that genes favoring high production tend to be dominant, and that not all genes have the same effect.

Crossbreeding experiments have yielded some information on the mode of inheritance of milk production. Three extensive crossbreeding experiments, in which dairy production was a prime object, have been planned and conducted in this country. They are the Bowlker herd (233), the Wisconsin crossbreeding experiments (35), and the Maine crossbreeding experiments (65). In other countries the most extensive and carefully planned one was the crossing of Jerseys and Red Danish at Tranekjaer in Denmark (227). In the first generation of such crosses production was nearly intermediate between that of the parent breeds with much individual variation, of course. The variation which occurs in the second generation indicates much segregation of factors and suggests that many genes are involved in the difference between the parent breeds. Gowen (65) concluded, from the fact that crossbreds between two differing levels of

production generally resembled the high parent more closely than the low parent, that there is partial dominance of factors for high production.

Smith and Robison (193) were of the opinion that milk production must be influenced by a fairly large number of factors. To determine whether or not sex-linkage plays an important part they compared the yield of half-cousins of two groups, those whose common ancestor is the paternal grandsire and those whose ancestor is the maternal grandsire. They conclude that sex-linkage does not play a large part in inheritance of milk yield, but they do believe that one or more of the factors for production are transmitted in a sex-linked manner. This they deduce from the significantly lower correlation to the paternal than to the maternal grandsire obtained in the work of Smith, Scott and Fowler (194) with correlations of Ayrshire cows to their ancestors. Madsen (131) furnished further evidence for sex linkage when he found that there was almost no correlation between the milking capacity of a bull (as measured by the average yield of his daughters) and the milking capacity of his paternal grandam, while the correlation between the bull's milking capacity and the milking capacity of his maternal grandam was significant. Correlations between relatives may, however, be unequally affected by environmental factors such as herd-to-herd differences, or by selection having been more intense for some kinds of relatives than for others. Thus, cows who are cousins through a maternal grandsire are more likely to have made their records contemporarily in the same herd than are cousins through a paternal grandsire. Likewise, paternal grandams are usually the survivors of a more intense selection than was applied to the maternal grandams. Hence, this method of testing for sex-linkage is open to serious pitfalls of interpretation.

The above theories as to the mode of inheritance of milk-producing ability have been presented to show how our knowledge has evolved to its present state. There are still many things concerning inheritance of milk producing capacity which are not understood. The majority of the evidence indicates that many genes are involved in the inheritance of milk characters. These genes apparently act mainly in an additive manner with little dominance or epistasis among them. No major genes for production have yet been identified separately from other such genes, and it seems unlikely that any genes will ever be known this well.

Other causes of variation in production besides genetic differences are important. These are the causes which some of the early investigators underestimated in their attempts to explain differences in production on the basis of simple Mendelian hypotheses.

By suitable statistical techniques it is possible to estimate what portion of the variance of quantitative characters, such as milk production,

is due to additively genetic differences within a given population. This fraction is called "heritability" in the narrow sense of the word; *i.e.*, it does not include variance due to the effects of dominance or of epistasis which may make the substitution of a gene for its allele produce in the presence of some genes an effect larger or smaller than the average effect of this substitution.

Estimates of heritability of intraherd differences in milk and butterfat production have been about .2 to .3 from most studies. Lush and Straus (129) have reported .17 for the heritability of butterfat production. Ward's (207) figure is about .3 for the heritability of the same character. Lush (123) reports that the figures obtained by Johansson and Hansson yield .3 to .4 for heritability of total milk or fat yield and .7 to .8 for heritability of fat percentage. Gowen (67) concluded from the Jersey Register of Merit records that inheritance accounts for about half of the variance in milk yield and about four-fifths of the variance in butterfat percentage. However, any general environmental differences which may have existed between herds were not measured and discounted in this study. If those were considerable, as probably was the case with milk yield and may have been true of test, these estimates are distinctly too high.

Whether variance due to dominance and to epistatic deviations are important in milk and fat production remains doubtful. They appear to be unimportant in the differences within breeds according to Seath and Lush (186) although their data were too scanty to be highly conclusive. Heizer *et al.* (82) reported some evidence for "nicking" being important, at least occasionally. Presumably epistatic effects might be more important in crosses between breeds or between inbred lines than they are within breeds.

2. *Evaluation of Breeding Worth*

Since the phenotypic expression of such a character as milk production certainly is much influenced by environment and may be influenced also by dominance and other nonadditive interactions of genes, means of evaluating the transmitting ability of males and females are highly important to breeders trying to improve the genetic level of their herds. All such evaluations, of course, are based on phenotypes of the animal being evaluated or of its relatives. When the animal's own phenotype is used alone, the environmental effects are often an important source of error. When averages of the phenotypes of several relatives are used, the truly random environmental effects may cancel each other into unimportance, but environmental effects which are prevailingly in one direction will remain as biased errors, which are often important. All evaluations of

breeding worth are relative to some set of environmental conditions, either specified or inferred.

As the economic character is not expressed in the male in dairy cattle, his breeding value can be estimated only from the performance of his female relatives. Among these his progeny permit the highest accuracy. For evaluating a female there is the additional evidence from her own phenotype. However, a cow's progeny cannot be numerous; therefore, the possible usefulness of the progeny test is more limited with females than with males. Lush (122) has pointed out that, on the average, a progeny test based on as few as four daughters cannot be as accurate an indicator of a cow's transmitting ability as her own performance. This applies to an unselected population of cows. If, as may happen, the cows with the poorest phenotypes have already been discarded, the relative usefulness of the progeny test for discriminating among the survivors is higher than that.

Because temporary environmental influences affect production so importantly, a cow's real producing ability is generally nearer to the breed or herd average than her record is (Berry (16)). An average of two or more records is usually closer to her real ability than a single record. The equation for predicting with least error a cow's real ability (Y) from (X), the average of the n records she has already made, is:

$$Y = A + \frac{nr}{1+(n-1)r}(X-A)$$

where A is the herd average and r is the repeatability of single records; *i.e.*, r is the correlation between records made by the same cow. This is useful in estimating the probable results of selection, especially when the choices are between cows with unequal numbers of records. Where the cow's transmitting ability, rather than her own future production, is being estimated from her own records, the r in the numerator of the above formula should be replaced by the heritability of differences between single records (Lush *et al* (128)).

Ways of estimating a sire's breeding worth from the phenotypes of his progeny have been studied extensively for dairy characteristics. Since each daughter gets half of her inheritance from her dam, evaluation of the sire's progeny test involves also some estimate of the breeding worth of his mates or some assumption, such as that those mates were a typical sample of their breed or herd (Lush *et al.* (128)). Definite "sire indexes" for expressing in a single figure the breeding value of a sire so that he could be compared with other sires were proposed at least as long ago as 1913 (Hansson (78)). Several have been proposed since. Nearly all of these are combinations of emphasis on the daughter average and emphasis on the differ-

ence between daughters and dams. They range from complete emphasis on the former to complete emphasis on the latter. One of the most widely used is simply the daughter average plus the amount by which the daughters exceed their dams. The validity of the various indexes has been reviewed by Lush (121). Since the raw data for sire indexes are phenotypic records susceptible to much modification by environment which may have been more favorable or less favorable than is known or assumed, the indexes will still contain those environmental errors except as the random ones have extinguished each other in the averaging or (in the case of the daughter-dam difference) a plus error in the daughters has been eliminated by subtracting a corresponding plus error in the dams. The averaging of many records is rather effective in reducing the truly random errors to unimportance but does not reduce those biased errors from environmental or other circumstances which tend to be in the same direction for all the daughters of one bull or for all their dams, but in the other direction or of a different size for the daughters and mates of other bulls. Subtracting the dams from their daughters corrects well for any biases which were alike for the daughters and for the dams pertaining to each bull but which varied from one bull to another. Any group of sire indexes will still contain at least a little of environmental error. If the indexes are constructed so that their range is comparable to that of the records of individual cows, those indexes (like the records of the cows) should be regressed considerably toward the breed or herd average before they are considered synonymous with most probable breeding value of that individual (Rice (172) and Lush (124)).

Expressing records as deviations from the contemporary herd average has been advocated extensively as a means of correcting for general environmental conditions applying to some herds but not to all (Von Patow (153) and Krüger (102)). This in effect assumes that all differences between herd averages are environmental. The opposite policy of using the actual record, without any reference to the contemporary herd average, assumes that general environmental differences between herds do not exist. It seems that some combination between these two extremes should be more accurate than either but no such combination is yet in general use (Lörtscher (114)).

The most extensive collection of data for proving sires appears to be that of the United States Department of Agriculture (29).

Besides the progeny test, the records of ancestors, of collateral relatives and (in the case of females) of the individual itself, should all be used for estimating breeding value. This amounts to using a multiple regression equation. However, the number and kind of relatives on which there is information and the amount of information on each vary so much that,

for maximum accuracy, each case would almost require a regression equation of its own. These complications have prevented any such method from becoming widely used as yet, although it seems likely that some approximation using only the closest relatives would increase accuracy considerably and yet would be simple enough for extensive use.

3. *Beef Qualities*

Beef qualities are apparently influenced by many genes with little dominance among them. Critical studies on their inheritance are very few. In crossbreeding experiments of beef and dairy breeds the first generation offspring are "in a general way, intermediate" according to Cole (35). The dairy parentage is reflected in the rear quarters of the crossbreds. Cole states that there seems to be a tendency for the crossbreds to resemble the breed of their sire more closely in conformation than the breed of their dam.

Lush (120) has reported a "duck-legged" condition which should be classified among beef qualities. A simple dominant gene produces an extreme shortening of the legs in Hereford cattle. No other change was found.

Stonaker and Tom (198) have studied the "compact" type in Shorthorns. This condition differs from the "duck-legged" condition reported by Lush in Herefords. The entire body is shortened and thickened. "Compact" calves are the specialty of several breeders. The character is apparently produced by a single dominant gene. The "comprest" type in the Hereford is very similar to the Shorthorn "compact" type in appearance, but it seems that the Herefords are more variable and cannot be divided definitely into two types, "comprest" and conventional. There appear to be many intermediates.

Attempts have been made to devise "record of performance" techniques for progeny testing beef bulls and cows for the practically important beef qualities of their offspring but these are still in the exploratory or experimental stage (Knapp *et al.* (94)). Some of the breed associations, such as the American Hereford Association, have adopted Record of Performance plans based on the prizes won by the descendants at prominent shows.

Pontecorvo (161) also reported high daughter-dam correlations for weight at 12 months but herd-to-herd environmental differences may have been included. In a later report (162) he concluded that the heritability of individual differences in weight and in growth rate is probably very low.

A distinctive "double muscled" condition has been reported by Weber and Ibsen (211). It occurred at a high frequency in a purebred Hereford herd in Nebraska and was also seen in some Shorthorns, Aberdeen-Angus

and Galloways. Double muscled animals have abnormally wide thighs, with this extreme width extending forward far enough to include the loin. Deep grooves between the muscles are conspicuous externally. There is little if any fat covering the outside of the muscles. The carcasses are so distinct from others in appearance that packers describe them by special terms such as "Yorkshires" or "Teeswaters." Wriedt (226) reported in some detail on this condition which he called "*Doppellender*," and stated that it occurs frequently in the black-and-white lowland cattle in the region along the Baltic and North Sea shores. It has been observed also in Ayrshires in Norway, Shorthorns in Denmark, Charolais cattle in France and Piedmont cattle in Italy. Wriedt concluded tentatively that the character is inherited as an incomplete dominant, with the homozygous dominants being low in fertility. Wriedt reports that some of the supposed heterozygotes were entirely normal in appearance. Weber and Ibsen say that the character behaves more as a recessive in their data and would be explained by Wriedt's hypothesis only if a very large fraction of the heterozygotes appear normal or nearly so. That is, it could be due to one main factor which has low penetrance. They mention the possibility of modifiers being involved because of the great variation in expression. Wriedt also emphasized this variability in expression. Kronacher (99) reviewed this case, including some earlier references, and used it as an example of how genetic analysis of anomalies found in breeders' herds must usually proceed. He inclined to interpret it as polymeric but not yet wholly and satisfactorily clarified.

Weber and Ibsen emphasized that the carcasses of double muscled animals are undesirable in the meat trade because the absence of a covering fat layer permits the meat to dry out before it reaches the customer. The junior author has been told by men in the meat trade (but with no measurements or other supporting evidence) that the meat is tender, richly flavored and highly esteemed by the few connoisseurs who really know it. However, it is admitted that the rarity of the carcasses, the irregularity of their occurrence on the market and the scarcity of such connoisseurs prevent taking economic advantage of this situation.

Double muscling appears to be an example of a characteristic kept in the breeds but at a low frequency, because the heterozygote is preferred over both homozygotes but one homozygote is very much preferred over the other. The almost universal desire of beef breeders to increase the thickness of muscling in their cattle leads them to select for breeding many of the heterozygotes which manifest this double muscling to only a slight or moderate degree. But the abnormal appearance and partial sterility of those which manifest the character extremely lead to intense selection against them. At least this conjecture is plausible, although

other explanations can hardly be excluded as long as the genetics of double muscling remains uncertain.

4. *Measurements*

Literally tens of thousands of measurements of live cattle have been made by scores of different workers but the net genetic information thus gained has been small indeed. In most such studies the objective was only remotely genetic. In many the measurements were taken primarily to learn what changes occurred with age or with changes in fatness. In some cases they were intended as a partial description of breeds and of the differences between these. In many cases the primary intention was to find any correlations which might exist between size or form and some physiological character, such as milk and fat production. In only a few cases was there the intention to study the inheritance of individual differences in dimensions. In most of these few cases the numbers of animals did not become large enough, or the investigator who had planned the work did not stay with the project long enough to make such an analysis.

In most cases the figures were not reduced farther than enough to present averages, although in a few an enormous amount of tedious work was done to reduce them to ratios or other forms which seemed to reveal more clearly the information contained in the figures.

Among those intended primarily to show the normal changes in dimensions and proportions with growth, that by Hansen (77) is one of the extreme examples of many measurements taken, much work spent in reducing them to ratios and pains taken to present the findings clearly. Another example is the work of Sciuchetti (184) who, however, used more refined biometrical methods and had a slightly different viewpoint, in that he was seeking to discover what measurements described the kinds of animals which the breeders admired highly. To this end he measured only cows which were scored officially at least three points higher than the average scores of all cows in the same canton. Hansen had measured all animals in the herd and, therefore, sought to describe that herd without selection. Among the less detailed studies of measurements which are more typical of the many published in the United States, may be mentioned that by Espe *et al.* (55) and that by Lush *et al.* (127).

The many attempts to find a close correlation between measurements, or ratios of measurements, and some physiological characters, such as milk and fat production, growth rates, feedlot performance, *etc.*, have not uncovered any really high correlations. Some of the correlations found have been large enough to be of some use (*i.e.*, of sizes as large as .2 to .3 or even a bit larger) but most of these seem, in considerable part, only to have reflected the effects of general size. This is evidenced by the partial

correlation generally being much nearer to zero when weight or some measurement closely related to general size (as chest girth, height over withers or body length) was held constant.

Gregory (69) discussed in some detail whether size factors in cattle were mostly general or specific. He compared measurements, indexes constructed from these and mature weights. He recommended expressing the conformation of an animal by an index which had for its numerator a measurement largely muscular (a measurement by tape held horizontally from stifle joint around to stifle joint) and for its denominator a measurement (height over withers) which is almost wholly skeletal.

Schutte (183) has studied the heritability of intrabreed differences in body measurements among range-reared cattle in South Africa. From the offspring-dam correlations he concluded that heritability of differences in height at withers was about three fourths; for width at hooks and width at thurls it was about 60%; for body length and live weight it was about 50%; for the chest measurements it was around 20–36%.

Gowen's study (66a) of weight and seven different measurements in Jersey cattle indicates a heritability of about 60% for individual differences (see his Table 10) within the breed, if environmental differences from herd to herd or differences in the measurer's technique from one herd or time to another contributed nothing to the correlation between sire and daughters. The much smaller resemblances which he found within herds (his Tables 12 to 14) mostly indicate heritabilities of the order of .20 to .30 for differences between members of the same herd. The known stratification in ideals in the Jersey breed at this time with respect to "Island type" and "American type" make it plausible that much of the variance between herd means was genetic. If so, the figures based on his Table 10 are more nearly correct for the heritability of individual differences within the whole breed. Yet it remains possible that some of the variance between herds was environmental and therefore that heritability actually is somewhat less than would be deduced from his Table 10 but more than his Tables 12 to 14 indicate.

Gowen found (66b) practically no relation between production of the daughter and any of seven measurements of the parents. Weight of parent was correlated a little higher than +.2 with milk and fat production of daughters but practically not with test. These findings seem to indicate no pleiotropic effects large enough to be important between production and any dimension except weight.

VII. Other Characters

1. *Antigenic Characters*

Nearly forty antigenic characters have now been isolated (Stormont and Cumley (199) and Owen (149)). Each of these antigens appears to be determined by single dominant genes. Some of the antigens appear to be genetically related and are probably linked. Linkage must certainly exist among some of them, since the number which have been established exceeds the haploid number of chromosomes in cattle cells. However, recent personal communications with Irwin indicate that some multiple allelism exists. There appear to be at least two sets of multiple alleles, each including several factors. This reduces the number of loci involved so that it is still possible that most of the known antigens are independently inherited. The breed differences in the frequencies of the genes responsible for these antigens are the most detailed information yet available for the genetics of cattle populations. Presumably they are a model of the genetics of breed differences in other characteristics too, except that most other characteristics as measured are probably affected by more than one pair of genes (Owen, Stormont and Irwin (150)).

2. *Aural Abnormalities*

A natural notched-ear condition has occurred in three widely separated regions. Lush (117) reports the Jersey case to be due to a dominant gene. The same explanation is presented for the inheritance of the Ayrshire condition by Yamane (232) and for the cases which Wriedt (224) found in Norwegian cattle.

A "double-ear" condition in Brahman cattle was reported by Lush (118). A thin flat piece of cartilage lies parallel to the longer axis of the ear and protrudes from the back surface. The factor for this is dominant to that for the normal ear.

3. *Udder Abnormalities*

In an Ohio Guernsey herd cows occurred with poorly shaped udders and only one teat on the left side. The condition occurs in males also, the left rudimentaries being hardly perceptible on some males who have normal right rudimentary teats. The condition appears to be inherited as a Mendelian recessive as it skips several generations, leaving the herd free for a few years, then it crops out again (Heizer (80)).

Johnson (89) has reported a fused-teat condition in Herefords. A front and rear teat on either the right or left side of the udder are fused together. The condition appears to be hereditary and is caused by a single Mendelian recessive. The supposed heterozygotes have a teat placement intermediate between the fused condition and the normal placement.

4. *Twinning*

Twins are rare in cattle. As there are significant breed differences, a general average has little meaning. Johansson (87) in a review of reports concerning nearly a million births finds that 1.9% of the births in "dairy breeds" are multiple births, while .4% of the births in "beef breeds" are multiple. Löwe (115), in a review of reports concerning 1,150,000 births in German breeds, finds breed averages ranging from .25% to 4.60%. Most of the highland breeds had averages higher than 2%, while all but one of the lowland breeds had averages lower than that. Triplets, quadruplets and quintuplets do occur but are so rare as to attract much attention. Richter (173) even listed 2 reported cases of septuplets. Johansson reported 25 triplets and 3 quadruplet births among his data. Among 14,111 births recorded in the Brown Swiss herdbook office in Switzerland, Engeler (53) reported 379 twin births (2.67% of the births), 4 sets of triplets, and one set of quadruplets.

Whether a particular birth will be a twin birth appears to be determined in small part by hereditary differences between the cows. The evidence for this includes, beside the existence of general breed differences, many cases in which individual cows or groups of several closely related cows have been known to have an unusually high frequency of twins among their calvings. Hayden (79) reported a cow which had five pairs of twins out of seven births. Since there were also twins born to cows closely related to her, it appears to have been hereditary. Atkeson *et al.* (4) reported an unusual case of twinning in Jerseys. Two full sisters were each twin to a bull, and yet both were fertile. They were born less than a year apart. Lush (130) presented extensive data from the Kansas Experiment Station to show that twinning factors of Hengerveld De Kol brought together in one sire seem to be responsible for much twinning.

Tandler and Keller (202a) and Lillie (108a) first explained on an endocrine basis the production of sexual abnormality in the female member of twin bovine embryos of opposite sex, the so-called "free-martin." Swett *et al.* (202) reviewed this subject and presented recent evidence, especially on the possibilities of early recognition of the free-martin condition.

An interesting development in the antigen work has recently been reported by Owen (149). Antigenic tests of the blood of more than 80 pairs of bovine twins have shown that a majority of these pairs have identical blood types, although few of them were identical twins. Identity of blood types between full sibs not twins is infrequent. The vascular anastomosis between bovine twins provides an explanation for the production of frequent phenotypic identities between twins who are genetically different. One twin sire failed to transmit to any of his twenty progeny

certain of the antigens he possessed phenotypically. In a case of twins of opposite sex by different sires identity of blood types was discovered, each twin possessing two antigens for which the genetic factors could not have come from his own sire or from the dam. It has been said that there is a mixture of two distinct types of erythrocytes in the blood of certain twins.

Identical twins occur in cattle but are rare. Johansson estimates from the sex ratio combinations that about 5–7% of the twin births are of identicals. Löwe's figures indicate more nearly 10–12%. Bonnier (20) estimates that 10% of the twins which are both heifers are identical. He and Skarman (21) describe methods of diagnosing monozygosis. Kronacher and Sanders (101) described in detail many pairs of identical twins and the methods they found useful for diagnosing monozygosis. Kronacher (100) indicated the high utility of identical twins for obtaining adequate controls in experiments on nutrition and physiology.

Sex ratio. It appears that slightly more males than females are born in cattle, although the various studies do not wholly agree on this. Johansson (87) reported 51.5% males among 124,000 births. Ward (207) found 52.2% males among 11,000 dairy calves in New Zealand. On the other hand, Roberts (180) reported 49.8% males among 4,912 calves, Roberts and Yapp (179) reported 49.1% males among 8,196 dairy calves and Engeler (53) reported 49.9% males among over 20,000 Brown Swiss calves. Gowen (68) found 50.5% males among 3,559 births recorded in such a manner as to insure a completely random sample. Reports on the sex ratio in premature births and abortions (87) make it clear that prenatal mortality is higher among the males than among the females.

5. *Birth Weights*

Breed differences in average birth weight are large. Naturally these parallel differences in mature size to a considerable degree, although not perfectly. Birth weight seems to be more largely a characteristic of the dam than of the fetus, although the genetic composition of the latter does have some effect. Rhoad *et al.* (171a) showed that the sire affects birth weight, at least in wide crosses. Knapp *et al.* (95) reported that characteristics of the individual cow determined 19% of the variance in birth weight among 770 range-raised Hereford calves. Much of the dam's influence — perhaps all of it although not certainly so — was accomplished through her characteristic gestation length and skeletal size. Among farm-raised Shorthorn calves at Beltsville, Knapp *et al.* (96) found that individual cow differences accounted for about 17% of the variance in birth weight within the beef and the dairy groups. Jordão and Veiga (91) reported an apparent, although small, effect of sire and also of dam but their numbers

were small. Davydoff *et al.* (42) have proposed preliminary proving of dairy sires on the birth weights of their calves. Straus (200) sought to test their proposal but his results were inconclusive. Clearly individual differences in birth weight are partly genetic but no definite genes have been identified and the situation is complicated by the strong maternal influence which makes the calf's birth weight partly a characteristic of its dam, as well as of the calf itself.

6. *Gestation Length*

The mean gestation length in cattle is generally given as about 282 or 283 days with a standard deviation of about five days for individual gestations. That there is some genetic basis for the variations is indicated by the fact that unmistakably significant breed differences occur. Some of these could be imagined to have been caused by differences in the general conditions under which the breeds are kept, but that will hardly explain all the breed differences, else findings such as the following would not occur.

Rife *et al.* (176) report significant differences between Herefords and Angus, with the F_1s being almost exactly intermediate. The crosses were made both ways with the same result. These were data from crossbreeding experiments, with all animals kept under the same conditions as far as could be controlled by the experimenters. Livesay and Bee (109) studied about 1,600 records from the West Virginia Station. They report that the dairy breeds averaged about 278 days which was significantly less than beef breeds, such as the Aberdeen-Angus with 282 days and the Herefords with 285 days. However, it does not appear that this is fundamentally a beef *vs.* dairy difference, for the Brown Swiss breed, which is dairy or dual purpose, has a longer average gestation length than most beef breeds. For example, a recent report (19) from Switzerland gives the average gestation length for Brown Swiss as $289.12 \pm .03$ days. Engeler (53) reported $291.0 \pm .2$ days for 1,000 Brown Swiss cows from the cantons of Luzern and St. Gallen. Johnson (88) reported that Aberdeen-Angus gestation periods were slightly (about 2.5 days), but significantly, shorter than those of Shorthorns and Herefords in the South Dakota State College herd. Jordão and Veiga (90, 91) reported averages of 286.9 days for the Caracu breed and 286.5 for the Mocho breed in Brazil. Analyses of variance between sires and between cows in both cases indicated a small (4–7% of the variance) repeatability for cows and a much smaller (statistically quite insignificant) effect of the sire of the calf. Knapp *et al.* (96) report an intrabreed repeatability of nearly .2 among Shorthorn cows which averaged 4 gestations each. Axelsson (7) reported a mean of 278.8 days for the Swedish lowland race and 285.2 days for the red-and-white breed.

Knott (97) reported a mean of 279.9±.06 days for 2,824 gestations of Holstein-Friesians in three herds in Washington. He concluded that there was evidence of some paternal influence and probably also of real differences between the cows.

It seems clear that there are genetic differences in this characteristic, but that is about as far as present knowledge of the genetics of gestation length goes. If gestation length is primarily a characteristic of the dam, the genetic element in the variance must be rather small, else gestation length would not vary as much as it does from one lactation to the next for the same cow. If, however, it is primarily a characteristic of the fetus, as the data of Rife *et al.* indicate, then the genetic element could be highly. important in determining the length of each individual gestation period.

7. *Artificial Insemination*

Artificial insemination is probably more widely used by cattle breeders especially for dairy cattle, than by breeders of any other kind of livestock During the past 10 years many coöperative artificial breeding associations have been organized in the United States. According to Perry *et al.* (155) approximately 225,000 cows were enrolled in 1945.

Many technical papers concerning the physiology and techniques involved have appeared, but few papers go into any detail about the genetic implications of artificial insemination. The most concise and complete compilation of information on the subject is the book by Perry *et al.* which includes descriptions of the techniques and physiology and discussions of the advantages and limitations of the practice.

IX. Inheritance in Species Crosses

1. *Zebus with Cattle of European Origin*

Probably this should not be called a species cross, although the zebu is sometimes referred to as *Bos indicus*. There is no more sterility among the crossbreds than among zebus or among European cattle. To call them distinct geographic races accords better with the differences between them and with what is done about the taxonomy of other animals than to call them different species. They differ widely in many kinds of characteristics, including things as different as horns, skull shape, dewlap, voice, size of digestive tract, resistance to some diseases and mental traits. Yet, in all these things, each group varies within itself and they are connected by at least a few intermediates. The center of radiation for the zebus is India. European cattle have moved out of Europe, rather than into it, since written records of cattle movements were kept but there is indirect evidence that cattle were brought into Europe from the east during prehistoric

times. Perhaps, also, many came with the armies and migrations that accompanied such wars as the conquests of Attila, of Genghis Khan and of the Mohammedans.

Shortly after the rediscovery of Mendelism there were several reports interpreting observations on zebu crosses in Mendelian terms. Nabours (145) reported that the F_1 individuals are resistant to ticks. This resistance is probably not simply inherited. The dewlap and sheath of F_1 animals appear much more like those of the zebu parent; hence, Nabours assumed these characters to be partly dominant. Such variable characters are probably not simply inherited. Nathusius (146) and Pucci (164) reported that the F_1 animals have a hump much like that of the Indian parent.

In recent years the observers of zebu crosses have turned their attention primarily to characteristics which appear likely to be of considerable economic importance. As these are likely to be complex genetically and easily modified by changes in environmental conditions, the reports are in more general terms and are concerned more with breeding systems, percentages of zebu blood, *etc.*, although definite genes are postulated whenever such are thought to describe the facts with reasonable completeness. Some of the more recent reports typical of current studies of zebu crosses will be mentioned.

Schneider (182) discussed methods of improving the dairy qualities of the cattle of India by some crossing with dairy breeds of European origin. Kelley (92) described the results of introducing zebu blood in various combinations to northern Australia for beef production. Phillips (157) described many of the breeds or local races of zebus in India. French (59) reported concerning the adaptability of zebu crosses in eastern Africa. Wilson (219) reported that in Nyassaland the interbreeding of Friesland-Hissar crosses had evolved a hardy type which is quick maturing, breeds regularly and has maintenance costs enough lower to more than compensate for its yielding less than purebred Friesians. Hammond (75) recommended for Jamaica and Trinidad enough zebu blood to secure adaptability to local conditions and yet enough blood from the specialized dairy breeds to get higher production than can be had from pure zebus. Black *et al.* (18) reported on the beef qualities of grade zebus as compared with Herefords and Shorthorns in Texas. The zebu grades had larger hide areas, smaller digestive tracts (and consequently higher dressing percentages), gained well in a short feeding period but not so well in the fifth and sixth months, had meat appraised a little lower but practically equal in desirability when cooked, and showed a number of other minor differences. Carneiro (31) has reported on the problems of dairying with grade zebus in Brazil. Too little zebu blood leads to poor adaptation while too much leads to low production, but the optimum proportion and the best method

of keeping the dairy population near that optimum were still a matter of considerable doubt.

Parr (152) described the general characteristics of the grade zebus called "Brahman cattle" in the Gulf Coast regions of the United States. Much of this applies to the other zebu crosses which have been made in so many tropical and subtropical regions, although there are some differences resulting from various races of zebus and various temperate-zone breeds having been used. Cattle with zebu blood generally have short and sparse hair, long ears, large humps in the uncastrated males, large dewlaps, longer legs with quicker and easier gait, better adaptability to hot weather and more resistance (although not complete immunity) to many insects and some diseases. These things certainly are not recessive but they do not seem completely dominant either. Whether they seem strictly intermediate or partially dominant depends much on the scale chosen for measuring them. There is not much really critical evidence as to whether the number of genes affecting each single characteristic is small or large. It is the almost unanimous opinion of those who have studied cattle in the tropics that some zebu blood is an important help in adapting cattle to thrive under tropical conditions. There is not nearly as much unanimity about whether the optimum fraction is as low as one quarter or higher than one half. Apparently that varies with whether the cattle are being used for dairy or for beef or work purposes, the altitude and the severity of the tropical conditions, the kind of feed and care which can economically be given them, the different breeds of zebu and of temperate-zone cattle which are being combined, *etc.* Neither is there agreement on the best system of breeding for producing cattle with the highest average practical merit. Commercial use of pure races from the temperate zone or the continual use of sires from such races for grading the native stock until it becomes practically the same as the temperate-zone races is still recommended by only a few, and those recommendations are for areas (such as some irrigated ones at moderately high altitudes or rather far from the equator) where the tropical conditions are not severe and where it is economical to give good feed and care. Elsewhere in the tropics the arguments mostly turn on whether it would be more advantageous really to form a new race by selecting the individual bulls and cows which have the highest practical merit within a population having about the optimum fraction of blood, or to continue producing the commercial cattle by some system of crossing alternately or in a more or less regular rotation, using males from relatively pure nucleus stocks maintained primarily to furnish such males. No decisive conclusions on that point seem to be justified by the evidence yet available, but this is at present the most generally impor-

tant question in applied cattle genetics in most tropical and many subtropical regions.

2. *Bison×Domestic Cattle*

Probably this should be called a generic or at least a subgeneric cross. Boyd (26) reported that hump is dominant to no hump and that the voice of bison is dominant to that of cattle. The inheritance of such a character as voice seems likely to be rather complex. Goodnight (63) reported that the F_1 animals are immune to ticks. Prenatal mortality among the males is very high, much of this being due to difficulties which the large hump of the male causes at parturition. The few F_1 males which have been reared have been sterile. The females are fertile. This is in accord with the general rule that, if sterility occurs in a species cross, it will be more extreme in the heterogametic sex. Backcrosses of the F_1 females to cattle and to bison have been used in a few small-scale efforts to combine desired traits of cattle and bison into a new race. The most extensive experiment is that reported by Deakin and Muir (43) concerning the descendants of the animals first produced by Boyd. They propose several Mendelian genes. Most of these concern color but some affect hair length, horns, voice, tail and muzzle shape. The conformation was intermediate with partial dominance of the domestic type. Other characteristics discussed were: wallowing, rising, hides, hybrid vigor, meat and body weights.

3. *Domestic Cattle×Yak*

These crosses have long been made to an extent which is practically important in Tibet and adjoining regions and in northern Mongolia. Phillips *et al.* (159, 159a) described the yak and its hybrids in China. The hybrids are intermediate in conformation but show hybrid vigor in being larger and stronger and probably producing more milk. The hair is intermediate in length but more like the yak in color. The hybrid males are believed to be sterile and it is also said that the males from both backcrosses are sterile.

Deakin and Muir (43) reported 54 calvings involving yak and domestic cattle parentage (and, in some cases, half or less of bison) in one way or another. Among these were 7 abortions, 6 stillbirths and 3 cases where the cow died. Obviously there was less mortality than in bison×domestic crosses but probably more than would be normal among domestic cattle. One F_1 male sired a calf, but this was a stillbirth. The yak×domestic hybrids did not appear to have any unusual values for beef production in western Canada. They report that the yak conformation was partially dominant over bison and over domestic cattle.

Lush (116) reported measurements of 89 yak cows and 34 hybrids.

The hybrids showed marked heterosis. The males were sterile. Some heterosis was still present in the backcrosses.

4. *Zebu×Yak*

Zawadowsky (236) reported crossing a zebu bull with yaks to produce 14 animals which were F_1s or backcrosses. All hybrid males were sterile, whether they were F_1s or backcrosses to the zebu. The three females which had reached sexual maturity all produced calves. Considerable heterosis was evident. The following characteristics were observed to show distinct variation which is explained as due to Mendelian segregation, although the numbers do not warrant postulating the specific genes involved: coat color, hair length, fringe, length of tail hair, shape of hump, shape of horns and shape of muzzle. The observations about these seven characteristics are reported in some detail. Kushner (105) investigated blood properties in yaks, cattle and their hybrids and reported that the hybrids greatly exceeded their parents in the basic indexes responsible for the oxidizing power, such as hemoglobin content, number of erythrocytes and alkalinity.

5. *Gayal×Cattle*

Nathusius (146) reported in detail the breeding records of the crosses which Kühn made at Halle in the two or three decades beginning about 1880. He did not give the details about other characteristics nor summarize the findings. Twenty-seven F_1s were born and 18 of these lived long enough to be tried for breeding. The European cattle were from several different breeds. Repeated and complex backcrosses were made. There was considerable sterility among the F_1 males but the females were normally fertile or nearly so. Only one of the seven F_1 males which were tried extensively for breeding sired a calf. Backcross males showed less sterility but probably were not fully fertile.

IX. Inbreeding

1. *General*

Comments concerning inbreeding are included even in the oldest agricultural literature. Inbreeding was used extensively in founding the pure breeds, at least as far back as Bakewell's time (54). Many detailed accounts of conspicuous instances have been reported in the agricultural press, but the usual absence of controls and the high degree of selectivity concerning the kinds of results which thus find their way into print make it difficult to appraise this evidence. Perfectly regular inbreeding systems with cattle are not possible (or at least cannot be continued for more than a generation or two) because each animal has so few full sibs that chance variations in the sex ratio alone would soon bring the regular lines to an

end. Hence, few people thought it worth while to institute planned inbreeding experiments with cattle until advances in genetics had clarified knowledge of the inbreeding process enough that the results of such experiments, involving irregular pedigrees, seemed likely to be interpretable. Genetics may be said roughly to have begun groping toward that stage about 1914, with Pearl's proposals for measuring inbreeding in livestock pedigrees, and to have reached it by 1922 when Wright proposed an inbreeding coefficient (230) which was strictly proportional to the probable loss in heterozygosis as a result of the inbreeding alone. Not enough time has elapsed since then to permit extensive and controlled inbreeding experiments with cattle to be planned, instituted and conducted for several generations. Nevertheless, a few reports have already appeared and deserve mention here.

2. *Experiments*

Woodward and Graves (222) inbred grade Guernsey and grade Holstein-Friesian cattle to determine whether they could breed a good dairy herd from an ordinary herd by continued use of only one good sire, followed by his inbred son when the older sire was no longer usable. Their report indicates that intense inbreeding caused a decrease in birth weight and a retardation of growth rate. They also suggest that inbreeding caused a reduction in mature weight. They emphasize that the nature of the results depends greatly on the genetic composition of the sires chosen.

The results of Bartlett, Reece and Mixner (12) indicate that dairy cattle can be inbred successfully if the breeder will practice rigid selection. No significant difference was seen between the growth of the closebred calves and heifers and that of outbred calves. Another study by Bartlett, Reece and Lepard (11) failed to show any significant difference between the growth rates of inbreds and outbreds. Margolin and Bartlett (134) conclude that Holstein-Friesian cattle can be inbred without decreasing weight or size, provided the inbreeding does not rise above 20%. Females more highly inbred than this develop normally to about the first calving but then their development becomes abnormal.

Nelson (147) analyzed data gathered from a closed Holstein herd at Iowa State College. The inbreeding at that time averaged only 5% but individual values ranged up to 28%. He found indications that inbreeding decreased the growth rate at ages under four years. After the fourth year the inbreeding seemed to have no effect on development and the mature size seemed unaffected, but the data on mature animals were few. Baker *et al.* (9) report a significant decrease of size with inbreeding.

Notable examples of the risk of uncovering undesirable recessives when practicing close mating are the four undesirable recessives which

have been brought to light in the inbred Jersey herd at the University of California and the gene for congenital cataract which was brought out with inbreeding in the Holsteins studied by Detlefsen and Yapp (45). All these genes were discussed at an earlier point in this paper.

3. *Analyses of the Inbreeding Which Has Been Done in Various Pure Breeds*

Samples of pedigrees from whole breeds or from special groups within a breed have been studied with the object of discovering what kind of breeding systems were used in forming the pure breeds originally and later in their continued development.

a. The Shorthorns. Wright and McPhee (231) developed the techniques for such studies and used them (137) in studying the Shorthorn breed in Britain from 1810 to 1920. The inbreeding coefficients they found were as follows:

Date	*Due to random mating*	*Observed*
1810	12.4	16.6
1825	15.5	19.9
1850	20.4	18.0
1875	23.3	27.4
1900	24.4	22.9
1920	24.6	26.0

Since most pedigree lines could be traced back only to about 1780–1795, it is evident that inbreeding was more intense during the formative period of the breed than later. The authors interpret the drop in inbreeding from 1825 to 1850 as the result of the leading herds being diffused through the breed at that time. The increase from 1850 to 1875 indicates renewed attention to building closely bred families.

In 1920 the relation of the breed to Favourite, a bull used extensively near 1800, was 55% and to Champion of England (a bull prominent in the ancestry of the modern beef or "Scotch" type) was 46%. These are the maximum relationships yet found between a whole breed and individual animals.

b. The British Dairy Shorthorns. (138) A random sample was compared with the 100 highest producers. The inbreeding of the high producers was 26.9% and of the random sample was 28.0%, the difference not being statistically significant. The two groups both had almost the same relationship as the whole breed did to Favourite and to Champion of England. The high producers were a little more closely related *inter se* than the random sample, but the difference was barely larger than its standard error. The British Dairy Shorthorns thus appear not to be a genetically distinct subgroup within the Shorthorn breed, except as very

recent selection may have caused the frequencies of some genes to be different among them.

c. Holstein-Friesians in the United States. The period 1881–1931 was studied by Lush *et al.* (126). The inbreeding was a little over 4% in 1931. The *inter se* relationship had risen to 3.4%. There was only a faint tendency for the breed to form into separate lines. The cow, De Kol 2d, has exerted the most influence. Her relationship to the breed is approximately that of a great grandam. She has probably furnished about 1/10 of the genes in the breed today. High producers and show winners do not differ much from the breed average in inbreeding or in relationship to remote ancestors. They do have a higher relationship to some recent ancestors. This is especially true of the show winners.

d. Brown Swiss in the United States. These were studied by Yoder and Lush (235). No one animal ever dominated the whole breed although the total number originally imported was small. The highest relationships between single animals and general breed samples were 9.2% (William Tell in 1909) and 9.1% (College Boy in 1929). The amount of inbreeding per generation was about the same as in other breeds. Sciuchetti (185) studied a small sample in Switzerland and found very little inbreeding and no tendency to separate into non-interbreeding families.

e. Herefords in the United States. Such cattle were studied by Willham (216). The inbreeding rose to 8.1% in the 12.9 generations from 1860 to 1930. The *inter se* relationship in 1930 was 8.8%. The inbreeding expected with this *inter se* relationship would be 4.6% if breeding within the breed were nearly random. Since the actual inbreeding was distinctly higher, there appears to have been a moderately strong tendency to form separate families or groups which only rarely interchange breeding stock. Nearly all the individuals who had high relationships to the breed were ancestors, descendants or mates of one bull, Anxiety 4th. The relationship of Anxiety 4th to the breed was 18.5% in 1930. Beau Brummel, grandson of Anxiety, had the highest relationship to the breed, 24.6% in 1930. Prize winners and Register of Merit samples had higher inbreeding and *inter se* relationships than the random samples. The ratio of the inbreeding to the *inter se* relationship was about the same as in the random group, indicating the same slight tendency to family formation within the special groups. Over 80% of the random ancestral lines in this study traced to foundation animals bred by only 20 English breeders. Three of these breeders bred 38% of the foundation animals.

f. Ayrshires in Scotland. Fowler (58) studied these for the period 1877–1927. The inbreeding rose to 5.3% in 1927. He found no difference between the inbreeding of bulls and of cows. Much of the inbreeding came from line-breeding to two foundation sires. High milk-yielding cows showed

markedly lower inbreeding than the breed average. A comparison of the inbreeding of high and low producing cows indicated no detrimental effect of inbreeding on milk production. In 1923, 39% of the inbreeding found in high milk-yielding cows was to ancestors which were in one herd, the Drumjoan herd.

g. Jerseys. Smith (192) studied Jersey cattle in Britain and found an average of 3.9% inbreeding for the period of 1916–1925. Ninety-eight contemporary cows giving over 1,000 gallons of milk in one lactation had an average inbreeding coefficient of only 1.8%. Smith suggests that the explanation for the lower inbreeding of the high producers might be that one or more of the genes affecting milk production are sex-linked.

h. Aberdeen-Angus in the United States. Stonaker (197) took sample pedigrees of those born in 1900, 1910, 1920, 1930 and 1939. The *inter se* relationships and inbreeding he found in each sample were as follows:

	1900	1910	1920	1930	1939
F=	8.9	12.7	10.8	14.2	11.3
R=	9.4	16.3	12.2	6.1	13.3

The inbreeding expected from random mating and the existing *inter se* relationship is only 62% of that observed. One-tenth of the .3% increase in inbreeding per generation appears to be due to isolation between herds because of distance. Black Prince of Tillyfour was 24.1% related to the breed, *i.e.*, he was approximately a grandsire of the breed. Other high individual relationships to the breed were: Hanton 21.3% and Grey Breasted Jock 15.5%. More than 60% of the genes probably came through foundation animals in only five herds in Scotland. Of these the Hugh Watson herd was most important.

i. Telemark registered bulls born in Norway from 1898 to 1921 were studied by Berge (14). During that time the average inbreeding rose from less than one to over 7%.

j. Summary of Breed Studies. Lush (125) has reviewed these from the standpoint of how much they indicate gene frequency to change merely from chance in the Mendelian sampling which takes place when one generation replaces another. Most of the studies show that the pure breeds lose something of the order of 0.5% of their heterozygosis per generation from this cause alone. Selection may either enlarge or reduce that loss of heterozygosis while mutation surely makes the net loss a bit less. The standard deviation of the differences in gene frequency from one generation to the next, due to chance alone, seem likely to be of the order of .02 to .03 for genes with frequencies between .1 and .9, although somewhat smaller for genes which are near extinction or complete fixation. Chance is thus of considerable importance in determining the changes in

the composition of a breed from one generation to the next. The causes for the chance changes being so much larger than would be expected to result merely from the finite size of the breed are not wholly clarified, but presumably they center mostly around the fact that only a few contemporary animals are famous at any one time and a considerable fraction of the breeders make some efforts to head their herds with close relatives of those currently famous animals.

X. Crossbreeding and Grading

1. *Crossbreeding*

Mendelian analysis of character transmission was the primary objective of the crossbreeding experiments already mentioned as conducted at Maine, Wisconsin and Iowa. Much crossbreeding has been carried on without this as its purpose and deserves a brief separate consideration. Some of these, such as the Tranekjaer (227) and Bowlker (233) herds were later studied from the Mendelian viewpoint but many others did not receive such attention or were not planned so that such study would yield interpretable results.

Crossbreeding for purely commercial reasons has long been practiced somewhat irregularly in various regions. The blue-gray cattle produced in Britain by crossing white Shorthorns with Aberdeen-Angus or with Galloway are an example. Crossbreeding is widely used in Ireland for producing the feeder steers shipped to Britain for fattening. In the United States grading steadily to one breed has been the more usual policy among producers of beef cattle, but there has always been some occasional crossbreeding and a few cases of systematic crossbreeding, such as the policy of the widely known SMS ranch (near Stamford, Texas) of turning into its pastures 10 Shorthorn bulls for each 90 Hereford bulls to maintain a "10% undercurrent of Shorthorn blood."

The widespread use of some zebu blood in many tropical and subtropical regions has already been mentioned but that crossing is done specifically to secure tick resistance or some other aspect of adaptation to tropical conditions, rather than for crossbreeding *per se*. In at least one case, the Santa Gertrudis breed formed on the King ranch at Kingsville, Texas, the crossbreds have been interbred closely enough and long enough to form a new breed. In this case the ancestry is about 3/8 zebu and 5/8 Shorthorn.

Planned experiments to measure heterosis or to study the practical results of crossbreeding have been conducted at several places. Phillips *et al.* (158), in reporting such an experiment from the Range Livestock Experiment Station at Miles City, Montana, reviewed many of the earlier

experiments. In their own comparisons of 57 crossbred and 67 purebred steers they found heterosis in more rapid gains, higher dressing per cent, fewer digestive disorders and lower variability. They did not find significant differences in efficiency of gain, slaughter grade and carcass grade. They did not have comparisons with purebred steers of the other breed in the cross, the Shorthorns. Baker and Quesenberry (8) reported that, among the heifers in the same experiment, the crossbreds were heavier than the Herefords at birth, weaning, 18 months and 30 months of age, and had higher body scores as yearlings. An experiment intended to measure heterosis in Angus×Hereford crosses began at the Ohio Station in 1939. Aside from the report on gestation length (176) formal reports have not yet been issued.

Longwell (113) did not find hybrid vigor in crosses between Aberdeen-Angus and Shorthorns at the North Dakota station. However, Shaw and MacEwan (189) in crosses between Galloway, Shorthorn, Hereford and Aberdeen-Angus breeds did find that the crossbreds definitely excelled the purebreds in rate of gain and carcass quality. Fuller (60) at the Wisconsin station compared Holstein, Angus and crossbred calves in the feedlot. He found that the crossbred calves gained nearly as well as the Angus. They were appraised nearer the Angus than the Holstein. The carcasses of the crossbreds "closely resembled those of the Angus, although they were not quite as refined in the forequarters nor as smooth in covering." The crossbred carcasses came closer to the Angus at younger ages and when covered with only a moderate amount of fat than when older or fatter. In most respects the crosses were intermediate, although not exactly midway between the parent breeds.

2. *Grading*

Grading consists of using purebred sires on females of unknown or mixed breeding and continuing to use purebred sires from the same breed on the descendants. Several experiments have been conducted to demonstrate the value of the practice.

Perhaps the most widely known experiment of this kind on beef cattle is that conducted at Sni-a-Bar Farms in Jackson County, Missouri. A thirty-year plan of continuous grading with purebred Shorthorn bulls on a foundation of scrub cows was instigated in 1913. Burch *et al.* (28) summarized ten years' data from the Sni-a-Bar experiments in 1926. The most improvement in any one generation occurred in the first cross. Further improvement was realized in subsequent crosses, but improvement per generation became progressively less. After the third or fourth cross the graded up progeny differed from purebreds very little in conformation,

and only exceptionally good sires could produce further noticeable improvement.

Semple and Dvorachek (188) reported results similar to those of the Sni-a-Bar experiments. They had data on three crops of purebred Aberdeen-Angus calves, native Arkansas calves, first-cross (Angus×native) calves and second-cross calves. The graded calves were much more profitable than the native calves.

Among grading experiments with dairy cattle, that conducted at the Iowa station is probably most widely known. McCandlish *et al.* (136) in 1919 reported on this work which was begun in 1907. Purebred Guernsey, Jersey and Holstein-Friesian sires were used in continuously grading up a foundation of females originally selected as inferior in appearance and showing no evidence of any blood of the dairy breeds. The increase in average milk production of the second generation over the original scrub cows was approximately 130%. There was also a noticeable improvement in type. Weaver *et al.* (210) later reported that the third generation showed further improvement but that the fourth generation did not equal the third in production. This corresponds to the results obtained with beef cattle at Sni-a-Bar, most of the improvement having been realized by the third cross.

An experiment similar to the Iowa grading experiment was conducted at the South Dakota station and was reported by Olson and Biggar (148). Purebred bulls of the same breeds as those used in the Iowa experiment were used in continuous grading up of grade Shorthorn and Hereford cows. The first generation produced approximately 60% more milk than the foundation females, but the average of the second generation declined somewhat, being only about 50% higher than the average of the grade foundation cows. The improvement in dairy type was striking, fourth-generation individuals very closely resembling purebred dairy stock.

The evidence is almost unanimous that the bulk of the improvement from grading is realized in the first two or three generations. Some of this improvement is due to heterosis as well as to meritorious genes possessed by the purebred sires. After the first few generations the genotypes of the graded up stock approach those of purebreds and heterosis no longer occurs.

XI. Vitality

1. *Climatic Adaptation*

Much of the general evidence concerning this quality has been presented in discussing the results of crossing zebu and temperate-zone cattle. Ways of measuring differences between individual cattle in their comfort or discomfort under varying temperatures, humidities and other

environmental factors which are important in making the differences between climates, have been developed only recently. It has hardly been possible as yet to explore the genetics of these differences between individual cattle, but the new techniques promise to open the way for rapid progress in that direction. An idea of the methods now being tried can be had from the articles of Rhoad (171), Bonsma (22 and 23), Villares (206), Bisschop (17) and Riemerschmid (174). Some other examples of findings already published which seem to have genetic implications are the following.

Manresa *et al.* (133) found that the body temperature of Holstein-Friesian cattle was consistently higher than that of Indian Nellore cattle and F_1 hybrids between the two. The temperature averages from an eight-month study were: Holstein-Friesian, 39.5° C.; Nellore, 38.7° C.; F_1 hybrids, 38.7° C. High air temperatures seem to be the limiting factor in adaptation in the Philippines where this study was conducted. The increases in air temperature raise the body temperature and upset the normal physico-chemical balance of the blood as shown by studies of the blood of the two pure types and the hybrids. These changes have an adverse effect on the general constitution of unadapted animals.

Rhoad (169) and Bonsma (23) have studied the reflection of solar heat from the coats of cattle. In general, their studies reveal that radiation of solar heat is inversely proportional to darkness of coat color. Absorption of solar heat and the extent to which it increases the burden of heat disposal is an important factor in adaptability. Rhoad (171) showed in another study that respirations per minute and body temperature are influenced to a greater degree by high atmospheric temperatures in Aberdeen-Angus cattle than in Guzerat cattle. A febrile condition was produced in the Aberdeen-Angus cattle but not in the Guzerats when exposed to direct solar radiation, indicating a greater efficiency of the Guzerats in disposing of excess body heat. Rhoad's results indicate that this greater efficiency of heat disposal is inherited and shows partial dominance. The backcross of Angus-Guzerat hybrids to the Angus parent produces animals with considerably lower efficiency of heat disposal than the F_1s have. Rhoad states that the time spent in grazing during daylight hours is directly proportional to the efficiency of heat disposal.

Evidence that differences in climatic adaptation are partly genetic in origin is plentiful and is exemplified by that given in a report by Rhoad (170) in which a method of testing for genetic differences in adaptability is described. Four genetic types were tested. It was shown that the purebred and three-quarter Angus are not physiologically adapted to high temperatures and intense solar radiation and that the differences in adaptability are genetic in origin.

2. *Resistance to Diseases and to Insects*

Only a little is known definitely about this in cattle. For some diseases there certainly are breed differences in susceptibility, even when the cattle are kept in the same pastures. The best known of these is splenetic or Texas fever which is a very serious disease for European cattle but is such a mild disease with zebus that many cattlemen think the zebus and their high grades are immune. Careful examination, including temperature readings after inoculation, shows that the immunity is not complete, as some at least among them do have the disease although in such a light form that they scarcely seem sick. Reports such as that of Zhurawok (237) are samples of the definite findings on this topic.

Foot-and-mouth disease in South America seems superficially to affect the zebus a little less seriously than it does cattle of European origin (personal observations of the junior author) but the difference is slight and might be only a consequence of the greater ease with which they normally move, or of their being less handicapped by the other tropic conditions. Other general observations of this nature have been reported from South Africa, India and northern Australia.

Bonsma (24) reported that Africander cattle and their crosses with exotic breeds resisted "heartwater" (one of the Rickettsia diseases) much better than exotic breeds. The Africander is not regarded by its breeders as belonging to the zebu group, although some of its outward characteristics are similar and perhaps there was some ancestral connection centuries ago through introductions of zebu blood to Africa before white settlement in the south began. He implies that the difference may arise largely from differences in the number of ticks the different types of cattle harbor, such differences themselves being partly genetic.

Ehrlich (50) and Schäper (181) presented reasons for concluding that differences in susceptibility to tuberculosis are in part hereditary. The latter included also brucellosis and some forms of sterility. They concede, however, that the then current programs for maintaining and improving the health of the cattle population in Germany ignored this aspect of the matter.

White and Ibsen (214) reported a case which suggests genetic differences in susceptibility to mastitis. Ward (207a) presented extensive data from New Zealand dairy herds strongly suggesting that heredity plays an important role in predisposition to clinical mastitis. The evidence is primarily daughter-dam resemblance within herds.

Knowledge concerning individual resistance to harmful insects is even less. It is apparent, even on casual inspection of mixed herds in subtropical or tropical regions, that those with a high percentage of zebu blood usually have fewer flies, ticks, larvae of the nuche fly, *etc.*, but there is no complete

freedom from these. Conceivably the observed differences may be entirely secondary results of differences in length and thickness of hair, in skin secretions, *etc.* Villares (206) reported that the infestation of cattle with the cattle fever tick was 4.7% for zebu, 6.7% for native cattle and 88.5% for European cattle in São Paulo, Brazil. He found some differences between the various European breeds, and between the various races of zebus, and distinct differences between individuals within breeds.

Practically nothing is known of the detailed genetics of such breed and individual differences in resistance to diseases and to insects. That most individuals having as little as one fourth zebu blood have considerable resistance to splenetic fever argues for several genes and some degree of dominance or else that the zebus have a liberal "factor of safety" against this disease. Again, however, part or all of this could conceivably be due indirectly to other physical characteristics of the zebus and of their crosses which adapt them better to tropical or subtropical conditions, so that they are in better condition to overcome the disease when they get it. That is, the resistance, even when real, may not be due to genes which act directly and solely or primarily to produce that.

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Recent Advances in the Genetics of Paramecium and Euplotes

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CONTENTS

I. Introduction

1. The genetics of the ciliated Protozoa entered a new phase in 1937 with the discovery (127) of mating types in *Paramecium aurelia.* For the first time, it became possible to cross-breed genetically diverse stocks as easily as in higher organisms, and thus pursue genetic studies by means of Mendelian methods. The genetic advances that have followed from this discovery, in every species in which mating types are now known, form the subject of this review. Unfortunately, these advances have been limited to several species of two genera, Paramecium and Euplotes. Prior to the modern period, a great amount of genetic work had been done on many ciliated Protozoa, with results of much general interest, in spite of the severe limitation of the studies (with few exceptions) to inheritance during vegetative reproduction and to inheritance following matings between identical individuals. This early work (and some of the later work) has been critically reviewed by Jennings (66, 71). The newer work, however, places much of the older work in a new light. For this reason, a complete review is presented of the genetics of the one species, *Paramecium aurelia,* which has been extensively investigated by both the older and the

newer methods. The results on this species illustrate well nearly all of the genetic phenomena known in the earlier work on the Ciliates. Therefore, familiarity with the cytology and genetics of *P. aurelia* provides an excellent introduction to the cytogenetics of the ciliated Protozoa.

2. The work on each species will be presented in a separate section. Within each section, there will be given, in sequence, accounts of the mating types and breeding system, of the basic cytogenetic processes forming the indispensable groundwork for discussion of the genetics and, finally, of the genetic results themselves.

II. Paramecium aurelia

1. Mating Types and the Breeding System

3. Present knowledge of mating types and the breeding system in *P. aurelia* is contained in a series of papers (127, 129, 130, 131, 132, 134, 141, 153, 154) which appeared between 1937 and 1946. The following account is confined to the present status of the subject, without attempting to develop it historically. Before proceeding, a few terms must be explained.

4. The term "stock" is used to designate the progeny of a single individual, usually an individual isolated from a pond or stream. The initial isolated individual reproduces by repeated fissions. Among the progeny produced in this way, there occurs at relatively short intervals the process of autogamy (paragraph 49), which is a fertilization occurring within unpaired individuals. In most stocks, after one or more autogamies have taken place, there may occur repeatedly thereafter matings between paired individuals (conjugation and cytogamy, paragraphs 24–29). Hence, although fertilizations may take place among the members of a single stock, their supply of genes is confined to those present in the original individual and any mutations that may subsequently arise.

5. For genetic work, the unit culture, which normally consists of animals of identical genotype and phenotype, is called a clone. It consists of the progeny of a single individual produced by successive fissions, in the complete absence of all forms of fertilization processes. In some genetic work, as will appear, the unit culture must be further limited to individuals having macronuclei descended by divisions from a single original macronucleus. Such a unit culture is called a "caryonide." As two new independently formed macronuclei arise in each fertilized animal and these segregate to the products of the first fission, the clone descended from a fertilized animal includes two caryonides stemming from the two products of the first fission.

6. When cultures of different caryonides of the same stock are mixed together, two at a time, in all possible combinations and under appropriate

conditions, most stocks show at once that they consist of two classes of caryonides with respect to mating. When the mixture consists of one caryonide of each of the two classes, the animals immediately agglutinate into clumps from which they later emerge as conjugated pairs, each pair consisting of one member of each of the two caryonides. These pairs then undergo reciprocal fertilization and later separate. On the other hand, when two caryonides of the same class are mixed together, neither agglutination nor conjugation occurs. With rare exceptions (to be dealt with later), every caryonide of the stock is classifiable as belonging to the one class or the other. These two classes of caryonides within a stock are called mating types. As a rule, all individuals of any one caryonide are of the same mating type. No stock contains more than two mating types.

7. Among the many stocks of *P. aurelia* that have been examined, only a few show relations different from those set forth in the preceding paragraph. Each of these exceptional stocks is found to consist of one mating type only, all the caryonides in any one of these stocks being alike in this respect. Mating types are thus of universal occurrence in *P. aurelia*.

8. The number of different mating types in the species and their breeding relations are discovered by mixing together in all possible combinations of two (under appropriate conditions) the mating types found in different stocks. When this is done, it becomes apparent that the stocks may be classified into 7 groups, in such a way that all the stocks of the same group contain one or both of the same two mating types and interbreed freely, while different groups of stocks contain different pairs of mating types and either do not interbreed at all or manifest in one way or another potent bars to free exchange and later recombination of genes. These 7 sexually isolated groups of stocks are effectively genetic species, but practical considerations have led to designating them as varieties. The varieties are numbered 1 to 7. The mating types in the first six varieties are numbered I and II in variety 1, III and IV in variety 2, V and VI in variety 3, and so on. Only one stock has been found in variety 7 and this contains only the mating type XIII. Stocks that contain two mating types always contain the two that occur in one variety. The breeding relations among these mating types and varieties are summarized in Table I. As shown in the table, under optimal breeding conditions the two mating types of the same variety give nearly 100% immediate agglutination and later mating when cultures of them are mixed together. This maximal reaction is obtained regardless of whether the two mating types mixed are derived from the same stock or from different stocks of the same variety. This is the basis for the present ease with which crosses of diverse stocks can be made for genetic analysis. The table also shows that thus far it has been impossible to obtain mating reactions between mating

TABLE I

The System of Breeding Relations in P. aurelia

The numbers in the body of the table give the maximum percentage of conjugant pairs formed in mixtures of mating types in the corresponding row and file. The abbreviation "Inc" stands for the "incomplete mating reaction" that never leads to conjugation; the number preceding "Inc" gives the maximum percentage of animals that give this reaction at any one time in a mixture of the two types.

Group			A				B			
	Variety		1	3	5	7	2	4	6	
		Mating type	I II	V VI	IX X	XIII	III IV	VII VIII	XI XII	General Type
A	1	I	0 95	0 0	0 40	0	0 0	0 0	0 0	−
		II	0	1 0	40 0	10	0 0	0 0	0 0	+
	3	V		0 95	0 0	0	0 0	0 0	0 0	−
		VI		0	0 0	3 Inc.	0 0	0 0	0 0	+
	5	IX			0 95	0	0 0	0 0	0 0	−
		X			0	1 Inc.	0 0	0 0	0 0	+
	7	XIII				0	0 0	0 0	0 0	−
B	2	III					0 95	0 0	0 0	
		IV					0	0 0	0 0	
	4	VII						0 95	0 0	
		VIII						0	0 0	
	6	XI							0 95	
		XII							0	

types of diverse varieties except in six of the 72 possible combinations; and in only four of these six exceptions does the mating reaction lead to completed conjugation. Aside from these four exceptions, the diverse varieties of *P. aurelia* seem to be sexually isolated from each other.

9. The intervarietal mating reactions at present known are confined to four of the seven varieties. These four varieties (1, 3, 5 and 7), which show many genetic and other features in common, form a group, designated as group A. They are clearly more closely related to each other than any one of them is to any of the other three varieties (2, 4 and 6), which form another closely knit group, designated as group B. This division of the 7 known varieties into the two groups A and B is fundamental, as will appear, with relation to the genetics of *P. aurelia.*

10. Among the four varieties of group A, intervarietal mating reactions occur most intensely between varieties 1 and 5; in mixtures of mating type I with mating type X and in mixtures of mating type II with mating type IX, as many as 40% of the animals may conjugate. Lesser percentages of

intervarietal mating take place in mixtures of type II (variety 1) with type V (variety 3) and of type II with type XIII (variety 7). Extremely feeble incomplete mating reactions (temporary adhesions between animals never leading to conjugation) also occur between mating type XIII and mating type VI (variety 3) and between mating type XIII and mating type X (variety 5). Aside from providing intervarietal hybrids, intervarietal reactions are important in showing that the mating types of group A varieties are homologous from variety to variety. Types II, VI and X are similar in that they all react with type XIII; types I, V, IX and XIII are similar in that they all react with type II. In general, all intervarietal reactions are limited to combinations between an even and an odd type. Thus, in the varieties of group A, the mating types are divisible into two classes that differ in the same way that the two mating types in any one variety differ: types that belong to the same class cannot interact sexually or mate with each other; mating reactions and conjugation occur only between types belonging to different classes. This seems to mean that each class of mating types is itself a mating type of a more general sort. The even types may therefore be designated as plus types and the odd types as minus types. Each variety of group A thus has one plus and one minus type. Parallels to the breeding relations of the sexes in closely related species of higher organisms are obvious. The success of DeGaris (25) in crossing *P. aurelia* with *P. caudatum* (in which there are also varieties with paired mating types, as in *P. aurelia*) suggests that plus and minus mating types may be comparable even in different species of this genus.

11. Even in the few cases in which intervarietal mating does occur, the possibilities for recombining the genes of diverse varieties are narrowly limited for, in certain crosses the F_1 is highly non-viable, and in all intervarietal crosses the later generations are so non-viable that apparently only those combinations survive which are like the parental varieties themselves or like the F_1. Thus, even the closely related varieties of group A are to a high degree genetically isolated.

12. The seven varieties of *P. aurelia* all conform exactly to the taxonomic description of this species, yet careful examination has revealed constant differences among them in addition to the differences in the mating types they contain. Full description of these differences has not yet been published, but it has been stated (141) that each variety shows a unique combination of the following characters: size, fission rate, the conditions of temperature and light under which mating reactions may occur, minimum lethal temperature, length of the period between successive self-fertilizations, antigenic constitution (2, 3), and certain basic rules of inheritance (150).

13. Although the knowledge of mating types and the breeding system in *P. aurelia*, set forth in the preceding paragraphs, is entirely the result of relatively recent researches, there are observations in the earlier literature which now find their explanation in the new work. As pointed out first by Jennings (61, 62, 79), when races of *P. aurelia* differing markedly in size are grown together in the same container, they never interbreed: the large ones conjugate only with the large ones and the small ones only with the small ones. Moreover, he found that the conditions under which conjugation would occur were different for such different races, for usually in such a mixture only one of the two races would conjugate at any one time. These results and others like them (58, 152) are now readily intelligible: the different races probably belonged to different varieties, as evidenced by their size difference, the difference in conditions for conjugation and the fact that they could not interbreed.

2. Cytological Basis of the Genetic System

A. INTRODUCTION

14. While *Paramecium aurelia* is extraordinarily favorable for genetic work, partly because of the variety and nature of the nuclear processes it undergoes, it has thus far proved unfavorable for direct cytological study of the chromosomes and certain details of nuclear behavior. As in other organisms, however, knowledge of these matters has been acquired in part by genetic methods. In the present section the results of both methods will be considered. For each of the cytogenetic processes there will be presented first the direct cytological observations, then the genetic tests to resolve conflicting observations and to acquire information hitherto not obtainable by cytological means alone. The processes with which we shall have to deal are: binary fission, conjugation, cytogamy, endomixis, autogamy, hemixis and macronuclear regeneration. Before entering into an account of these, however, we shall discuss a cytological matter which underlies the whole cytogenetics of the organism, namely, the phenomenon of nuclear dimorphism encountered in all but the most primitive Ciliates and found in no other organisms.

B. NUCLEAR DIMORPHISM: MICRONUCLEUS AND MACRONUCLEUS

15. The two kinds of nuclei, macronuclei and micronuclei, that exist in every normal individual of *P. aurelia*, have a common origin (Fig. 2) during certain nuclear reorganization processes (conjugation, cytogamy, endomixis, autogamy). At one stage of each of these processes, a single nucleus (of micronuclear origin) undergoes two divisions and two of the resulting nuclei transform into micronuclei, two into macronuclei. The

micronuclei divide at every fission, maintaining regularly two micronuclei per animal. The two macronuclei, however, do not divide at the first fission, but are segregated so that each animal gets one. Thereafter the macronucleus also divides at every fission, so that there is regularly one per animal.

16. The two kinds of nuclei are very different in structure and in behavior. Micronuclei, in general, are like the nuclei of higher organisms, containing chromosomes and undergoing mitosis. The micronuclei of *P. aurelia* are very small (diameter 3 μ during interkinesis) and the chromosome details at mitosis are obscure. Current knowledge is based on the study of species with fewer and larger chromosomes. In the macronucleus, although it arises from a micronuclear derivative and becomes greatly enlarged, chromosomes are never seen. It seems to divide amitotically. These peculiarities of the macronucleus are illuminated by recent discoveries to be discussed later (paragraphs 54, 55).

17. The two kinds of nuclei have very different fates and potencies. The macronucleus is unable to produce micronuclei and regularly disintegrates and disappears at times of nuclear reorganization. The micronuclei, however, regularly produce new macronuclei at the time the old macronucleus disappears. The differentiation into macronuclei is thus an irreversible differentiation, but micronuclei retain the capacity to produce both kinds of nuclei.

18. To a certain extent, the diverse functions of the two kinds of nuclei are well known. In the absence of a macronucleus, animals cannot long survive or undergo more than one or two fissions. But without micronuclei, animals can reproduce for long periods and are even capable of mating (131). It is not yet clear whether their vitality is completely unimpaired by the loss of micronuclei, although in *P. aurelia* it seems clear that life without micronuclei would necessarily be limited to a few hundred fissions at most. This is a consequence of the fact that an amicronucleate animal is unable to replace its macronucleus by a new one; and it is known (84, 123, 164, 165) that the macronucleus must be renewed periodically in order to continue life indefinitely. From this sort of evidence, it is clear that the macronucleus is essential for life, while the micronucleus — at least for long periods — is not. It has been further supposed that the micronucleus is without direct effect on the characters of the organism, the macronucleus maintaining this control. This has recently been demonstrated (151) by arranging (see paragraph 55) to have different alleles in the two kinds of nuclei in the same animal and showing that the clone manifests characters corresponding to the macronuclear genes and independent of the micronuclear genes. The micronuclei thus serve, mainly or entirely, as germinal nuclei, taking part in fertilization processes and

giving rise to somatic nuclei (macronuclei), but having little or no direct effects on the organisms, while the macronuclei are the somatic and physiologically active nuclei.

19. This unique nuclear dimorphism raises questions important from a cytogenetic point of view. What determines the initial differentiation of the 4 products of one nucleus so that two become micronuclei, two macronuclei? The macronuclei, controlling the characters of the organisms and being derived from micronuclei that contain chromosomes and genes, must also contain chromosomes and genes; but what has become of the chromosomes and what is the genic constitution of the macronucleus? How can its apparent amitosis be accounted for? Finally, how can one of the two kinds of nuclei in every animal remain physiologically inactive and what are the genetic consequences of this inactivity? On some of these questions, recent work has provided information which will be presented later.

C. BINARY FISSION

20. Binary fission is the one and only process by which paramecia make more paramecia. It has been described for *P. aurelia* by R. Hertwig (55). The two micronuclei prior to division are about 3 μ in diameter and lie close to the macronucleus. Each consists of a thin membrane, a hollow achromatic sphere and a central chromatic core (Fig. 1a). In preparation for division, they enlarge into spindles about 7 μ in diameter. In the spindle appears a group of chromatic granules, presumably the chromosomes, so small as to be near the limit of resolving power (Fig. 1b, c). These granules form a metaphase plate (Fig. 1d). During late anaphase (Fig. 1e) and telophase (Fig. 1f, g, h), the spindle is enormously elongated, finally extending most of the length of the long axis of the body. The chromosomes in this stage are aggregated into two dense terminal knobs; between the knobs is a long middle piece which is characteristically swollen in the middle region. The terminal knobs break off (Fig. 1i) and reconstruct resting nuclei; the middle piece is rapidly resorbed. The two micronuclei divide simultaneously and one product of each goes to each daughter animal. Micronuclear division is almost completed before the animal as a whole begins to show transverse constriction. A remarkable feature of micronuclear division in all Ciliates is the failure of the nuclear membrane to break down at any stage in the division cycle. Although detailed chromosome studies are lacking, micronuclear division is certainly mitotic and precise. This is demonstrated cytologically by the succession of recognizable prophase, metaphase, anaphase and telophase figures in *P. aurelia* and by detailed chromosome studies on dividing micronuclei of other species. The same conclusion is required by genetic results: when many members

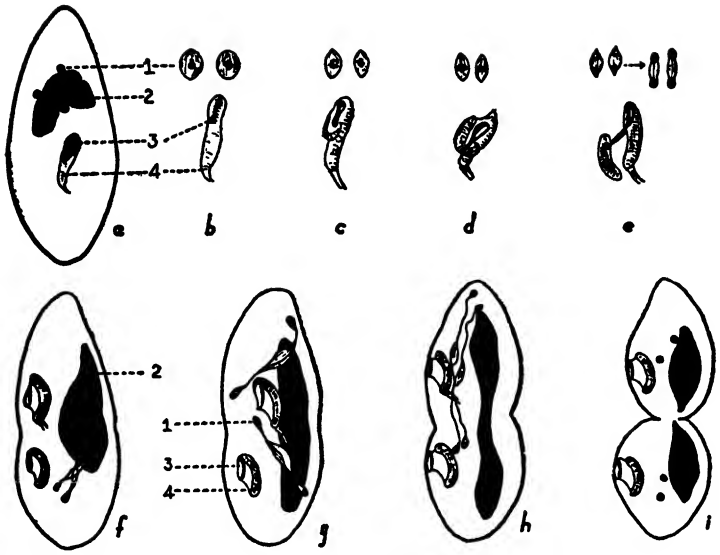

FIG. 1

Binary Fission in *P. aurelia*

Whole animals shown in a, f, g, h, i; only micronuclei, mouth and gullet shown in figures b, c, d, e. All figures redrawn after Hertwig (55), except a and i. Approximate magnifications: 250 diameters for figures a, f, g, h, i; 500 diameters for figures b, c, d, e. Symbols: 1, micronucleus; 2, macronucleus; 3, mouth; 4, gullet. a — resting animal; b — early micronuclear spindles, mouth almost closed; c — later prophase, mouth and gullet beginning to divide; d — metaphase, new gullet and mouth enlarging; e — early and late anaphase, new mouth and gullet about to separate from old ones; f — early telophase, mouths and gullets separated, macronucleus beginning to elongate; g — middle telophase, macronucleus more elongated; h — late telophase, body and macronucleus beginning to constrict; i — division of micronuclei and macronucleus complete, body nearly divided.

of a clone are subjected to tests for genotype by appropriate crosses, they are found (132, 145) to agree in genic constitution. This could occur only if the micronuclei underwent exact equational division at every fission, since the micronuclei are the only ones that function in crosses.

21. Hertwig reports that the macronucleus passes to the side of the body away from the mouth (Fig. 1f), elongates greatly and becomes narrower as it elongates (Fig. 1g), divides transversely (Fig. 1h, i) at about the time the parent animal is constricting into two, and then reverts to the vegetative form. Diller (29) remarks that the macronucleus undergoes complicated changes preparatory to division, but these have never been fully described. The macronuclear membrane (like the micronuclear membrane) remains intact throughout division. No part of the division figure

is cast into the cytoplasm in *P. aurelia*, though this has been reported in a number of other genera. In the absence of any recognizable chromosomes or mitotic stages, the division of the macronucleus is considered to be amitotic. On the other hand, genetic evidence requires that the division of the macronucleus be precise and effectively equal. Many investigators have confirmed the pioneer discovery of Jennings (60, 61) that the members of a clone are normally genetically identical. This fact, together with the evidence (paragraph 18) showing that the macronucleus controls the phenotype, requires the genic content of the products of macronuclear division to be normally as identical as the products of micronuclear division. Recent studies (paragraphs 54, 55) provide an explanation of how equal division takes place during what seems to be amitosis.

22. Except for Hertwig's account of the changes in the mouth and gullet, almost nothing has been reported for *P. aurelia* on the non-nuclear processes during fission. In brief, the mouth and gullet of the posterior daughter animal arise by being budded off from the preexisting mouth and gullet, the old structures passing to the anterior daughter animal (Fig. 1). Of the processes that occur in the remainder of the animal, nothing has been directly reported for *P. aurelia*, but these processes are probably essentially the same as in closely related species. Thus, the oral groove ordinarily disappears during fission and new oral grooves arise in each daughter animal; likewise the body form is radically altered (Fig. 1a, f-i) during fission, so that a complete remodelling is required to restore the characteristic form. Of the contractile vacuoles, one passes to each of the two daughter animals, a new anterior vacuole forming in the posterior daughter and a new posterior vacuole forming in the anterior daughter. It is suspected that the cilia may be resorbed and replaced by new ones, their basal granules probably undergoing division. Much is still unknown concerning the detailed processes involved in binary fission. Enough is known, however, to emphasize two things with direct bearing on genetics. First, some structures other than nuclei undergo division and so manifest direct genetic continuity. Second, parts of the daughter animals are entirely reformed at every fission, the previous counterparts of some of these disappearing. Reformation and redifferentiation of parts thus occur in binary fission just as in the sexual reproduction of higher organisms. Because of this, Jennings (60) insisted that the perpetuation, during prolonged reproduction by fission, of differences between clones kept under effectively the same conditions is an example of hereditary differences in the same sense as is the maintenance of differences in the sexual reproduction of higher organisms. He further showed that there is consequently no reason to expect the passive transmission of acquired characters

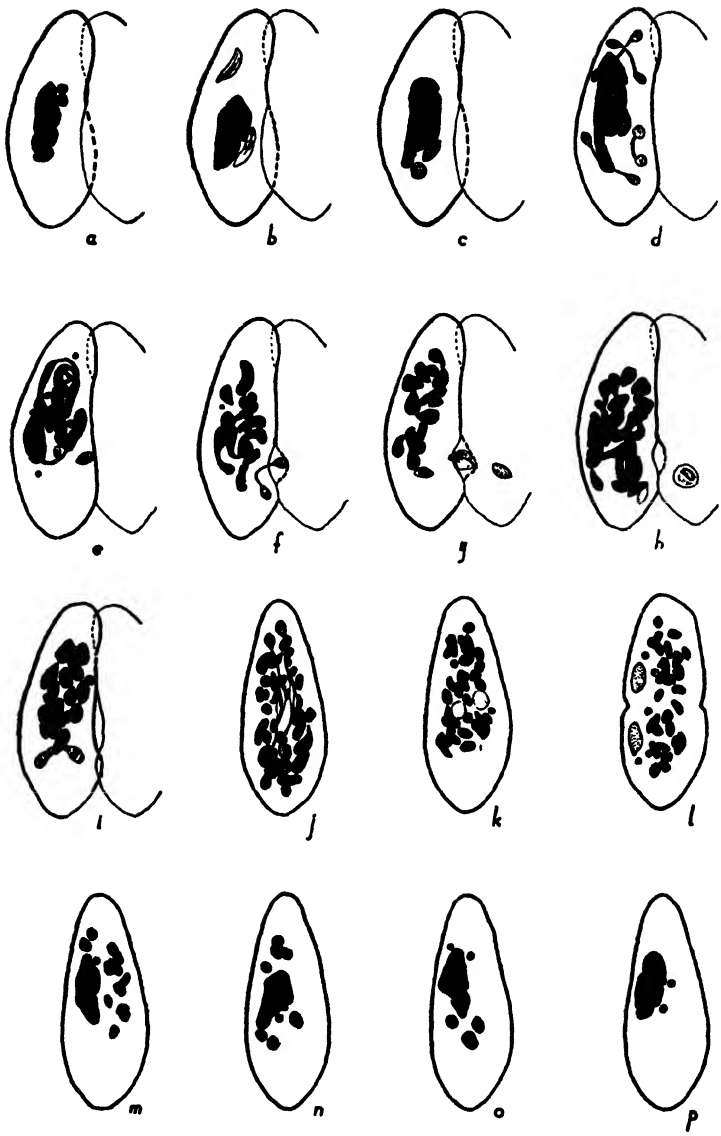

FIG. 2

Conjugation of *P. aurelia*

Figures a through j based on Hertwig (55). Figures a through i show mating pairs; j through p show exconjugants and early progeny. Nuclei shown in left conjugant only, except in two figures (g and h) in which the micronuclei are shown in both mates.

to both products of fission and that, in fact, such transmission does not ordinarily occur.

D. MATING: CONJUGATION AND CYTOGAMY

a. Cytological Observations

23. The nuclear and other processes during mating have been described by Maupas (106), R. Hertwig (55), and Diller (29). In most respects, these three accounts agree. The points on which they differ will be pointed out. We shall discuss in sequence the macronuclear changes, the micronuclear changes and certain other features of mating. As the two mates of each pair normally undergo the same developments at approximately the same time, an account of what takes place in one of them will suffice.

24. During the second half of the period of mating, the macronucleus unravels into a complex long skein (Fig. 2e) that develops constrictions (Fig. 2f, g, h) resulting in the formation of 20 to 40 separate pieces (Fig. 2k), Hertwig believed that a considerable increase in volume of macronuclear material occurs during the unraveling of the skein. The products of macronuclear disintegration are ultimately resorbed in the cytoplasm. According to Hertwig, this takes place in large measure before the first post-zygotic fission; but Diller states that it occurs only after several

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Otherwise, right conjugant indicated by part of outline only; it is to be understood that the same nuclear processes occur in both mates. Approximate magnification: 325 diameters. a — Shortly after union of mates: micronuclei enlarging. b — First maturation division: middle prophase, micronuclei in form of crescents. c — First maturation division: late prophase (polar view) in one micronucleus (about 35 chromosomes); metaphase (side view) in other micronucleus. d — Second maturation division: 4 spindles in telophase, macronucleus beginning to develop skein. e — Third maturation division: spindle near border next to mate; the other 7 micronuclei disintegrating; macronucleus in skein. f — Third maturation division: telophase, with one end in paroral cone; one disintegrating product of second division still visible. g — Gamete nuclei (products of third division): two in each conjugant; one from each conjugant in paroral cone. h — Syncaryon formation in each conjugant after exchange of migratory gamete nuclei. i — First postzygotic division: late anaphase; macronucleus fragmented. j — Second postzygotic division: two spindles extending nearly whole length of animal. k — Differentiation of the four products of the second division: two have become micronuclei and two are enlarged and developing into macronuclei. l — First fission after conjugation: the micronuclei have divided; the developing macronuclei do not divide; one of these and about half the fragments of the old macronucleus pass to each daughter animal. m — Animal after first fission: new macronucleus nearly fully developed. n — Animal after second fission: new macronucleus, now fully developed, has divided for first time. At this and later fissions, whatever fragments of the old macronucleus may be present are about equally segregated (without dividing) to each daughter animal. o — Animal after third fission: only 3 fragments of old macronucleus. p — Animal after fourth fission: no fragments of macronucleus remain.

fissions, during which the macronuclear fragments are segregated among the products of fission (Fig. 2l–p). This difference in reports is probably correlated with differences in nutritive condition (Sonneborn, unpublished). Under poor nutritive conditions, when growth and division are retarded or prevented, the pieces of the old nucleus are resorbed in part before fissions begin; under better nutritive conditions, resorption takes place later.

25. Micronuclear activity begins soon after the animals become joined in the normal mating position. The first micronuclear division is very complex and occupies about two-thirds of the time the mates are joined. The two original micronuclei grow from a diameter of 3 μ to a length of 20 μ or more, assuming the form of crescents or sickles with the chromatin parallel to the long axis near the concave edge (Fig. 2b). The crescents transform into sub-spherical spindles (Fig. 2c) and the chromosomes now appear as granules and short rods. This is the stage at which they are most readily observed and Diller has estimated that there are "in the vicinity of 30 or 40 chromosomes" at this time. Unpublished observations from this laboratory agree with Diller's estimate (Fig. 2c). Hertwig supposed there were 8 to 10 chromosomes in the diploid condition, 4 or 5 haploid; but this is certainly an error based on counts of spindle fibers, which, as Hertwig remarks, were probably groups of fibers with several chromatin granules (chromosomes) on each group of fibers. The spindle now rapidly completes division. Hertwig believed that each of the two anaphase groups contains the same number of chromosomes as the earlier metaphase group. The second micronuclear division (Fig. 2d) quickly follows the first without an intervening resting stage. These first two micronuclear divisions are usually considered to be the two meiotic divisions.

26. At this point there appear important discrepancies in the accounts of different observers. The generally accepted account is the one agreed upon by Maupas and Hertwig. They hold that 7 of the 8 nuclei disintegrate (Fig. 2e), the remaining one undergoing a third division (Fig. 2e, f) to form the two gamete nuclei (Fig. 2g). One of these, the migratory or "male" gamete nucleus, is located near the border where the mates are joined by a newly formed protrusion (called by Diller (29), the "paroral cone," Fig. 2g). The paroral cones of the two mates overlap and the migratory gamete nucleus of each mate passes into the other through the cones (Fig. 2h). Each migratory nucleus quickly unites (Fig. 2h) with the stationary ("female") gamete nucleus of the mate into which it has migrated, forming a fertilization nucleus or syncaryon and thus achieving reciprocal fertilization. From this account Diller dissents in several respects. In the first place, he holds that from 2 to 5 of the 8 nuclei resulting from the first two divisions begin to undergo a third division and that

usually two or more proceed to complete this third division. Of the 4 or more potential gamete nuclei thus formed, only two are functional, the others disintegrating. Diller is inclined to feel that the two functional gamete nuclei may sometimes be sister nuclei (the two products of division of a single nucleus) and sometimes non-sister nuclei (being derived from different nuclei). Further, he reports finding no evidence for the migration of gamete nuclei from one mate to the other during mating and he, therefore, suggests the possibility that the syncaryon is formed by the union of the two gamete nuclei produced in the same individual. According to this view, mating would involve not cross-fertilization (conjugation, *sensu strictu*), but double self-fertilization, or cytogamy, as this alternative is called by Wichterman (163).

27. All three observers agree that the fertilization nucleus normally divides twice (Fig. 2i, j, k) to form 4 nuclei. Before the second division is completed, usually before it begins, the mates separate. Two of these four nuclei transform into macronuclei, two into micronuclei (Fig. 2k). In a considerable proportion of the fertilized individuals of certain stocks, the number of nuclei formed from the syncaryon and the number that become macronuclei vary, up to 10 macronuclei forming in some exconjugants (131, 132). In the normal case, the spindles of the second postzygotic nuclear division are so arranged (Fig. 2j) that two of the resulting nuclei are located near each end of the animal. Maupas believed that the two in the anterior end of the animal become the macronuclei, but Hertwig maintains that it is seldom possible to recognize which two of the four nuclei transform into macronuclei before they migrate toward the center of the animal, when it is no longer possible to know which two came from the anterior region.

28. The normal macronuclear condition is restored after mating, according to Maupas and Diller, by the passage of one of the two new macronuclei, without fusion or division, to each of the two daughter animals formed at the first fission (Fig. 2, l). Hertwig, never observing this, believed the two macronuclei fused before the first cell division. Balbiani (1) had earlier reported that this does occur under starvation conditions in other species and I have made similar (unpublished) observations on *P. aurelia* supporting Balbiani's view. As Hertwig states his animals were not fed, this seems to account for his observations. Beginning with the second fission, the macronucleus divides at every fission (Fig. 2n–p). As to the micronuclei, all agree that normally both of them divide at every fission (Fig. 2l–p).

29. The question of the intimacy of union between the mates and the allied question of whether cytoplasm is exchanged along with the gamete nuclei remain to be considered. In agreement with his failure to observe

exchange of gamete nuclei between the mates, Diller was also unable to find evidence that the limiting membranes between the conjugants ever break down at any point during any stage of conjugation. Hertwig, on the other hand, states that a small cytoplasmic bridge develops between the mates at about the time of the third prezygotic nuclear division and persists until the first division of the syncaryon is completed. According to Hertwig, this bridge connects the two paroral cones and through this bridge the two migratory gamete nuclei pass during the process of nuclear exchange prior to fertilization. Hertwig further specifies that the bridge can only be demonstrated by certain special methods of observation, which he fully describes, the difficulty of observation being due to the fact that the position in which the mates ordinarily lie obscures the connecting bridge. On the important question of whether cytoplasm passes across this bridge, neither Maupas nor Hertwig makes any comment. The bridge could simply be a point at which the contiguous pellicles have fused, forming a solid bridge through which the nuclei can pass only by dissolving a path as they proceed and thus perhaps effectively barring exchange of cytoplasm. Recent observations (145, 146, 147, 149) show that in exceptional cases the bridge can serve as the path for exchange of cytoplasm (see paragraphs 42–44).

b. Genetic Observations

30. The preceding account of the observations of different investigators on the cytological processes involved in mating brings out a number of conflicting reports on matters of importance for genetics and is conspicuous for its absence of comment on other equally important matters. The following five questions, which are in these categories, have been answered by means of genetic experiments.

(1) Which of the three prezygotic micronuclear divisions are reductional and which equational? Among Ciliates in general, the first two prezygotic nuclear divisions have usually been considered the meiotic divisions during which reduction takes place, while the third division is usually considered an equational one. Cytological observations show that the first division is unique in many respects and it would seem likely that this is the heterotypic division. But some students (121, 168) have maintained that the third division is regularly, or at least sometimes, reductional.

(2) Are the two functional gamete nuclei, produced at the third prezygotic division, sister nuclei or may they arise from different products of the second division? Diller, it will be recalled, is inclined to answer this question differently from Maupas and Hertwig.

(3) Does mating involve conjugation (reciprocal fertilization) as

Maupas and Hertwig maintain, or does it involve cytogamy (double self-fertilization) as Diller implies?

(4) Is cytoplasm normally or only exceptionally exchanged between the two mates?

(5) · Is the macronucleus haploid, diploid or polyploid? Although the macronucleus arises from a diploid nucleus, it has been suggested that it may be haploid (127, 131) or polyploid (98). Direct evidence from chromosome counts has thus far been impossible to obtain, as chromosomes have never been recognized in the macronucleus.

It is inconvenient to present the genetic evidences on these five questions separately, for frequently the same experiment bears on several. Discussion of the pertinent genetic material will necessarily include an account of many of the normal genetic phenomena in *P. aurelia* and will thereby serve as essential background for later special discussions of general genetic problems.

31. The first approach to these problems by genetic techniques was made by Jennings (65) in one of the pioneer papers on Protozoan genetics. In point of view and in methods it was imitated with little or no improvement for 20 years. The point of view was this: in higher organisms, mating involves recombinations of chromosomes and genes and leads to the production of hereditarily diverse organisms; if the mating of paramecia leads to the same result, it is probably due to the same cause. The method of attack was to compare the variability among the members of a single stock produced in the absence of mating, with the variability of a group of clones descended from members of the same stock after mating; and to ascertain whether the diversities found within the two groups being compared are inherited (*i.e.*, perpetuated during vegetative reproduction). Jennings examined these matters on a large scale and came to three general results: (1) with respect to the characters examined, groups of exconjugant clones are regularly more variable than the parent stock from which they were derived; (2) the diversities found among the members of the parent stock are not hereditary, but those found among their descendant exconjugant clones are; (3) the two members of a pair of conjugants tend to produce similar exconjugant clones: if one differs from the parent stock, the other tends to differ in the same way. On the basis of these results, Jennings drew two conclusions. (1) In accordance with the point of view set forth above, hereditary diversities arising at conjugation are probably the result of nuclear recombinations similar to those occurring in the fertilization of higher organisms. (2) The tendency of a pair of conjugants to produce similar clones implies that they tend to have similarly recombined nuclei, a result that must be due to the formation of the fertilization nuclei of both conjugants of a pair from the fusion of one nucleus from each

mate. In other words, each exconjugant clone shows biparental inheritance: it derives its genetic constitution from both conjugant parents. In this first paper on the genetic consequences of mating in the ciliated Protozoa, Jennings thus answered one of the five questions raised in paragraph 30: during the mating of *P. aurelia*, there does occur reciprocal cross-fertilization.

32. The results of Jennings were quickly seized upon by Jollos (85) and subjected both to criticism and to further analysis in terms of chromosome behavior. The chief criticism of Jollos was that it is inadmissible to interpret hereditary diversities arising after mating as being due to nuclear recombinations when the mating takes place in a branch of a stock that has undergone repeated inbreedings, as had been the case in many of Jennings' experiments. Jennings (64) himself had worked out formulae for the rate at which homozygosis is achieved by successive inbreedings and was fully aware of this difficulty, which led him to remark that "Mendelian recombinations may not be the whole secret of the matter." Jollos, however, went further than Jennings, maintaining that these seemingly hereditary variations were either pathological phenomena of a special type (Dauermodifikationen) or mutations (89) induced by environmental conditions at a sensitive period after conjugation. On the other hand, Jollos did not exclude recombinations entirely. However, he maintained that they appeared only at the first inbreeding among the progeny of an individual brought in from nature. As Jennings' main and most critical experiment was apparently performed under just such conditions, the hereditary diversities appearing in this experiment were admitted by Jollos to be true recombinations; while those appearing after many inbreedings (up to 11 in some of Jennings' experiments) were held to be of the other kinds.

33. Jollos' analysis of Jennings' data on biparental inheritance has been more fruitful, having led to much further work. He pointed out that the production of similar clones by the two members of a pair of conjugants implies not only that the two mates acquire identical nuclei at fertilization, but, further, that the first two nuclear divisions are the reducing divisions, while the third division is equational. The reasoning here is illustrated by the diagram in Fig. 3. For a single pair of original heterozygotic genes, if the third division is equational, with reduction occurring at preceding divisions, one obtains identical heterozygotes in both mates of half the pairs, identical dominant homozygotes in both mates of ¼ of the pairs and identical recessive homozygotes in both mates of ¼ the pairs. If the third division is, on the other hand, reductional, one obtains identical heterozygotes in both mates of ½ the pairs, but diverse homozygotes in the two mates in the other ½ of the pairs. Thus, reduction at the third

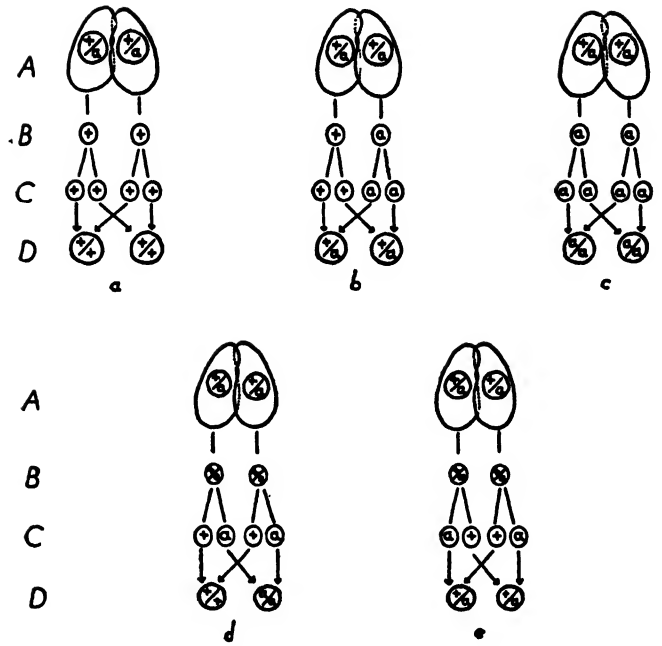

FIG. 3

Genetic Consequences of Alternative Chromosomal Behaviors at the Third Maturation Division of Conjugation

Cross of $+/a \times +/a$. The upper series of diagrams shows the three possibilities if the third division is equational, with reduction at the earlier maturation divisions. The lower series of diagrams shows the two possible alternatives if the third division is reductional, the earlier divisions equational. (On both alternatives, the gamete nuclei are assumed to arise from a single product of the second maturation division.) In both series of diagrams, row A shows the conjugants and their micronuclear genotypes at the start of conjugation; the remaining rows show the functional micronuclei only. Row B represents the single functional product of the second maturation division; row C represents the gamete nuclei; row D represents the syncaryons.

division yields diverse clones from the two members of ½ the pairs, but equational third division requires that the two members of a pair of conjugants always yield genically identical clones. The latter is moreover true, regardless of how many heterozygotic loci are involved in the original mating and regardless of whether the mates were genically alike or different before the mating. This analysis tacitly assumes further that the two functional gamete nuclei are sister nuclei arising from a single haploid nucleus, for without this assumption one cannot regularly obtain genic identity between mates.

34. The first three cytological questions under examination are thus answered if the two mates of a pair regularly yield genetically identical clones. However, Jennings' data showed merely that the two members of a pair of exconjugants "tended" to yield similar clones, as indicated by significant coefficients of correlation between them. The correlation was not perfect, indicating that the pairs of clones were not always alike. To account for the exceptions to biparental inheritance, Jollos held that there were three possibilities: (1) reduction in these cases might have taken place at the third prezygotic division (a possibility which Jollos considered unlikely); (2) determination of the differences in characters by cytoplasmic differences (implying therefore no exchange of cytoplasm between the mates); or (3) the occurrence of a mutation in one mate after the third nuclear division. In later papers (88, 89), Jollos maintained that probably both alternatives 2 and 3 occurred.

35. Nearly all of the work of the next 20 years confirmed the results of Jennings. The production of inherited diversities following conjugation within a stock has been found by almost all who have looked into the matter (80, 81, 83, 86, 88, 89, 118, 119, 120, 121, 155, 156). In only a few of the many experiments of Sonneborn and Lynch (155) did conjugation result in no appreciable increase in variation. It is surprising that this result was so seldom obtained in such highly inbred material, for most of the conjugations investigated took place after a number of successive inbreedings, when little or no heterozygosity might be expected in the stocks.

36. Regarding the question of similarity between the two clones from the two members of a conjugant pair, Raffel (118, 121) maintained his results did not confirm those of Jennings. He found no significant correlation in fission rate between the two clones from a pair of conjugants when he included records of all clones for the entire period covered by the experiment, entering as zero the record for every day after a line died. On the other hand, when he employed the more usual procedure of including only the fission rate records of those pairs in which both members survived throughout the course of the experiment, he obtained coefficients of correlation of .3 to .4. From these data, Raffel concluded that the third prezygotic division was, at least sometimes, reductional.

37. Thus, the earlier work, limited to the study of mating within a stock, served to focus attention on the cytological problems involved in the mating of *P. aurelia*, but was unable to provide a decisive solution for most of them. Even the first study on crossing diverse clones (156) left much to be desired. In this work, the two exconjugants from every viable hybrid pair always gave rise to two clones with identical characteristics, thus indicating (1) the occurrence of reciprocal cross-fertilization, (2) the

origin of the gamete nuclei of each conjugant from a single haploid nucleus, and (3) the equational nature of the third prezygotic division. However, only a small number of hybrid pairs was examined and the further genetic analysis indicated complex differences between the parent clones. Consequently, the generality of the results with the hybrids and the behavior of single pairs of alleles remained unproved. Definite resolution of the problems came only with the study of crosses involving single gene differences. With such material, it was found (132, 145) that, although the detailed rules of inheritance differ in the two groups of varieties (A and B, paragraph 9), the basic nuclear processes are the same in both. Inferences concerning the latter are founded on the mode of inheritance of a pair of characters in variety 1 of group A (132) and a pair of characters in variety 4 of group B (145).

38. The alternative characters investigated in variety 1 are diversities with respect to the mating types I and II. Some stocks are pure for mating type I, others contain both mating types I and II. In stocks of the latter sort, any clone — regardless of its mating type — can produce at uniparental nuclear reorganization some clones of each of the two mating types

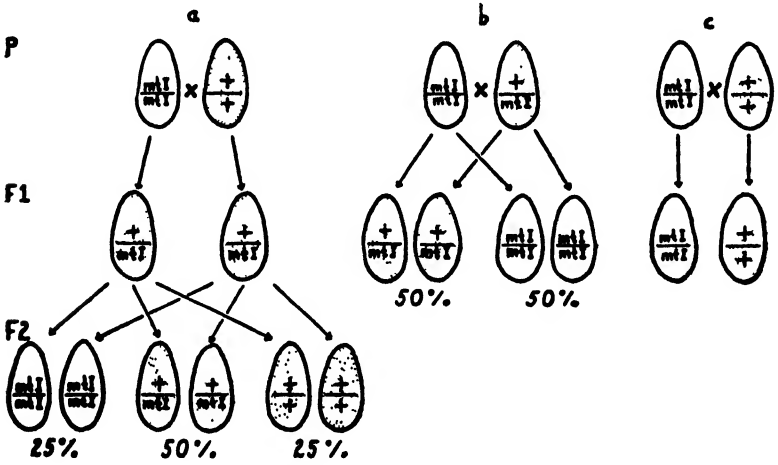

FIG. 4

Inheritance at Conjugation and Cytogamy in Variety 1 of *P. aurelia*

Two-type condition: phenotype represented by stippling; gene, by +. Pure type I condition: phenotype represented by absence of stippling: gene, by *mtI*. Cross of *mtI*/*mtI*×+/+. In column *a* are shown the results of conjugation in F_1 and F_2; the F_2 consists of 3 kinds of pairs of exconjugant clones in a 1:2:1 ratio. In column *b* are shown the results of backcrossing the F_1 to the pure type I parent; two kinds of pairs are produced in a 1:1 ratio. In column *c* are shown the results of the original mating if cytogamy occurs instead of conjugation: each mate produces a clone like itself.

(With the mode of inheritance of the two mating types in these "two-type" stocks, we shall deal later.) The difference between pure type I stocks and two-type stocks is determined by a single pair of chromosomes, presumably by a single pair of alleles, with the common two-type condition (+) dominant and the rare pure type I condition (*mtI*) recessive. The details of inheritance (Fig. 4) are simple and clear cut: the F_1 conjugants regularly show the dominant two-type condition in the clones obtained from both members of a mated pair and further breeding tests show that both clones of the pair are heterozygotic (+/*mtI*). The F_2 generation yields the expected ¾ dominants: ¼ recessives, the two members of each mated pair again agrecing in phenotype and genotype. The F_2 dominants consist of homozygous dominants (+/+) and heterozygotes (+/*mtI*) in the expected proportions. Backcrosses of the F_1 to the recessive pure type I parent (+/*mtI*×*mtI*/*mtI*) yields the expected 1:1 ratio and again the two members of a mated pair agree in phenotype and genotype. The same results, so far as the distribution of genes is concerned, were reported (145) for the inheritance of a difference between stocks of variety 4 in group B: here the cross was made between a stock homozygous for the dominant character killer (+/+), and a stock homozygous for the contrasting recessive character non-killer (*k*/*k*). (The phenotypic expression of the + gene depends also on a cytoplasmic factor; see paragraphs 42, 43.)

39. The preceding results demonstrate that mates exchange gamete nuclei and therefore undergo true conjugation, or reciprocal cross-fertilization. Further, the two members of a conjugating pair emerge from conjugation with the same genotype, regardless of whether they were alike or different before conjugation. As Fig. 3 shows, this can occur only if the third prezygotic nuclear division is equational, reduction taking place during the two earlier divisions; and the two functional gamete nuclei formed in each conjugant arise by the equational division of a single haploid nucleus. The scheme represented in Fig. 3a, b, c is therefore correct, and the transmission of genes at conjugation may be represented as in Fig. 5. The results also permit a partial answer to the fifth question raised in paragraph 30: the macronuclei cannot be haploid, for all heterozygotic clones (under appropriate conditions) manifest the dominant phenotype. As the macronucleus controls the phenotype (paragraph 18), the macronuclei must always contain the dominant allele although they have arisen from heterozygotic micronuclei. Therefore, the macronucleus could be haploid only if recessive genes were regularly eliminated from it in heterozygotes, and this is, of course, a practically impossible situation.

40. On the questions that have just been discussed, certain other phenomena were at first thought to bear, but these now appear to be of a totally different nature. In varieties of group A, when crosses are made

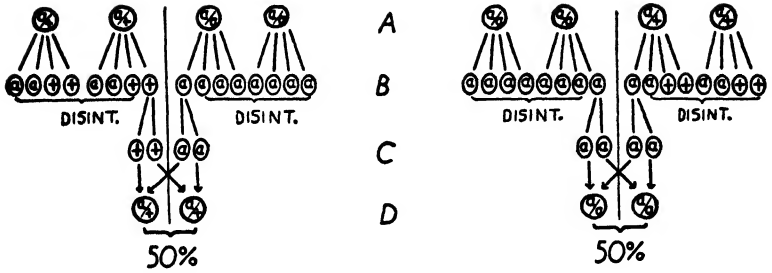

FIG. 5

Genic Recombinations at Conjugation in *P. aurelia*

Cross of $a/+ \times a/a$. The two possibilities, which occur with equal frequency, are shown in two diagrams, one to the left of the column A, B, C, D and one to the right. In each diagram, the vertical line separates the nuclei of one conjugant from those of the other conjugant of the pair. Row A represents the two nuclei in each conjugant, with the genic content at the start of conjugation. Row B represents the four reduced nuclei descended from each original nucleus, at the end of the two meiotic divisions; nuclei that disintegrate are marked "Disint." Row C represents the two gamete nuclei produced by the third division of one of the reduced nuclei in each conjugant. Row D shows the syncaryons formed by fusion of the gamete nuclei after exchange of the migratory gamete nuclei between the two conjugants of a pair. The syncaryons in both mates of half the conjugant pairs acquire the genotype $a/+$; the syncaryons in both mates of the remaining conjugant pairs acquire the genotype a/a.

between clones of different mating type and both clones belong to two-type stocks (paragraph 38), the two exconjugants sometimes produce clones of the same mating type, sometimes clones of different mating type, and sometimes one or both exconjugants produce a caryonide of each of the two mating types. Here, therefore, pairs of exconjugant clones frequently differ in phenotype. At first these results were interpreted (127, 131) as indicating that the third prezygotic division was reductional and the macronuclei haploid. However, the results in the other crosses discussed in the preceding paragraph seem to preclude that interpretation. Inheritance of mating type in two-type stocks is discussed further in paragraphs 79–84,

41. In the experiments discussed in paragraph 38, on the other hand there did occur exceptional pairs of conjugants in which the two mates did not agree in genotype after mating. The matter was further tested in a special set of experiments (137), using the genes *mtI* and + mentioned above. It was then found (Fig. 4c) that the exceptional pairs had not undergone reciprocal fertilization, but each mate had retained its own genes, receiving none from its partner. Such exceptional pairs apparently underwent double self-fertilization or cytogamy, the condition which Diller was inclined to consider normal on cytological grounds. These experiments showed, however, that the relative frequency of the two alternative pro-

cesses varied greatly with temperature. At a temperature of 17° C. during mating, conjugation occurred in about 95% of the pairs, cytogamy in about 5%; but as the temperature during conjugation was either increased or decreased, the frequency of cytogamy increased to 47% at 10° C. and 60% at 27° C. There was also some indication that the addition of sodium or calcium to the culture medium affected the relative frequency with which the two alternative processes occurred. The occurrence of cytogamy in addition to conjugation was also detected in the experiments with variety 4 (145), although here its possible dependence on environmental conditions was not examined. It thus appears that both reciprocal cross-fertilization (conjugation) and double self-fertilization (cytogamy) may occur during mating in varieties of both groups A and B and that due allowance and checks for this must be made in all genetic work with this material.

42. The question of exchange of cytoplasm during conjugation remains

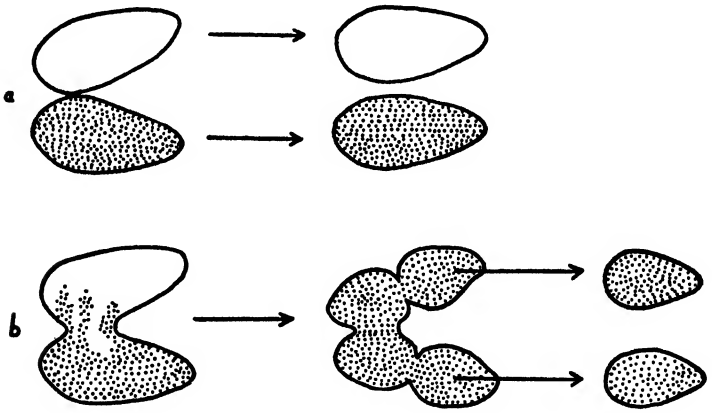

FIG. 6

The Behavior of Cytoplasm in the Conjugation of Variety 4 of *P. aurelia*

The normal behavior is shown in (a). At the end of conjugation, the conjugants remain joined last in the region of the paroral cone, but this connection is broken usually within one minute. Under these conditions, no cytoplasm is exchanged between the mates. The extreme abnormal behavior is shown in (b). The paroral cone regions fuse and cytoplasmic continuity develops, with exchange of cytoplasm. The region of fusion enlarges. At the first fission, normal single animals are cut off from the anterior ends; these contain cytoplasm from both parents. If the connecting strand shown in (a) persists more than 3½ minutes, cytoplasm may be exchanged even if the mates then separate. The two diagrams also illustrate the inheritance of the killer character, which is represented by stippling. In the cross of killer×sensitive, non-killer, each mate retains its character when cytoplasm is not exchanged (a); both mates produce killer clones if cytoplasm is exchanged (b).

to be considered. Work on varieties of group B indicates that cytoplasm is normally not exchanged during conjugation, at least not in effective amounts. This is shown by the fact that, although cross-fertilization occurs (paragraph 38), when diverse clones are crossed, the two clones from the two members of each conjugant pair are usually diverse, each clone resembling the parent from which it derived its cytoplasm. This is the ordinary result in crosses of mating types III and IV in variety 2, VII and VIII in variety 4, XI and XII in variety 6, as well as for all other characters in these varieties (145, 149). That the difference between the mates and their progeny is in fact due to a difference in cytoplasm and that cytoplasm may in exceptional cases be exchanged during conjugation was shown (147) by the following observations. Occasionally, conjugants in variety 4 are unable to separate after fertilization, but remain united by a thin strand in the region of the paroral cones. In some pairs, this fine strand later enlarges to form a broad connecting band through which cytoplasm is free to flow from one mate to the other. The first fission that takes place now cuts off normal single animals from each of the "parabiotic twins" (Fig. 6b) and when these two animals (derived from different mates) are separately cultivated they are found commonly to yield clones of the same mating type. This result is clearly correlated with exchange of cytoplasm in the exceptional pairs of conjugants and shows further that in the normal pairs of conjugants, from which clones diverse in mating type normally arise, there is no effective exchange of cytoplasm.

43. Results (145, 149) with the killer character in variety 4 are similar, but simpler, and the details of cytoplasmic exchange have been followed more fully. Normally (Fig. 6a) the cross of a killer to a non-killer yields a clone of killers from the killer parent, a clone of non-killers from the non-killer parent. When "parabiotic twins" are produced after conjugation (Fig. 6b) with visible interchange of cytoplasm, the single animals given off from both the killer and the non-killer twins produce clones of killers. The same result follows when "parabiotic twins" are not formed, if the thin connecting strand between the mates persists for as much as 30 minutes after the mates have separated elsewhere, and it may happen if this strand persists for as little as 4 minutes; but it never happens when the strand breaks in less than 3½ minutes. Thus, effective exchange of cytoplasm is correlated with persistence of the strand for more than 3½ minutes. In the great majority of conjugant pairs, it breaks in a shorter time, usually within a minute. Hence, cytoplasm is normally not exchanged in effective amounts at conjugation, but exceptionally it may be. (The relation of the cytoplasmic determination of the killer character to the genic determination is set forth in paragraphs 59–64).

44. The evidence just given for the transfer of cytoplasm at conjuga-

tion in cases in which the paroral strand persists abnormally long, and for the absence of such transfer when this strand dissolves at the usual time, is all derived from studies on the varieties of group B. No comparable phenomena have ever been reported for the varieties of group A. In them, there is as yet no evidence for cytoplasmic determination of characters and no data on the question of whether or not cytoplasm is exchanged at conjugation. Persistent paroral strands have not yet been searched for in that material. On the basis of results with variety 4 and other varieties of group B, it may be supposed that normally there is no exchange of cytoplasm in the varieties of group A also; but this must remain a surmise for the present.

45. To summarize, so far as the questions raised in paragraph 30 are concerned, the genetic work has shown: (1) meiosis and reduction occur regularly during the first two nuclear divisions of conjugation; (2) the third division is regularly equational; (3) the two functional gamete nuclei arise from the division of a single reduced nucleus; (4) both reciprocal cross-fertilization (conjugation, *sensu strictu*) and double self-fertilization (cytogamy) occur during mating, their relative frequency of occurrence varying with environmental conditions; (5) certainly in varieties of group B and possibly also in varieties of group A, exchange of cytoplasm at conjugation does not normally occur, but occurs as an exception when the paroral strand connecting the mates persists more than 3½ minutes after they have separated elsewhere; (6) the macronucleus is not a reduced haploid nucleus. In the course of establishing the preceding cytological facts, the genetic experiments have demonstrated the occurrence of simple Mendelian inheritance in varieties of both groups A and B. This operates so as to give the two members of each pair of conjugants identical genotypes, except when cytogamy occurs. In varieties of group B, however, the phenotypic results are complicated by the additional fact that the characters depend not only upon the genes, but also upon cytoplasmic factors. The system of interaction between genes and cytoplasmic factors will be dealt with in full in paragraphs 104–117.

E. UNIPARENTAL NUCLEAR REORGANIZATIONS: ENDOMIXIS, AUTOGAMY, HEMIXIS

46. The mating of two paramecia is such a striking phenomenon that it early commanded attention. The occurrence of similar internal cytological processes at other times, not being signalized by the conspicuous joining of two animals, was not even suspected until 1889, when R. Hertwig (55) briefly recorded important observations on it. It was not intensively studied until 1914, when Woodruff and Erdmann (168) described the periodic occurrence of endomixis, and was not finally brought to a head

until 1936, when Diller (29) published his remarkable study of autogamy and hemixis. Concerning the nature of the cytological processes occurring during uniparental nuclear reorganization, there is fundamental disagreement in the literature. As in the case of the less fundamental disagreements concerning the cytology of conjugation, genetic experiments have, at least in part, provided crucial tests for alternative interpretations. We shall present in sequence: (1) an account of endomixis, (2) an account of autogamy, (3) the genetic evidence bearing on these two processes, and (4) an account of hemixis.

47. Woodruff and Erdmann (168) reported that there occurred at intervals of about a month, in all stocks of *P. aurelia* examined, a process of nuclear reorganization which they called endomixis. Briefly, the essential features of this process are the following. The macronucleus disintegrates and is resorbed. After its disintegration is far advanced, the two micronuclei divide twice to form eight micronuclei. All but one or two of these eight disintegrate and disappear. If two persist, there is a fission without nuclear division; if only one persists, there is no fission. The single remaining nucleus divides twice to form four and, as in conjugation, two of these develop into new macronuclei and two remain micronuclei. The restoration to the normal vegetative condition takes place exactly as it does after conjugation.

48. Woodruff and Erdmann emphasize that endomixis differs from conjugation in the following ways. (1) The entire process takes place without the joining of two animals. (2) Instead of disintegrating by forming skeins and then small fragments, as a rule the macronucleus directly extrudes chromatin balls until only an empty membrane is left. Woodruff and Spencer (169) later reported that macronuclear disintegration by skein formation also occurs, but infrequently, at endomixis. (3) The micronuclear divisions are of the ordinary vegetative sort and are not the kind that occurs at conjugation. The typical meiotic enlargement of the nuclei, with the characteristic long crescent stage of the first meiotic division, are lacking. (4) The third prezygotic nuclear division, that gives rise to the two gamete nuclei at conjugation, is entirely lacking in endomixis. (5) Consequently, there is in endomixis no fusion of nuclei, no fertilization. Woodruff (167) has given an excellent review of the status of endomixis.

49. According to Diller (29), the cytological processes in unpaired animals are identical with those taking place in conjugation; he therefore employs the term autogamy instead of endomixis. (See Figs. 2 and 5 for nuclear and genic changes during conjugation. The same figures would represent autogamy if the processes in a single mate are followed, except that the syncaryon is formed by the union of the two gamete nuclei that

arise in one individual, instead of by the union of two from diverse individuals. For behavior of genes in autogamy, see Fig. 7.) Long before this, Hertwig (55) observed, in unpaired animals, meiotic phenomena and reconstruction of a new nuclear apparatus according to the conjugation scheme. His observations, however, were fragmentary and he did not find the critical fertilization stage, so he was inclined to consider the process a parthenogenesis. After the publication of Woodruff and Erdmann's paper, Hertwig reported (56) his observations in more detail and suggested that the process he found might be autogamy. Jollos (87) also reported that the micronuclei at uniparental nuclear reorganization underwent typical meiotic divisions and that the macronucleus disintegrated by skein formation, as in conjugation. I have made extensive cytological studies (unpublished) on many stocks and varieties of *P. aurelia* and have never been able to find critical evidence of endomixis, though autogamy has appeared in every race examined.

50. As soon as the first pair of genes was discovered in *P. aurelia*, they were used (132) as a tool to test the question of whether endomixis or autogamy was occurring. This pair of genes (*mtI* and +; paragraph 38) controls the difference between stocks of variety 1 pure for mating type I and stocks in which both mating types are producible (the two-type stocks). The two kinds of homozygous stocks were crossed and the heterozygous F_1 dominants were then induced to undergo uniparental nuclear reorganiza-

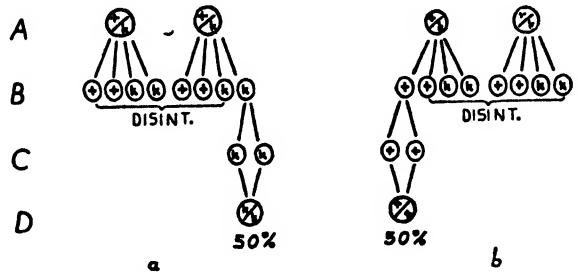

FIG. 7

Genic Recombinations in a Heterozygote (+/*k*) at Autogamy in *P. aurelia*

The two possibilities, which occur with equal frequency are shown in diagrams *a* and *b*. Row A represents the two original nuclei (in each animal) with their genes at the start of autogamy. Row B shows the 4 reduced nuclei produced by the two meiotic divisions from each original nucleus; nuclei that disintegrate are marked "Disint." Row C shows the two gamete nuclei produced at the third division from the surviving reduced nucleus. Row D shows the syncaryon formed by the fusion of the gamete nuclei. Diagrams *a* and *b* differ in the genic content of the surviving reduced nucleus (row B); as half the nuclei contain *k* and half contain +, each of these genes has a 50–50 chance of being in the surviving nucleus.

tion. The clones developed from reorganized individuals were tested for their phenotypes and genotypes. It was found that the progenitors of all the clones had undergone genic recombination: half of them were homozygous dominants and half homozygous recessives. As recombination of genes is impossible at endomixis with its absence of meiosis and fertilization, this demonstrated that endomixis could not have occurred in any of the reorganized individuals, for none of them retained the heterozygotic condition. On the other hand, the genetic results are those required if the reorganized animals underwent autogamy, involving the regular formation of gamete nuclei as sister haploid nuclei. Diller (29) was inclined to feel that sometimes the gamete nuclei were derived from different reduced nuclei, but the failure to find heterozygotes after autogamy shows that this either does not occur or occurs so rarely as to have escaped detection. A similar genetic study has also been reported (145) on variety 4, using the killer character as material (Fig. 7). Heterozygous ($+/k$) killers were induced to undergo uniparental nuclear reorganization and the resulting individuals were grown into clones and tested for phenotype and genotype. Again, half proved to be homozygous ($+/+$) killers and half homozygous (k/k) non-killers (sensitives). Thus, both in a variety of group A and in a variety of group B, the process of uniparental nuclear reorganization appears to be exclusively autogamy under the conditions employed.

51. The cytological observations of Hertwig, Jollos, Diller and Sonneborn and the genetic results of Sonneborn all agree in demonstrating the normal occurrence of autogamy. On the other hand, the question as to the existence of endomixis in *P. aurelia* may be fairly raised. There has as yet been neither cytological nor genetic confirmation of it. Two possibilities have been suggested (132, 167): (1) endomixis might occur in certain stocks, autogamy in others; (2) endomixis might occur under certain cultural conditions, autogamy under others. The first possibility now seems excluded, for both Diller and Sonneborn found only autogamy in cytological study of the main stock investigated by Woodruff and Erdmann. Moreover, Woodruff and Erdmann found only endomixis in all the stocks they examined, whereas only autogamy has been found in all the stocks investigated by others. The possibility that different cultural conditions call forth the two processes is more difficult to exclude. In attempts (133) to duplicate, in so far as possible, the conditions employed by Woodruff and Erdmann, the genetic results again indicated the exclusive occurrence of autogamy. While the possibility still remains that endomixis may occur under as yet unknown conditions, it seems clear that it does not occur under the conditions employed in all of the genetic studies with which we shall deal, except possibly those reported by Erdmann and by others working in the Yale laboratory. Therefore, we shall assume that ordinarily

the process of uniparental nuclear reorganization, which takes place periodically in *P. aurelia*, is autogamy.

52. The process of autogamy, in the form in which it is now known to occur, is of such surpassing importance in the genetics of *P. aurelia* that this aspect of it must here be emphasized. As has been pointed out (132, 139), the periodic occurrence of autogamy at relatively short intervals renders the homozygotic condition normal for this species. The fact that, at autogamy, fertilization regularly is accomplished (Fig. 7) by the fusion of two sister haploid nuclei, necessarily brings about homozygosis for all genes. The heterozygotic condition can be brought about only by crosses of diverse stocks or by mutation; it can persist, in the absence of further crosses and mutations, only until the first autogamy, which normally occurs within a few weeks or even sooner. Thus, although *P. aurelia* is a diploid organism, it is as favorable for genetic work as haploids, for recessive genes cannot long remain hidden. Finally, the regular occurrence of autogamy enormously simplifies genetic analysis. The simplest way to discover the genotype of a clone is to induce autogamy; each heterozygotic locus then segregates into the two homozygous classes in a 1:1 ratio. In modern genetic work on *P. aurelia*, therefore, autogamy rather than conjugation is the method of choice for genetic analysis following hybridization of diverse stocks. Finally, it has been pointed out (139) that the knowledge of autogamy explains and confirms the view of Jollos (85) that the hereditary variations appearing after conjugation within a single stock can be recombinations only when the conjugation is one of the first to occur after the stock has been brought in from nature.

53. In addition to autogamy, Diller (29) found a number of peculiarities in the behavior of the macronucleus during uniparental reproduction, but all of these were unaccompanied by any special activity of the micronuclei. He grouped these peculiar macronuclear activities together under the name "hemixis." The main feature of these processes is either the presence of two or more macronuclei (or major parts of macronuclei) instead of the usual single macronucleus, or the extrusion from the macronucleus of chromatin balls, or a combination of the two. In some hemictic animals, micronuclei are lacking. The diverse forms of hemixis need not concern us in detail. Their genetic significance, if any, is at present unknown, mainly because they are of sporadic occurrence and have not yet been brought under experimental control. The observations of DeLamater (27) indicate that experimental control may be achieved, for she observed that cultivation of *P. aurelia* on *B. coli* resulted in the occurrence of one of the forms of hemixis.

F. MACRONUCLEAR REGENERATION

54. Sonneborn (135, 136) discovered that animals of variety 1 can reorganize their nuclear apparatus, both after conjugation and after autogamy, by developing new macronuclei from the disintegrated (and normally destined to be resorbed) products of the old macronucleus, instead of from products of the syncaryon (Fig. 8, compare with Fig. 2). In this process, which was called macronuclear regeneration, the many (20 to 40 or more) pieces of the old macronucleus segregate in approximately equal numbers at each fission until there is only one per animal. Meanwhile, during these segregating divisions (Fig. 8, fissions 1 to 5), the pieces of the old macronucleus grow but do not divide. When there is only one piece per animal, the piece has regenerated to full macronuclear size and thereafter it divides at each fission (Fig. 8, fission 6 and thereafter). The same process was later found (149, 150) in variety 4 and probably occurs in all stocks of *P. aurelia*. Although it was stated that the process can be experi-

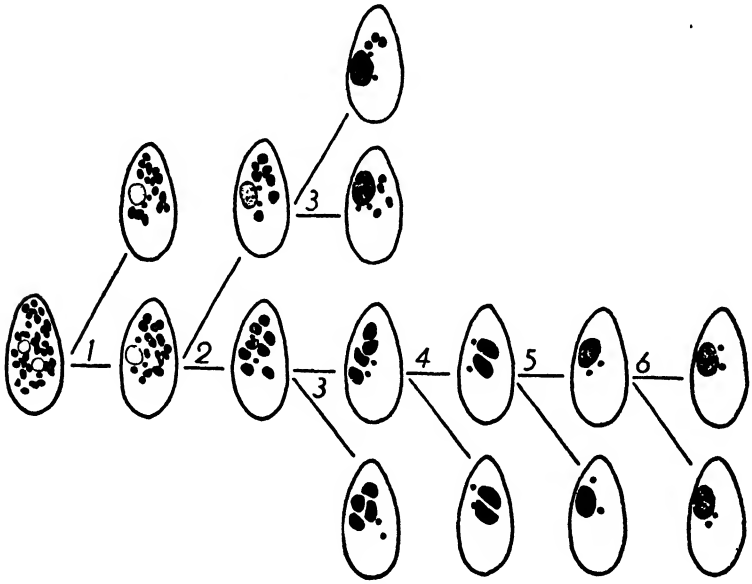

FIG. 8

Macronuclear Regeneration in *P. aurelia*

The animal on the left shows the nuclear condition before the first fission following fertilization (conjugation, cytogamy or autogamy): the solid black spheres represent the 30 or more pieces of the old disintegrated macronucleus; the two stippled circles represent the developing new macronuclei; the small circles containing a single dot represent the two micronuclei. The numbers represent successive fissions. For further explanation, see text, paragraph 54.

mentally induced, the method of inducing it has never been published. It consists of heating the organisms to 38° C. during the processes of fertilization and subsequent reconstruction of the nuclear apparatus. When exposed to this temperature during most of the period of meiosis and fertilization and up to the time of the first fission, the micronuclei are often aberrant, failing commonly to begin differentiation into macronuclei and often being lost entirely. However, when the exposure to 38° C. is limited to the period after fertilization, but starts before the second post-zygotic nuclear division (that is, before the division which normally results in the differentiation of the new macronuclei), the micronuclei remain normal and two of them as usual begin differentiating into macronuclei. These developing macronuclei, however, often have their growth arrested (Fig. 8, columns 2 and upper 3) after a short time and do not resume growth and development until after two or more fissions have been completed. Arrested macronuclei do not divide until their growth has been resumed (Fig. 8, after upper fission 3). Animals therefore arise (Fig. 8, lower animal after fission 2, and its progeny) which lack the new macronuclei, but, of course, contain many pieces of the old disintegrated macronucleus. The latter, when removed from the presence of the new macronuclei, proceed to regenerate into complete macronuclei in the way described above.

55. The process of macronuclear regeneration has both cytological and genetic significance. The fact that each piece of the old macronucleus can regenerate a complete macronucleus capable of controlling the normal functioning of the animal, even in the absence of micronuclei, shows that the macronucleus is a compound structure composed of many units of which each contains a full set of genes. It has been stated (98) that these observations show the macronucleus to be polyploid; on the contrary, they merely show that it is compound, consisting of many discrete nuclei which may be, and probably are, diploid. The gross amitotic division of the macronucleus is now understandable: it is merely a segregation of one group of discrete and genically complete subnuclei into two groups. The place to look for mitotic activity is in the individual component subnuclei and this has never been done. The genetic importance of macronuclear regeneration lies in the many uses to which it may be applied in experimental work. Some of these will be brought out in later sections. Here may be mentioned the possibility it provides for getting different genes into the micronucleus and macronucleus of the same animal. If homozygotes for diverse alleles are crossed, the syncaryons and both kinds of derived nuclei will normally be heterozygous; but, by inducing macronuclear regeneration at the time of the cross, the regenerated macronuclei remain genically the same as before the cross. Thus, the micronuclei are

heterozygotic, the regenerated macronuclei homozygotic. This was the method employed for demonstrating (paragraph 18) that micronuclear genes have no effect on the phenotype, and that the phenotype is controlled exclusively by the macronuclear genes.

3. Genetics

A. INTRODUCTION

56. In higher organisms all hereditary diversities are believed to be due to recombination of genes, chromosomal mutations, gene mutations and, in a few cases, cytoplasmic differences. Except for chromosomal mutations, which are as yet unknown, the same classes of genetic diversities occur also in *P. aurelia.* In addition, however, there are in this organism genetic diversities of several kinds showing features unlike those commonly recognized in higher organisms. These are the ones that make the genetics of this organism of special interest. One of the main purposes of this review is to see how far these unique features can be related to the general body of genetic knowledge and theory and to consider the extent to which new concepts are indicated. This is attempted in parts E*e* and F of this section. The main observations are set forth in parts B through E*d.* These observations are classified according to the magnitude of the genetic diversities, because different genetic phenomena appear in the different categories. Successive parts, therefore, deal with the inheritance of differences between varieties, stocks, clones within a stock, and lines of descent within a clone.

B. INHERITANCE OF VARIETAL DIFFERENCES

57. The seven known varieties of *P. aurelia* differ in many ways. Some of these diversities have been mentioned briefly in paragraph 12, but a full account of them has not yet been published. For present purposes, these varietal differences are of limited value for genetic work because the varieties either cannot be interbred or, if they can, the further analysis is prevented by other blocks to later recombination of the genes. Thus far, only one study has been made on the genetics of varietal differences. This (154) is on the inheritance of mating types in crosses between variety 1 and the other three varieties (3, 5 and 7) of group A; but the results have not yet been published.

C. INHERITANCE OF STOCK DIFFERENCES

58. Analysis of the basis of diversities between stocks of the same variety is one of the main contributions of recent work on *P. aurelia,* as this had never been feasible prior to the discovery of mating types. Thus

far, however, relatively few of these diversities have been analyzed. Only in variety 1, among the varieties of group A, has cross-breeding analysis been carried out, and for only a few character differences: antigenic differences (143) and mating type differences (132). For these, typical Mendelian inheritance has been reported. The details for the case of the alleles *mtI*, determining the pure type I condition, and +, determining the two-type condition, have been given in paragraph 38 and Fig. 4. So far as present knowledge goes, differences among the stocks of variety 1 are determined by genes showing ordinary Mendelian behavior.

59. Of the 3 varieties (2, 4 and 6) of group B, variety 6 cannot yet be subjected to crossing analysis because only one stock is now known in this variety. Many stock diversities are known, however, in varieties 2 and 4 and a number of these have been analyzed, some fully, others less so. There is one outstanding feature of crosses between stocks of variety 2 or 4: as a rule, the two members of each pair of conjugants produce clones showing the same diversity as the parents, each producing a clone of the same character as the stock from which it derived its cytoplasm (Fig. 6a). This result was obtained (132, 139, 145, 146, 147, 149, 150) for many stock differences: in variety 2, for mating types, two kinds of killers, and various grades of resistance to one of the killers; in variety 4, for mating types, a different kind of killer, antigenic differences and various grades of resistance to the killer. Two alternatives were apparent. Either cytogamy (double self-fertilization) regularly takes place during the mating of diverse stocks in this group of varieties or the stock differences are determined by differences of cytoplasm, instead of differences of genes. For a number of years, no method was available for distinguishing between these radically different interpretations, so the detailed data were not published.

60. Observations on exceptions to the rule finally made possible a choice between the alternative interpretations. Sometimes, one or both mates of a conjugant pair produced a clone with altered characters. This was observed in the inheritance of mating types, killers and antigens. As set forth in paragraphs 42, 43 (Fig. 6b), the exceptions were later found (145–149) to be due to exceptional exchange of cytoplasm between conjugants. In most stock crosses, it was clear that the diversities under examination were due entirely to cytoplasmic differences, no genic difference being involved; for example, genic differences controlling differences of mating type have never been detected in either variety 2 or variety 4. Even with respect to characters known to be determinable by genes, certain stocks proved to be genically alike, differing only in cytoplasm (146). Thus, stocks 51 and 47 of variety 4 are both homozygous for the killer gene, +, yet stock 51 is a killer and stock 47 is a sensitive, non-killer. When these two stocks are crossed, the genetic results depend entirely

upon whether effective amounts of cytoplasm are transferred during conjugation. When effectively no cytoplasm is transferred (as indicated by the rapid separation of the mates after fertilization), each conjugant produces a clone like itself: the killer produces a killer clone; the non-killer, a non-killer clone. When effective amounts of cytoplasm are exchanged (as indicated by prolonged paroral connection between the mates, paragraph 43), both exconjugants produce killer clones. If only these two stocks were known, the killer character would appear to be cytoplasmically determined and inherited independently of the genes, in agreement with the Plasmon concept.

61. The discovery (145) of a gene for the sensitive, non-killer character in stock 32 completely changed the interpretation and raised the question of whether other examples of Plasmon inheritance might not have a similar explanation. Stock 32 is homozygous for the non-killer gene (k/k) and crosses between this stock and the killer stock 51 ($+/+$) show that the genes are transmitted according to the typical simple Mendelian scheme (Figs. 4, 5; paragraph 38). The phenotypes, however, do not necessarily parallel the genotypes; in the absence of cytoplasmic transfer during conjugation, they follow the scheme represented in Fig. 9. The two clones produced from an F_1 pair of conjugants, although both are genotypically heterozygous ($+/k$), differ in phenotype: the killer conjugant produces a killer clone and the non-killer conjugant a non-killer clone. The non-killer

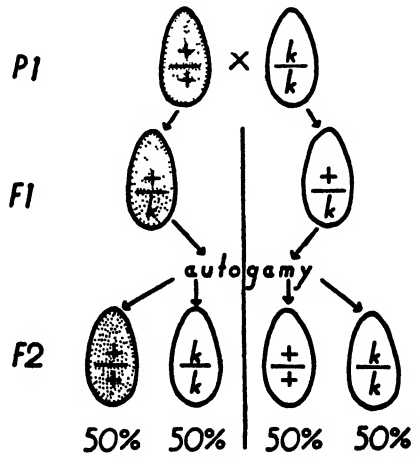

FIG. 9

Inheritance of the Killer Character in Variety 4 of *P. aurelia*

The killer character and the presence of kappa, the killer cytoplasmic factor, are represented by stippling; the non-killer, sensitive animals, lacking kappa, are unshaded. The vertical line separates the cytoplasmic descendants from the two parents.

F_1 produces exclusively non-killer progeny at autogamy, but breeding tests show that some are now homozygous for the killer gene (+/+), like stock 47 (paragraph 60), and some are homozygous for the non-killer gene (*k*/*k*). On the other hand, the killer F_1 clones produce two kinds of F_2 at autogamy: half are homozygous for the killer gene (+/+) and are killers, the other half are homozygous for the non-killer gene (*k*/*k*) and are non-killers.

62. These results show that there are two factors involved in determining the difference between killers and non-killers: one of these is a cytoplasmic factor for killer, called "kappa", the other is a pair of alleles, + for killer and *k* for non-killer. Both kappa and the gene + must be present for the killer character to appear; either the absence of kappa or the replacement of + by *k* results in the non-killer character. Stocks 51 and 47 differ only in the presence or absence of kappa; they are alike with respect to gene +. Stocks 51 and 32 differ with respect to both factors: 51 has kappa and the + gene; 32 lacks kappa and has the *k* gene. The results also show that kappa is not directly producible by gene +, for the non-killer stock 47 (+/+) lacks kappa, and introduction of + into stock 32 does not result in the production of kappa or the development of the killer character.

63. By means of exceptional pairs of conjugants in which cytoplasm is transferred (Fig. 6b), it was shown that any non-killer containing + in homozygous or heterozygous condition could produce a killer clone if it received effective amounts of cytoplasm (containing kappa) from a killer mate, but not if it were homozygous for the non-killer gene *k*. Thus, in the presence of gene +, kappa is maintained and increased, but not in the presence of homozygous *k*. The latter is further shown by reintroducing + into F_2 non-killers (*k*/*k*) derived by autogamy (Fig. 9) from F_1 killers (+/*k*). If the gene + is reintroduced quickly, within 5 fissions after +/*k* became *k*/*k*, the clone may maintain kappa and the killer character; but if + is reintroduced after more than 5 fissions, kappa is lost and the progeny are non-killers. Hence, kappa is not an independently self-multiplying plasmagene; its maintenance depends on the genotype.

64. This analysis of the determination and inheritance of the killer character thus reveals an intimate relation between genes and cytoplasmic factors. Its most important feature is that, although the cytoplasmic factors are dependent on genes for their maintenance and increase, the genes cannot accomplish this unless the cytoplasmic factors are already present. Therefore, cytoplasmic factors have been called primers (149). The gene + is like a pump that will not work without being primed; the cytoplasmic factor, kappa, is its primer. In the presence of kappa, + now works: it controls the production of more kappa. For further analysis of

the relations of cytoplasmic factor to gene, see paragraphs 104–126. If, as seems probable, the relations discovered for kappa and + are typical for the varieties of group B, then it may be concluded that the other stock differences known in this group, which appear to be exclusively determined by cytoplasmic factors, appear so only because allelic differences in the controlling genes have not yet been discovered. They are probably differences like the one between killer stock 51 and non-killer stock 47.

65. In sum, the stock differences in varieties of group A appear to be due entirely to genic differences; but stock differences in the varieties of group B are mainly due, not to genic differences, but to differences in cytoplasmic factors or primers under the control of genes which are identical in most stocks.

D. INHERITANCE OF DIFFERENCES WITHIN A STOCK

a. Introduction

66. By far the largest number of hereditary diversities reported in *P. aurelia* are diversities between different branches of the same stock. As yet, however, relatively little material of this sort has been subjected to thorough breeding analysis. Among the hereditary diversities within a stock, some are apparently permanent, persisting indefinitely through fertilization as well as through vegetative reproduction; others are impermanent, though long-lasting; others are cumulative, increasing with time. Among the permanent diversities, some are due to recombinations, others to mutations, and others to some unknown feature of macronuclear regeneration. Among the long-lasting but impermanent diversities are found two remarkable phenomena: caryonidal inheritance and Dauermodifikationen. Finally, the cumulative diversities are due either to inherent aging or to cumulative effects of suboptimal conditions. These varied phenomena will be dealt with in the following paragraphs.

b. Persistent Differences

67. (1) *Recombinations.* In the literature on *P. aurelia* there is not a single adequate demonstration that an animal collected in nature produced a heterozygotic clone from which recombinations were later obtainable. In view of present knowledge, however, this is not surprising for, as pointed out above (paragraph 52), the frequent occurrence of autogamy has the effect of narrowly limiting the persistence of the heterozygotic condition from the time of a mutation or a cross-breeding until the next autogamy — a matter of, at most, a few weeks. Although improbable, it is still theoretically possible to collect heterozygotes in nature and there is at least one instance in which this may have happened. Jollos (89) found

what he believed to be a recombination among the exconjugant clones produced at the first conjugation among the progeny of a wild individual. The recombination showed a higher resistance to As_2O_3 than the original stock and the new character was followed for a year. There was no reversion after conjugation and many autogamies. Attempts to extract similar clones from the parent stock at later conjugations failed. As expected, the lines selected to perpetuate the stock had presumably become homozygous. The necessary genetic details, however, are lacking: there was no cross-breeding analysis of the new type and the proportions in which the two characters appeared among the first group of exconjugants are unknown.

68. Many of the experiments on conjugation, from the earliest ones of Jennings (65) until the discovery of autogamy (28), implied or presumed that the hereditary variations arising after conjugation between members of the same stock were, at least in part, due to recombinations. In no case, however, was the genetic analysis required to demonstrate this performed; and the recombination interpretation now seems highly improbable for most of the cases, in view of knowledge of autogamy and the fact that the conjugations must have occurred after many autogamies and inbreedings, with repeated selections of single individuals to perpetuate the stocks.

69. (2) *Mutations.* Jollos (89, 90) and Raffel (120) have extensively discussed the occurrence of mutations within stocks of *P. aurelia*, and others (23, 24, 53, 107, 142, 150) have found what may have been single conspicuous mutations arising in previously known material. As with recombinations, not one of these presumed mutations has been subjected to adequate genetic analysis. In the main, this is due to limitations of knowledge and available techniques at the time the investigations were performed. For the same reason, even the discussions of mutations (particularly detailed in the paper of Raffel) are necessarily erroneous. There is consequently a need for the following restatement of the situation with respect to the detection of mutations, taking present knowledge into account.

70. Detection of micronuclear mutations is conditioned by three facts: (1) any particular new mutation will normally be confined to one of the two micronuclei in an individual; (2) the mutation will not come to phenotypic expression so long as it is confined to the micronucleus (paragraph 18); (3) conjugation cannot normally be obtained between two individuals of the same clone, carrying the same mutation, because the members of a clone are alike in mating type. Therefore, the simplest way to detect a micronuclear mutation is to bring about autogamy in a line of descent bearing it. Regardless of whether it is dominant or recessive, ¼ of the autogamous animals will produce clones homozygous for the mutation, because the gamete nuclei will arise from the mutated nucleus in ½ of

them and in ½ of these they will bear the mutated gene. The origin of a mutation in a previously heterozygous locus is practically too remote a possibility to consider, in view of the normally homozygous condition of the organism. Recessive micronuclear mutations will not ordinarily be brought to light by conjugation of two heterozygotes because of the improbability of having the same mutation arise independently in two different clones; and, to obtain conjugation among the progeny of an animal bearing a new mutation, autogamy must take place before the complementary mating type arises and makes conjugation possible. At this autogamy, the mutation is either lost or comes into homozygous condition and so appears phenotypically before the cross is made. On the other hand, a dominant micronuclear mutation will appear in heterozygous condition in ¼ of the hybrids between the heterozygous mutant clone and an unmutated clone, for the gamete nuclei of the mutant mate will arise from the mutated micronucleus in ½ the cases and in ½ of these they will bear the mutant dominant gene.

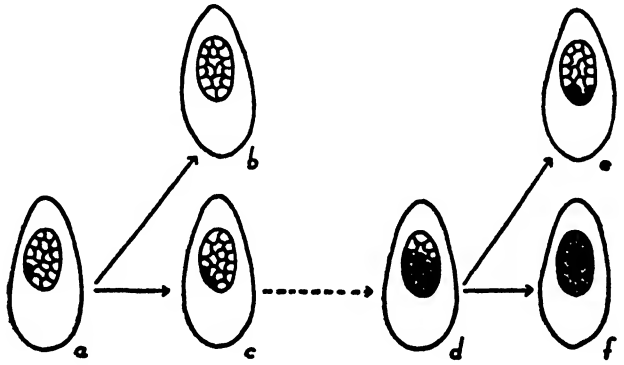

FIG. 10

P. aurelia

Diagrammatic representation of the macronuclear condition in vegetative descendants of an individual in which a macronuclear mutation occurs. The subdivisions of the macronuclei represent the component subnuclei of which the compound macronucleus is composed. In animal *a*, one subnucleus of the macronucleus is shown in black to indicate the presence in it of a mutation. At fission, each subnucleus divides into two, but by amitosis the 2 resulting mutated subnuclei pass to individual *c*, while the mutation is lost from individual *b*. A number of fissions intervene between animal *c* and animal *d*, in which the accumulating products of division of the mutated subnucleus now constitute a majority of the macronuclear subnuclei. Therefore, at the next fission, individual *f* receives a macronucleus with *all* of its subnuclei mutated, while *e* receives both mutated and unmutated subnuclei. Stable unmutated lines of descent thus arise from individuals like *b*, stable mutated lines from individuals like *f*, and unstable lines (producing occasionally both sorts of stable lines) arise from individuals like *a*, *c*, *d* and *e*. Micronuclei are omitted from the diagram.

71. The detection of macronuclear mutations is conditioned by (1) the compound structure of the macronucleus (paragraph 55), and (2) its amitotic division as a whole (paragraph 16). Recessive mutations in the macronucleus cannot be detected at all except in the unlikely case of mutation of the dominant allele at a heterozygous locus. In this case, as in the case of a dominant mutation, the change in an adult macronucleus will occur in only one component subnucleus of the compound macronucleus (Fig. 10). As a result of the duplication and amitotic segregation of the component subnuclei in the course of repeated fissions, some animals will arise with exclusively mutated component subnuclei in the macronucleus. (The earlier the mutation occurs, if it occurs at the time of macronuclear development from a micronucleus, the sooner entirely mutated macronuclei will segregate during fission.) In general, macronuclear mutations can appear only as altered lines segregating within a clone in no predictable order or proportions. However, three kinds of lines (Fig. 10) must arise within such a clone: (1) stable lines with entirely unmutated macronuclei; (2) stable lines with entirely mutated macronuclei; and (3) unstable lines capable of producing both kinds of stable lines.

72. Although no one has yet used the new knowledge and techniques for the investigation of mutations in *P. aurelia*, some of the results of the earlier work need to be reevaluated. Jollos (89) was the first to maintain that certain persistent variants in Paramecium were mutations. He further attempted to show that environmental conditions (high temperature and subjection to dilute solutions of As_2O_3) could induce mutations (in fission rate, minimum lethal temperature, resistence to arsenic) when applied during a sensitive period immediately following fertilization. He claimed mutations were produced in this way in two experiments out of more than 50 performed, recording one mutant in each of these two experiments. He states that no mutations occurred among controls, but gives no figures for the control experiments. The persistence of the altered characters for years and through many fertilizations indicated they were mutations, although neither cross-breeding analysis nor adequate information concerning the origin of the mutations is given. Induction of the mutations by the treatment is not clearly demonstrated.

73. Raffel (120) attempted to demonstrate (1) that mutations occur with high frequency in *P. aurelia*, (2) that practically all known variants in this species were mutations, the regular reversion of many of them being considered reverse mutations, and (3) that the commonly observed high mortality following conjugation and endomixis (autogamy) was due to rapidly accumulating lethal mutations in the micronuclei. Jollos (90) pointed out the formal, unconvincing and improbable nature of Raffel's arguments. As mentioned above, Raffel's assumptions and reasoning are

to a large extent invalidated by newer knowledge. Nevertheless, Raffel's contribution is valuable in emphasizing the need for an analysis of this sort and in pointing out the general approach to the problems involved in it. This work was unfortunate in having been done a number of years before the knowledge and techniques required for its satisfactory performance were available.

74. In addition to the extensive studies of Jollos and Raffel, a number of cases of apparently permanent alteration of a branch of a stock are recorded. Dawson (23, 24) found in a normal stock a notched and grooved variant that persisted during the 4 years he followed it through repeated autogamies and conjugation. Moore (107) reported the independent origin, in two branches of a normal stock, of lines in which the animals had a lump on the posterior oral surface; and this was said to persist through one endomixis (autogamy?). Sonneborn and Lynch (156) reported the origin of a line of reduced size and fission rate and attempted to discover by cross-breeding how it differed from the parent stock; but the results did not lend themselves to a simple and convincing genic interpretation. Sonneborn (142) found a single exconjugant (one member of a pair of conjugants, the other member of which remained unaltered) that produced a clone with modified morphology. Further, this clone was capable of giving the mating reaction with mating type II, but it was incapable of consummating conjugation. This "mutant type I" was followed for over a year, but could not be analyzed because of its inability to conjugate. Harrison and Fowler (53) report a number of antigenic variations arising within various stocks and persisting for up to 1½ years.

75. Finally, there is a large group of cases in which hereditary variations of many kinds appear commonly within a stock following either autogamy or conjugation. The genetic nature of these variants is by no means clear as yet. It was pointed out above (paragraph 68) that these variants were earlier thought by some to be ordinary recombinations but that this seems improbable in view of recent knowledge. Jollos (89) and Raffel (120) have suggested some or all of them may have been newly arisen mutations. Jollos (89) has also suggested many of them may be Dauermodifikationen. Although they have been found more often (5, 34, 65, 80, 81, 83, 89, 110, 111, 118, 120, 121, 139, 155, 156) and studied more extensively than any other sort of hereditary variation in *P. aurelia*, their nature still remains obscure. No one has yet undertaken to analyze them, taking advantage of the new knowledge and techniques now available. This is one of the great present needs.

76. (3) *Characters Associated with Macronuclear Regeneration.* In certain cases (135, 136), lines descended from individuals that have undergone macronuclear regeneration (paragraph 54) maintain indefinitely a

well-defined complex of characters. The animals are reduced in size and fission rate; they show high mortality at autogamy and conjugation, many animals being unable to form new macronuclei; the regenerated macronuclei may show peculiarities of structure during vegetative life and at subsequent nuclear reorganizations; the animals undergo repeated nuclear reorganizations at unusually brief intervals; and they show unusually intense mating reactions when mixed with animals of the opposite mating type. In those lines in which micronuclei are lacking, all subsequent successful nuclear reorganizations involve macronuclear regeneration and the whole set of characters persists indefinitely. Some lines of this sort have been followed over a year. This matter requires further investigation in varieties of both group A and group B (in which these phenomena may differ), before the basis for the character complex can be known. The possibility of a direct connection between absence of micronuclei and the character complex is not yet excluded; on the other hand, it is possible that the regenerated macronuclei are in some cases not fully normal.

77. Many of the characters associated with macronuclear regeneration have been described (5, 83, 119, 155, 156) in a number of variants reported before macronuclear regeneration was known and several of these variants persisted for periods up to 1½ years. Raffel (119) believed a mutation was involved. In his material, as in some of the others, reversion to normal continually took place and he presumed this was due to reverse mutations. In view of later knowledge, the reversions might have been due simply to later normal nuclear reorganizations, yielding normal new macronuclei from micronuclei instead of regenerated macronuclei. As macronuclear regeneration occurs occasionally, even in the absence of heat treatments, this possibility must be kept in mind in relation to the origin of variants showing characters of this sort. Experience with macronuclear regeneration shows how unsound it is to presume that variants are mutations (with the implication of genic mutations) simply because they persist for long periods. In the Protozoa, as elsewhere, breeding analysis is the only sound basis for such presumptions.

c. Long-Lasting, but Impermanent, Differences

78. (i) *Introduction.* One of the most characteristic and remarkable facts about the hereditary diversities reported in *P. aurelia* is that so large a proportion of them are transient. They often arise at one fertilization, persist during vegetative reproduction (even for very long periods), and then disappear at a later self-fertilization, sometimes at the very next one. The familiar hide and seek cropping out of recessives cannot explain these results, for the organisms would already have to be homozygous when the trait first appears; its later disappearance at self-fertilization could not,

therefore, be due to further recombinations. The genetic mechanisms involved in these cases are still obscure, but enough is known about them to exclude certain interpretations and indicate the general nature of the probable mechanisms. One case may be referred to as "caryonidal inheritance" and is illustrated by the inheritance of mating type in two-type stocks (paragraph 38). The distinctive feature of this sort of inheritance is that phenotypically diverse, but constant, caryonides may arise from a single fertilized animal. Another group of cases form the class of Dauermodifikationen, or temporarily inherited effects of environmental action, discovered and analyzed by Jollos.

79. (2) *Caryonidal Inheritance.* Caryonidal inheritance appears regularly in the inheritance of mating type in the two-type stocks of the varieties of group A (91, 127, 131, 132, 133, 138, 154); it also appears to some extent in varieties of group B (149) and in another case to be discussed later (paragraph 86). In the two-type stocks of group A, the main facts are as follows. (1) Normally all the animals in any one caryonide have the same mating type. (2) In any one stock, some caryonides are of one mating type and the remaining caryonides are of the other mating type of the same variety, *e.g.*, in variety 1, some are mating type I, others are mating type II; or, in variety 3, some are mating type V, others are mating type VI. (3) At fertilization, either autogamy or conjugation, the new caryonides that arise are redetermined at random as to mating type, independently of the mating type of the parent or of the sister caryonides. Thus, regardless of whether the parent caryonide was type I or type II, when autogamy occurs in a caryonide, the two caryonides produced from a single autogamous individual may be either both type I, or both type II or one of each type, and the frequencies of these three combinations are determined by the overall frequency with which the two types are produced. For example, if in one experiment 2/3 of the new autogamous caryonides are of type II, $(2/3)^2$ of the autogamous individuals produce both caryonides of type II, $(1/3)^2$ produce both of type I and $2(2/3)(1/3)$ produce one caryonide of each type. In precisely the same way, chance determines the frequencies of the combinations found among the four caryonides produced from a pair of conjugants (two caryonides from each exconjugant). (4) The overall frequency of the two types of caryonides produced at fertilization varies from experiment to experiment, even in different experiments on the same caryonide. In general, the frequency of the "plus" mating types (types II, VI and X in varieties 1, 3 and 5, respectively) increases with increase of temperature during a sensitive stage of the nuclear processes associated with fertilization. The random combination of mating types in the two caryonides from an autogamous individual and among

the four caryonides from a pair of conjugants holds, whatever may be the overall frequencies of the two mating types.

80. Although attempts to discover the mechanism of mating type determination in these two-type stocks have not as yet led to a completely satisfactory explanation, two important conclusions are warranted by the available evidence. The first of these is that there is no directly or indirectly determining genic difference between the micronuclei in caryonides differing in mating types within two-type stocks of one variety. For this, there are the following evidences. (1) The two caryonides from a single fertilized animal, as pointed out above, frequently differ in mating type; yet their micronuclei are derived from the same syncaryon by mitotic divisions. (2) Micronuclei are known (paragraph 52) to become entirely homozygous at autogamy, making genic recombination at subsequent autogamies impossible. Nevertheless, any caryonide arising at one autogamy produces some caryonides of each mating type at every subsequent autogamy. (3) Repeated selection of the same mating type at successive autogamies can neither establish a line that remains pure for mating type through autogamy nor lessen the frequency with which the other mating type arises at subsequent autogamies under the same conditions. These three lines of evidence seem to preclude the possibility of micronuclear genic differences correlated with differences of mating type within two-type stocks.

81. For the second conclusion, namely, that the differences of mating type are determined by macronuclear differences, there are the following evidences. (1) In general (paragraph 18), it is known that the macronuclei control the phenotype, the micronuclei having no direct phenotypic effect. (2) Specifically, mating types are manifested even in amicronucleate clones (131). (3) Mating type normally remains constant within a caryonide, *i.e.*, among individuals having macronuclei descended by division from the same ancestral macronucleus. This results both in the usual uniformity of caryonides and in the maintenance of mating type through macronuclear regeneration, when new micronuclear combinations may be formed but the old macronuclear constitution persists. (4) Mating type changes only when new macronuclei arise from micronuclei, at autogamy, cytogamy and conjugation. (5) Mating type segregates at fission only when independently formed macronuclei segregate. This normally occurs at the first fission after fertilization, when the two newly produced macronuclei segregate. In some stocks, however, a considerable proportion of the fertilized animals form 3 to 10 new macronuclei instead of just the normal two, and in these stocks there is a corresponding frequency of segregation of mating type at the second or third fission (131). These five lines of evidence agree in showing that mating type inheritance in two-type stocks is determined by the macronuclei.

82. The evidences presented in the two preceding paragraphs raise a difficult question that goes to the heart of the problem of the genetic mechanism involved in this type of inheritance. How can diverse macronuclei arise from identical micronuclei? To this question no positive answer can yet be given. In the early efforts to answer this question, various hypotheses were suggested (127, 131, 132); but they have all been shown to be untenable as a result of later discoveries (29, 91, 131, 132, 133) concerning autogamy, macronuclear constitution and the ratios in which the mating types arise at fertilization. There seems to remain but one possibility (138). A nuclear change (a mutation?) must take place in the very process of macronuclear development from the micronucleus. The nature of this change, however, remains unknown and, as breeding methods for testing macronuclear constitution are apparently impossible, methods for testing directly the nature of the macronuclear changes have not yet been forthcoming.

83. Whatever the mechanism may be by which the diverse macronuclei arise from identical micronuclei, it is in some unknown way affected by the environmental conditions. For, as mentioned above (paragraph 79), increase of temperature during the nuclear processes associated with fertilization increases the proportion of caryonides with plus mating types. In variety 1, the percentage of caryonides of mating type II is $18.4+(t/0.51)$ where t is the temperature in degrees Centigrade (138). This relation holds through the range 10°-35° C., yielding from 38% to 88% caryonides of type II. The attempts thus far made (132) to delimit the temperature-sensitive period indicate merely that it is close to the time at which the conjugants separate. It may be the time of the second post-zygotic nuclear division, which takes place usually within ½ hour after the mates separate; this is the nuclear division which gives rise to the prospective new macronuclei. If macronuclear differentiation involves a mutation, as suggested above, the mutation frequency is proportional to temperature, directly if the mutated condition determines type II, inversely if it determines type I.

84. A remarkable feature of the temperature work on variety 3 is that the ordinary caryonidal mode of inheritance seems to disappear at very low temperatures (132). When the processes of mating and subsequent nuclear reconstruction both take place at 10° C., the exconjugants show but little change of mating type: the type V parent produces two caryonides that are mainly or exclusively type V and the type VI parent produces two caryonides that are mainly or exclusively type VI. Many of the caryonides show some selfing, indicating the presence of animals of both mating types, though the predominant type is regularly the same as that of the cytoplasmic parent. This exceptional result in this variety of group A is similar to what happens normally in the inheritance of mating type

at conjugation in the varieties of group B. As has been pointed out (paragraph 42), mating type in group B is determined by cytoplasmic factors. Further, when small amounts of a mating type cytoplasmic factor are transferred at conjugation, the two caryonides from a single fertilized animal may differ in mating type. These resemblances between the phenomena in the two diverse groups of varieties, under exceptional conditions, may lead eventually to an elucidation of the mechanism by which effectively unlike macronuclei arise from identical micronuclei in the two-type stocks of the varieties of group A.

85. (3) *Dauermodifikationen.* The concept of "Dauermodifikationen" was developed by Jollos (85, 86, 88, 89, 90) on the basis of studies on Paramecium and was later extended by him and others to other Protozoa and to other organisms, including Drosophila. Hämmerling (51) has reviewed the whole field. The work on *P. aurelia* illustrates well the sort of results obtained in the Protozoa. By the term Dauermodifikationen, Jollos means temporarily inherited alterations producible by environmental means, the degree of effect or the duration of its persistence often being increased by repeated or long-lasting environmental treatment. The effects persist for hundreds or even thousands of fissions, but sooner or later they invariably disappear, the disappearance being hastened by changes of environment and by fertilization. The physical basis of Dauermodifikationen is presumed to be in the cytoplasm or in the macronucleus or in both. The reversion of Dauermodifikationen is not a matter of reverse mutation because it occurs invariably in all lines of descent and in each independently at nearly the same time. Reversion may take place progressively in a series of steps. Many or all of the hereditary diversities in Paramecium that eventually revert to normal were considered by Jollos to be Dauermodifikationen.

86. The Dauermodifikation Jollos found most complex and difficult to explain was obtained by subjecting his stock *h* to a temperature of 31° C. for three years. This resulted in the following changes of character: the fission rate was increased to 50% above normal, the minimum lethal dose of As_2O_3 was decreased nearly 20%, and the minimum lethal temperature was increased from 35° to 37° C. The new characters persisted during vegetative reproduction when the animals were cultivated at room temperature, but disappeared in some lines after conjugation or autogamy. The lines in which they persisted through one fertilization lost them at the second or third fertilization. Peculiarly, some of the lines which reverted at one fertilization reacquired the Dauermodifikation at a later fertilization without further treatment; but those that reacquired it lost it again at the next fertilization. From these results, Jollos concluded that the high temperature had altered the cytoplasm so as to make it determine

the observed effects, and that the modified cytoplasm was reproduced for a long time, but eventually reverted to the normal condition. The fact that reversions occurred only after fertilization Jollos attributed to an effect of the altered cytoplasm on the new macronuclei formed at such times: when the cytoplasm was in the modified condition, it caused the newly formed macronuclei to develop in such a way as to determine thereafter the altered characters; when the cytoplasm lost its modification, the new macronuclei formed in the reverted cytoplasm lacked the capacity to control the modified characters; when the cytoplasm was about to lose its modification, it might fail to affect new macronuclei formed in it at this time, but affect the ones formed at a slightly later time (thus accounting for the reappearance of the effects in apparently reverted lines).

87. These results of Jollos are, however, subject to a very different interpretation in view of newer knowledge. Examination of Jollos' data shows that the mode of inheritance of the modified and normal conditions is essentially the same as the mode of inheritance of the two mating types in the two-type stocks of the varieties of group A (paragraphs 79–83). In the first place both the modified and the normal lines produced at fertilization the two kinds of progeny in the same proportions. Jollos followed individually 66 caryonides, of which 48 were normal and 18 showed the modification; that is, 72.5% of all caryonides were normal. Of these 66 caryonides, 49 were derived at fertilization from modified lines and 37 of the 49 had the normal characters; 12 remained modified. Of the 17 caryonides derived at fertilization from normal (reverted) lines, 11 were normal, 6 modified. The proportions are practically the same in the two cases: 72.5% of 49 is 35.5; 72.5% of 17 is 12.3. Other parallels between the two cases are: (1) inheritance is constant within the caryonide; (2) the two caryonides from the same fertilized animal may be diverse.

88. The only apparent difference between the two cases is Jollos' statement that the altered lines all eventually reverted to the normal condition after a few fertilizations. However, the complete transformation of all lines of type I into type II would also be obtained if the lines were selected in the way Jollos did. From each line he obtained only one or a few autogamous progeny and when they reverted to normal they were usually discarded. He thus selected his material in such a way as to keep for study mainly those which had not changed, and he followed so few lines of descent from each that, by chance, he was bound to get all of the more frequent type in a large proportion of the cases. Under such conditions, he would have to find all the lines reverting in a few autogamies. The parallel to the inheritance of mating type in two-type stocks of varieties of group A is so remarkable that a similar mechanism in the two cases can scarcely be doubted.

89. There remains the question of how to account for the difference between the so-called Dauermodified branch and the parent stock from which it was derived. The parent stock did not yield these two kinds of caryonides; the heat-treated line did. The difference between the original stock and the heat-treated branch is comparable to the difference between stocks pure for mating type I and two-type stocks. The latter difference is known (paragraph 38) to be due to a genic difference; the former difference may likewise have had a similar genic basis. This suggests that during the three years of cultivation at 31° C., there may have occurred a mutation which would readily have been selected by reason of its observed higher fission rate and its observed better adaptation to higher temperatures. It is even possible — and some of Jollos' observations indicate this — that, during the three years of selection at high temperature, more than one mutation was selected. In four of the 66 caryonides observed, two of the altered characters were independently segregated, *i.e.*, one of them changed in a particular caryonide while the other did not. These by no means improbable possibilities would then fully account for Jollos' results and remove this case from the category of Dauermodifikationen.

90. The preceding case, however, is in a class by itself among the studies of Jollos. It is the only one that shows the mating type mode of inheritance and it is the only one in which the interpretation of selected mutations is readily applied. There are two other examples reported for *P. aurelia* which seem to be of a different sort. One of these deals with changes of fission rate induced by subjecting the animals of race *h* to $CaCl_2$ or $Ca(NO_3)_2$. After subjecting the organisms for 3 months to Ca^{++}, the fission rate remained low for 2 months in Ca-free medium; after 6 to 18 months of subjection to the Ca^{++}, the fission rate remained low for 6 to 8 months; but in all cultures in Ca-free medium, the fission rate eventually returned to normal. Prior to reversion, 7 successive autogamies took place. Jollos reports he was able to hasten the reversion by frequent changes of culture method and by allowing conjugation to recur, the Dauermodifikation reverting to normal after, at most, two conjugations. The final loss of the Dauermodifikation took place sometimes in the midst of an interautogamous interval. Jollos therefore concluded that the basis of the Dauermodifikation lay in the cytoplasm: it could disappear in the course of fissions alone and it could persist through autogamy and fertilization. Why conjugation should be more effective than autogamy in causing reversion — indeed, why either should cause reversion of cytoplasmic modifications — is not at all clear.

91. Jollos' other case of a Dauermodifikation in *P. aurelia* involves the use of specific antisera against race *h* (89). The antiserum immobilized the paramecia and then killed them, when applied in dilutions of 1:100

to 1:500. By subjecting the paramecia to gradually increasing concentrations of antiserum and selecting the most resistant animals to perpetuate the line, he was able to isolate lines that could resist the antiserum in concentrations of 1:25 to 1:40. The acquired resistance lasted from a few weeks to 2 months during vegetative reproduction, but was regularly lost at the first occurrence of autogamy. In one experiment, he isolated a line resistant to 1:25 concentration of antiserum after only one exposure for 24 hours to a 1:100 concentration; previously, this line was affected by 1:500. This change behaved exactly like the others.

92. Among recent investigations there are two that provide evidence on the production of antiserum-resistant Dauermodifikationen. Sonneborn (143, 144) attempted to obtain antiserum-resistant paramecia by selecting resistant animals after subjection to antiserum. He found that diverse stocks showed very different relations. In some stocks, all clones and all animals were uniformly susceptible to the antiserum and selection in these stocks was without effect. In other stocks, however, certain clones consisted of two sorts of animals: some were susceptible, others resistant. When a number of animals of such a clone (stock 60, variety 1) were isolated and allowed to divide into two, and one product of the fission of each animal was tested with antiserum, about 80% were found to be susceptible, 20% resistant. If now the untested sister animals were allowed to reproduce for a day or two and the progeny tested with antiserum, all of the cultures — both those from the sisters of the susceptible and those from the sisters of the resistant animals — were found to react alike: the great majority of the animals in every culture were affected by the antiserum. The resistance was, therefore, transient and not inherited. On the other hand, if animals subjected to antiserum, and found to be resistant, were removed from the antiserum and cultivated, they were found to yield clones resistant to the antiserum: exposure to antiserum thus makes otherwise temporarily resistant animals capable of producing resistant clones.

93. In these clones, the resistance persists for variable periods during purely vegetative reproduction: in some lines of a clone it disappeared within a week or so, after 25 to 30 fissions; but in others it persisted for longer periods, up to 75 days (more than 279 fissions). In the latter, autogamy was completely avoided by methods previously devised (128). As has been shown (123), under such conditions, lines gradually weaken and die. That is why lines in which resistance persisted could be followed no longer than about 300 fissions in the absence of autogamy. Attempts to analyze the genetic basis of the acquired resistance led always to the same result: in crosses of resistant×susceptible, resistant×reverted, resistant× resistant, and even after autogamy in resistant lines, *all* the progeny promptly lost their acquired resistance. The resistance, capable of main-

tenance for hundreds of fissions, thus disappeared invariably at the first fertilization. Moreover, reversion often occurred (both at fertilization and during vegetative reproduction) even while the organisms were being cultivated in dilute antiserum; hence reversion is not due to the action of serum-free medium. In a few cases, however, some caryonides that arose under these conditions (fertilization and cultivation in dilute antiserum) retained resistance, even when removed to serum-free medium. Presumably these were the few new caryonides that included transiently resistant animals capable of being transformed into enduringly resistant lines by exposure to antiserum. This fact and the failure of attempts to select serum-resistant lines in the absence of exposure to antiserum show that the modification must have been a consequence of the treatment. The fact that it invariably disappeared at the first fertilization (in the absence of additional treatment) shows that it is not a normal property of the stock and could, therefore, hardly be expected to appear by selection alone. The results, therefore, confirm and extend those of Jollos; they agree with his conception of "Dauermodifikationen." But the most important question remains unanswered: what is the physical basis of the Dauermodifikation? On the answer to this question probably depends the answer to two other questions. (1) How does it happen that some lines of a caryonide retain the modification for nearly 300 fissions, while other lines of the same caryonide may lose it in 25 to 30 fissions? (2) Why is it that exposure to antiserum is effective in causing Dauermodifikationen only when transiently resistant animals are exposed?

94. The second recent work bearing on the question of serum-resistant Dauermodifikationen is that of Harrison and Fowler (53). Bernheimer and Harrison (2, 3) had previously observed that subjection to antisera resulted in immobilization of some, but not of other individuals in certain stocks. Harrison and Fowler chose such stocks for special and very extensive study. They practiced selection by exposing a sample of the stock to the antiserum and isolating extremely resistant and extremely susceptible individuals. These selected animals were then allowed to reproduce and their progeny were subjected to similar treatment and similar selection. Of 43 such experiments on many different stocks, involving up to 15 successive selections of resistant animals, 13 gave cultures with no change of resistance, and 30 gave more resistant cultures. In the successful experiments, 4 or more selections were usually required before resistance increased; but in 8 experiments, resistance increased after a single selection. In some of the experiments, resistance increased progressively with repeated exposures and selections; in others, there was no change of resistance in the early selections, then at one selection a fully resistant clone developed. In addition to these experiments, there were 4 others in which the animals

selected for resistance gave cultures that were more sensitive than the original. Finally, in one stock, resistant lines were isolated without exposure to antiserum. The reverse type of selection proved more difficult, as animals selected for sensitivity frequently died after exposure to the antiserum; but 35 such experiments yielded viable progeny. In 24 of these, in which only one selection was made, the resulting culture showed no change in sensitivity. In 11 that were repeatedly treated and selected, only two showed alteration: one showed increasing sensitivity up to a certain stable level; the other showed no change until the third selection, when it became markedly less, not more, sensitive. The authors proceeded to find out whether these variations were inherited and, if so, how long. For this study, they chose 10 lines in which selection had been effective. These were grown in mass cultures in serum-free medium and repeatedly tested for titer during a period of 17 months. Two of the cultures reverted to the original condition in less than 3 months, two reverted between 7 and 14 months, and six showed relatively little change in 15 to 17 months. Some of the experiments were repeated with similar results. In some experiments, resistant cultures grown in dilute antiserum also lost their resistance. The authors made a few efforts to discover whether change of antigenic constitution was correlated with the occurrence of autogamy. There was some indication that autogamy occurred several days before variants appeared in a mass culture.

95. Although their primary interest was in other than genetic problems and their data are therefore not presented in a form suitable for critical genetic evaluation, Harrison and Fowler's results are full of genetic interest. They conclude that exposure to antiserum merely selected changes that occurred spontaneously and independently of exposure to antiserum. This conclusion is supported by many of their observations, particularly by their success in isolating variants of one stock without exposure to antiserum. Their very diverse results with different materials, however, indicate that more than one genetic mechanism may be involved. In some of the material, selection was ineffective and its effect in other material was not necessarily in the direction of selection, in agreement with their conclusion of the spontaneous, non-induced nature of the changes. On the other hand, the variations must have been of diverse kinds, because the period through which the variations persisted in mass cultures varied greatly from no persistence at all to persistence through the entire 17-month observation. These facts indicate that some of the variants might well have been genuine mutations and causally unrelated to the treatment with antiserum. On the other hand, the variants, mass cultures of which reverted in from "less than 3 months" (how much less is not indicated) to 14 months, were clearly of another kind or kinds. Some of these seem

to be like the antiserum Dauermodifikationen described in paragraphs 92–93. The lead of Harrison and Fowler needs to be followed by others with genetic interests, using genetic methods, before the full significance of their remarkably varied results is known.

96. The present status of Dauermodifikationen in *P. aurelia* appears to be as follows. One of the three main series of experiments presented by Jollos in support of the concept of Dauermodifikationen in this species seems to be a case of selection of probably spontaneous mutations. His other two series of experiments do not seem to be subject to this or any other obvious fatal criticism. Further, his essential observations in one of these series (the one dealing with antiserum resistance) have been confirmed by Sonneborn and probably also by some of the results of Harrison and Fowler, although the latter authors also reported cases that may be interpreted as selected spontaneous mutations. With respect to observations of a superficially quite different kind, Jollos (85–90) was inclined to interpret, partly as Dauermodifikationen and partly as mutations, the hereditary diversities arising at fertilization (conjugation or autogamy) in highly inbred, homozygous cultures (*e.g.*, those reported by Jennings (65), some of Caldwell's (5) and many others of like nature). He believed (88) that the sensitive period for mutation induction, just after the separation of conjugated mates, probably occurred also at the corresponding stage of autogamy; and that Dauermodifikationen were much more readily induced at this sensitive period than mutations. Jollos' sensitive period coincides in time with the temperature-sensitive period for mating-type determination in the two-type stocks of group A varieties (paragraph 83), and in both cases action on the macronucleus may be involved. This connection suggests that, as knowledge of macronuclear cytogenetics develops, the physical basis and mechanisms involved in so-called Dauermodifikationen may yet be demonstrated. Until that occurs, however, and particularly in view of the recurrent spectre of selected spontaneous mutations, alternative interpretations of cases presumed to be Dauermodifikationen will generally be sought by geneticists.

97. (4) *"Life cycle" changes.* It has been shown (45, 84, 113, 123, 164, 165) that, with the passage of fissions following fertilization, the members of a clone undergo progressive changes; the fission rate declines, the readiness with which autogamy may be induced at first rapidly increases and then slowly decreases, the ability to survive autogamy decreases, the organisms later become incapable of undergoing autogamy, and finally they die. This series of changes has been held by some to be the expression of an intrinsically determined life cycle, by others to be the manifestation of cumulative effects of suboptimal conditions. So far as the evidence from *P. aurelia* is concerned, no decision between these alternative views

is now possible. No conditions of culture are known which eliminate decline and death in the absence of fertilization. In certain other Ciliates, however, conditions have been found which seem to eliminate the "life cycle" and it is at least possible that the same may eventually be discovered for *P. aurelia*. From a strictly genetic point of view, however, prevention of decline or restoration of vitality as a consequence of fertilization (autogamy or conjugation) may be of great interest, particularly if the "life cycle" is a result of prolonged suboptimal environmental conditions. For an extensive treatment of this matter from a genetic point of view, the reader should consult Jennings' (66) general review of Protozoan Genetics. A suggestion as to the possible mechanism of the fertilization effect is given in paragraph 118. The question of the life cycle is dealt with again in the section on *P. bursaria*.

E. INHERITANCE OF DIFFERENCES WITHIN A CLONE OR CARYONIDE

a. Doubtful Cases

98. Work of subsequent years has mainly served to confirm and extend the basic observation of Jennings (60, 61) that hereditary characters normally remain constant during vegetative reproduction. There are, however, on record a number of reports of exceptional results: a pair of contrasting or alternative characters, known to be genetically determined and usually found only in different caryonides, may under certain conditions be found in different individuals of the same caryonide. Some of these reports seem to be misinterpretations and the facts may be consistent with clonal constancy. In one group of studies, autogamy may have occurred within the clones, thus producing new clones; the diversities, therefore, may have been between different clones, not between different branches of the same clone. This may well be the explanation of three reports (83, 119, 120) that some clones repeatedly produce diverse sublines. In one of these (83), the authors raise the unexamined possibility that autogamy preceded the diversification of the culture; in the other two papers, the author attempted to note the occurrence of autogamy, but his methods were based on the assumption that autogamy occurs with fixed periodicity, taking place in all lines of descent at about the same time. This was later shown (125, 126, 127) to be a false assumption. Further, there is internal evidence (temporary fission rate decrease; origin of the opposite mating type) in his data indicating that the variations did in fact arise at autogamy, not within a clone.

b. Cytoplasmic Lag or Maternal Effect

99. In another group of studies, although there is no doubt that diversities exist within a caryonide, it is also clear that they exist only for

a relatively small number of fissions and then disappear. These transient diversities are examples of "cytoplasmic lag," as it is called in the Protozoa, or "maternal effect." This cytoplasmic effect was discovered (156) in Protozoa in the first crosses made between diverse clones of *P. aurelia*. The parent clones differed markedly in size and fission rate, and similar differences persisted for a time between the two F_1 clones produced from the two members of each hybrid pair of exconjugants: each clone manifested characters similar to those of the parent from which it received its cytoplasm. For example, in one hybrid pair the clone cytoplasmically derived from the normal parent underwent 18 fissions in the first 10 days, while its partner clone, cytoplasmically derived from the less vigorous parent, underwent only 4 fissions in the same period. Likewise, differences in size distinguished these two clones. At a later period, however, both clones underwent 16 fissions in a 10 day period and the individuals were of practically identical size. In most cases, the lines of descent in one or both clones of such a pair suddenly changed and became alike after relatively few fissions; sometimes the change occurred more gradually and somewhat later. During the period of change, different lines of descent within the same clone may change at slightly different times, so that there are transient diversities within the clone, but eventually all the lines of a clone assume the same character and the two clones of such a pair become identical. It appears, therefore, that the characters prior to the change are determined by cytoplasm directly transmitted from the parents (cytoplasm which was itself determined by the nuclear constitution of the parents); but the new hybrid genotype eventually controls the character of the cytoplasm in the hybrids, though phenotypic expression of this control commonly appears first at slightly different times in different lines of descent within the clone.

100. Subsequently, similar cytoplasmic lag phenomena have been reported in other species of Ciliates and for other characters in *P. aurelia*. The three following examples will illustrate the main relations. (1) Kimball (91) found in a two-type stock of variety 1 that, when mating type II animals transform into type I at autogamy, they remain phenotypically type II for several fissions after fertilization before developing the type I phenotype. Moreover, the transformation into mating type I occurs at slightly different times in different animals of a caryonide, some still remaining type II while others have already become type I. In the reverse change at autogamy, from type I to type II, no cytoplasmic lag could be detected. These observations have been confirmed (132) for other two-type stocks of variety 1, have been extended to transformations at conjugation in the same material, and have also been extended to variety 3 for the transformation of type VI animals into type V at autogamy and conju-

gation. As in variety 1, no lag was detectable in variety 3 in the reverse transformation. (2) Antigens in variety 1 were detectable (143) for 4 to 8 fissions, but not longer, in new clones that had just lost the genes for these antigens. On the other hand, when a gene for an antigen was introduced into an animal lacking this antigen, the new antigen could not be detected for several fissions, after which the antigen titer gradually increased during the course of many fissions until it reached the normal level. This result indicates that loss of a cytoplasmic condition following removal of the controlling gene proceeds rapidly (4 to 8 fissions), while full acquirement of a cytoplasmic condition following introduction of the controlling gene may proceed more slowly and gradually. Similar results were reported (145, 149, 150) for the loss and gain of the killer character in variety 4: replacement of + by *k* in a killer was followed by maintenance of kappa and the killer character for about 5 fissions; introduction of either kappa alone (into + non-killers), or kappa and + (into *k* non-killers), was followed by up to 80 or more fissions before the killer character developed.

101. The study of cytoplasmic lag in organisms like Paramecium has certain advantages over the study of maternal effect in higher organisms. (1) In higher organisms, the effect of the maternal cytoplasm usually wears off during the many cell divisions intervening between fertilization and differentiation of the characters under examination and, consequently, maternal effects are seldom observable. In organisms like Paramecium, on the other hand, each fission yields fully formed "adults" with all characters available for observation and, as a result, maternal effects are the rule rather than the exception. (2) For the same reason, the interaction between maternal cytoplasm and newly introduced genes can be examined and analyzed in detail at every stage. An example of this has been given already in paragraph 63.

c. Persistent Diversities; Basis Unknown

102. There are several reports in *P. aurelia* of the origin within a clone or caryonide of genetically diverse lines of descent. One of these has to do with the mating types, which are ordinarily constant within a caryonide, particularly among group A varieties. In all two-type stocks of these varieties, however, there occur (130) exceptional caryonides in which a certain amount of selfing takes place, indicating diversity of mating type among the members of a caryonide, and such exceptional caryonides are much more common among the varieties of group B, particularly after conjugation. Kimball (93) has made a thorough study (in stock S, variety 1) of such selfing or unstable caryonides, which occur with a frequency of about 4%. He demonstrated directly that two animals which conjugate in such caryonides are regularly different in mating type. Kimball care-

fully excluded autogamy as a possible explanation for the origin of the diverse mating types; they were undoubtedly arising within a single caryonide. He further showed that animals of both mating types could arise again among the vegetative progeny of an animal of either mating type. The changes, therefore, occurred repeatedly in both directions: from type I to II and from type II to I. Kimball showed, on the other hand, that the changes were relatively infrequent in these unstable caryonides, less than one animal in 100 undergoing a change. Hence, the mating type diversities were inherited to some extent but not completely. He also found that there occasionally arose in such an unstable caryonide, animals whose vegetative descendants never again changed: *i.e.*, stable lines reproducing true to one mating type arose within unstable caryonides. In the four caryonides examined with regard to this point, the stable lines were always of the same mating type as the parent clone from which the selfing caryonide had arisen at autogamy. Finally, he showed that the unstable condition was confined to particular caryonides and was not inherited through autogamy: when the animals of an unstable caryonide underwent autogamy, they, like the animals of stable caryonides, also produced more than 96% stable one-type caryonides and less than 4% selfer caryonides. Kimball suggested that the determination of mating type in selfing caryonides might be either environmental or due to unequal nuclear or cytoplasmic division. No evidence on which to base a choice among these alternatives was available.

103. Another group of cases of hereditary diversity within a caryonide are those reported in connection with the studies of Dauermodifikationen: within a single caryonide, some lines of descent may revert to normal while others retain the Dauermodifikation. For example (paragraph 93), after serum-resistant clones arose following exposure of temporarily resistant animals to antiserum, these clones produced two kinds of lines, some resistant and others susceptible. All of the susceptible lines remained so; some of the resistant lines remained resistant and some produced susceptible branches.

d. Persistent Diversities Determined by Cytoplasmic Factors: Classes of Observations

104. The role of cytoplasmic factors in the determination and inheritance of characters was pointed out in paragraphs 59–64. In varieties 2 and 4, detailed study of inheritance of the killer characters, which involve cytoplasmic factors, has brought to light four instructive cases of hereditary diversities arising within the clone. Together they provide the basis for a new and promising field of study. In this section, the main facts are set forth; in the following section, they are analyzed and interpreted.

105. The first case of the origin within a clone of hereditary diversities due to a cytoplasmic factor was reported by Sonneborn (149). Very small amounts of the killer cytoplasmic factor, kappa, were introduced into +/+ sensitive animals by mating them to killers and selecting for study those conjugant pairs in which the cytoplasmic connecting bridge between the separating mates was maintained for periods only slightly longer than normal (3½ to 30 minutes, see paragraph 43). Under such condition, some of the sensitives receive no detectible kappa, others receive enough kappa for them to produce killer clones, and others receive so little kappa that they do not produce killer clones. The latter clones thus manifest a new condition: although both kappa and gene + are present, the animals are not killers, but remain sensitive non-killers. The presence of kappa is demonstrated by the facts that animals of these clones produce killer clones when they undergo autogamy and that they are also capable of transferring kappa to non-killers (and thus transforming them into killers), during conjugations that involve transfer of cytoplasm.

106. Clones started with sensitive +/+ exconjugants, into which small amounts of kappa had been introduced, showed a definite pattern of instability. When the four animals resulting from the first two fissions of each conjugant were used to start separate daily isolation lines of descent, it was discovered that some animals lost kappa at the first fission, some at the second and some at later fissions up to the 88th. When an animal that had lost kappa was, by chance, selected to continue the isolation line, all subsequent progeny, of course, lacked kappa and were pure sensitives. In the majority of lines, however, this did not happen; kappa was permanently retained. Nevertheless, these lines remained sensitives for long periods of vegetative reproduction, usually for 60 to 80 fissions; then they became killers. In the original report (149), it was not absolutely clear that this transformation occurred in the absence of autogamy; but later studies (151b) proved autogamy to be unnecessary for the transformation of old lines into killers. Clones of sensitives that contain both gene + and small amounts of kappa thus give rise during vegetative reproduction to two permanently diverse types of progeny: some lose kappa and become pure sensitives, others retain kappa and eventually become pure killers. These two diverse conditions are thereafter permanently inherited in both vegetative and sexual reproduction.

107. The second case of the origin within a clone of hereditary diversities was observed (149) when the pure killer stock 51 was induced to undergo macronuclear regeneration (paragraph 54). The animals produced at the first five or six fissions (by which stage each of the macronuclear fragments had segregated into a different animal) were isolated, grown into cultures and later tested. It was found that about 25% of such

cultures produced some sensitive animals. When sensitive animals arising in this way were isolated and grown into cultures, some reverted to the killer character, but others remained permanently pure sensitives. Thus again pure killers and pure sensitives arose within single clones.

108. The third case may, as will appear, be related to the second; it was reported very recently (151b). Exposure to a temperature of 38.5° C. for 3 to 3½ days kills variety 4 animals; during this period they go through not more than two fissions, often none. When pure killers are exposed to this temperature for 12 hours or more they turn into sensitives. If the exposure is for 36 hours or more they are all transformed into permanently pure sensitives. With shorter exposures, the proportion transformed into pure sensitives decreases until it reaches zero at exposures of 12 hours. Though all the animals become sensitive after an exposure of only 12 hours, they are all capable of producing killer progeny when they undergo autogamy. As the exposure increases, the proportion of sensitives capable of producing killer progeny decreases until it reaches zero at a 36 hour exposure. As the ability to produce killer progeny at autogamy is the test for the presence of kappa in sensitives, exposure to 38.5° C. for 36 hours or more results in the complete destruction of kappa in all animals; and exposure for shorter periods results in the complete destruction of kappa in decreasing proportions of animals until no animals have kappa completely destroyed after exposures of 12 hours or less. However, as in the situation described in paragraph 105, when small amounts of kappa are present, the animals are sensitives instead of killers; in other words, exposure for 12 hours has reduced the amount of kappa in all animals and longer exposures invariably reduced the amount of kappa when they did not destroy it entirely. These results suggest that the average amount of kappa destroyed is proportional to the length of the exposure to 38.5° C.; this implication will be taken up later. From the present point of view, the main result is that pure killer and pure sensitive lines may be isolated from the same clone by keeping one part of the clone continually at 27° C. and exposing another part of the clone to 38.5° C. for 36 hours only.

109. The fourth condition under which killer and sensitive animals may be isolated from the same clone has recently been discovered by Preer (117), working on the killer stocks G, H, 36 and 50 of variety 2. These four killers differ from each other and from the variety 4 killers in the types of effects produced on sensitive animals before the sensitives are killed. Killer characters in variety 2 are determined by cytoplasmic factors (Sonneborn, unpublished), though the relations of these factors to genes remain unknown as yet. Each of these cytoplasmic factors for killer is referred to as kappa, without implying necessary identity between the kappas in different stocks and varieties. Preer observed that when the

variety 2 killers were grown as rapidly as possible, they soon transformed into sensitives. At first these sensitives could be made to revert to killers if their growth was stopped or sufficiently retarded by controlling the food supply; but as the period of rapid fission increased, a higher and higher percentage of the resulting animals became irreversibly pure sensitives. Thus, pure sensitive and pure killer lines could be obtained from the same clone by growing some lines rapidly, others slowly. Although variety 4 grows twice as rapidly as variety 2, sensitives have not been produced in variety 4 by this technique.

e. Persistent Diversities Determined by Cytoplasmic Factors: Analysis and Interpretation

110. Interpretation of the origin of hereditary diversities with respect to the killer character within a clone was first attempted by Sonneborn (148, 149, 150) when only the first two investigations (paragraphs 105–107) on this subject had been performed. Two different possible interpretations were suggested. One may be called the hypothesis of the union of kappa and gene + in the macronucleus, the other the concentration hypothesis. On the basis of the facts available in 1945, Sonneborn, although stating that the correct interpretation was still undecided, was inclined to favor the former hypothesis, which has subsequently been accepted in the main and further developed along different lines by Lindegren (105). An essential feature of this hypothesis, as applied to Paramecium, was the assumption that kappa was present not only in the cytoplasm but also in the macronucleus and that in the macronucleus kappa was bound so firmly to gene + that it could not get into the cytoplasm except when the macronucleus disintegrated at the time of fertilization. The work of Preer (paragraph 109) shows that this is not true in variety 2, for kappa was completely removed from normal killer animals, in the absence of macronuclear disintegration, simply by letting them reproduce rapidly. Hence, either kappa was not in the macronucleus or it passed freely from the intact macronucleus to the cytoplasm. On either alternative the hypothesis of the union of kappa with gene + in the macronucleus would be inapplicable. Sonneborn (151b) has pointed out the grounds for rejecting the hypothesis of the union of cytoplasmic factor and gene, and has shown how the alternative concentration hypothesis explains and unifies both the older and new studies on intraclonal hereditary cytoplasmic diversities. This hypothesis is developed and applied in the following paragraphs.

111. When originally proposed by Sonneborn (149), the concentration hypothesis was suggested as a means of understanding the observations on sensitives that contained kappa (paragraphs 105–106). As these arose only when very small amounts of killer cytoplasm (and therefore also very

small amounts of kappa) were introduced into +/+ sensitives, the animals quickly becoming killers when larger amounts of kappa were introduced, the concentration of kappa in an animal must determine its character: killers have a higher concentration of kappa, sensitives have sometimes a lower concentration, sometimes none at all. It therefore follows that any process which converts sensitives with kappa into killers must be a process which increases the concentration of kappa. As set forth above, autogamy is a process that has this effect, hence autogamy increases the concentration of kappa. Assuming that autogamy produces an approximately constant increase in the concentration of kappa, Sonneborn (149) concluded that different animals in clones of kappa-bearing sensitives contained different concentrations of kappa because they had different concentrations of kappa after autogamy. Some animals produced after autogamy pure kappaless sensitives; hence, they had no kappa before autogamy. Others produced after autogamy kappa-bearing clones of sensitives; hence, they had very low concentrations of kappa before autogamy. Others produced after autogamy clones of killers; hence, they had higher concentrations of kappa before autogamy. As all three kinds of animals arose by fission from the same common ancestor, this implies that kappa may be unequally divided at fission, in agreement with the lack of a precise mechanism for partitioning of cytoplasm at fission. It was further concluded that the rate of increase of kappa must be very nearly or exactly the same as the rate of increase of the animals because the animals that retained kappa remained sensitive for 60 to 80 fissions or more and this implies that the concentration of kappa remains low for long periods of vegetative reproduction. Finally, one of the most significant conclusions reached by Sonneborn, in this first attempt to formulate and apply the concentration hypothesis, was that the proportion of animals that yield killer clones at autogamy is a measure of the average concentration of kappa in the parent clone. This mode of interpreting the phenomena has turned out to be very fruitful, though it was not followed up and developed at this time because the alternative hypothesis then seemed preferable. When the latter was proved untenable by Preer's discoveries on variety 2, the concentration hypothesis was taken up again.

112. Preer's discovery (paragraph 109) that killers of variety 2 became sensitive when they multiplied rapidly means, on the concentration hypothesis, that the concentration of kappa decreases during rapid reproduction. His observation that some of the sensitives are incapable of producing killer progeny means that some lose kappa entirely. Reasoning as in the preceding paragraph, the average concentration of kappa in the clone at any time is correlated positively with the fraction of animals containing kappa and negatively with the fraction containing no kappa

at that time. Assuming that kappa is distributed at random at fission, Preer (117) calculated the mean number of particles of kappa per animal by employing the first term of the Poisson series, $P_o = e^{-m}$, where P_o is the fraction containing no kappa, e is the base of the natural logarithms and m is the mean number of particles of kappa per animal. From the calculated mean number of particles of kappa after various periods of rapid growth, Preer then calculated that the particles of kappa in stock G of variety 2 increase at a rate equivalent to 1.9 doublings per day when the animals reproduce at a rate of 3.4 fissions per day. Using this information, he computed that the original killer animal at the start of the period of rapid reproduction contained 180 particles of kappa. When this calculation was corrected to compensate for the fact that the particles are increasing during the process of sampling, the number of particles in the original animal comes to 256, though their rate of increase remains the same. These calculations, while probably not strictly accurate, show that the number of kappa particles in a stock G killer is of the order of magnitude of 150 to 300. By demonstrating the predictive value of the Poisson method of analysis, Preer proved the correctness of his assumption that the particles of kappa are distributed essentially at random. He further proved by an ingenious experiment that a sensitive animal with only one particle of kappa transforms eventually into a killer if its growth is stopped long enough for the kappa to increase up to the required concentration.

113. Sonneborn (151b) then applied the Poisson technique to the cases in which small amounts of kappa had been introduced into +/+ sensitives of variety 4 (paragraphs 105–106). The fraction of animals lacking kappa is given by the fraction incapable of producing killer progeny after autogamies and this was equated to e^{-m}. Calculations of this sort gave means of 2 to 4 particles of kappa per animal. The means remained constantly at about this same level so long as the animals multiplied at a rate of 6 fissions per day. This demonstrates that, under the conditions employed, kappa in variety 4 increases at a rate equal to 6 doublings per day, confirming quantitatively the earlier (149) conclusion that kappa and the animals increased at about the same rate for long periods of vegetative reproduction.

114. As set forth in paragraph 106, however, kappa-bearing sensitives transformed into killers usually between the 60th and 80th fissions. At this stage the fission rate begins to decline; hence, kappa must multiply more rapidly than the animals when the fission rate of the latter declines during middle and old age. Assuming that the rate of increase of kappa remains at its high level, calculations could be made of the concentration of kappa when the sensitives first transform into killers: each day on which the animals divide less than 6 times would result in one doubling of kappa

concentration for each fission less than 6. Thus, if the animals divide 4 times on one day and kappa increases to an extent equivalent to 6 doublings. the kappa concentration rises by 2 doublings or four-fold. On this assumption, it was shown that sensitives would transform to killers when the concentration of kappa reached a level of the order of 256 particles per animal, agreeing with Preer's estimate for stock G of variety 2. To the extent that the rate of increase of kappa varies with the rate of fission of the animals, the estimate of kappa concentration in killers would be in error. Probably any error in the assumption, however, would give a lower rather than a higher figure than 256 particles, for there is reason to believe that the rate of kappa production would vary directly with the fission rate of the animals rather than inversely.

115. The other condition under which sensitives containing kappa transform into killers is autogamy and this too can be explained in the same way. The animals divide not more than twice on the day they undergo autogamy. If kappa reproduces at the usual rate, an increase of kappa concentration of $(2)^4$, or 16-fold, would result. This agrees with the observation that when kappa concentration is low, sometimes two autogamies are required to bring about transformation into killers. If the minimum of one particle of kappa were present, one autogamy would raise the concentration to 16, two autogamies to 256; the former would not transform to killer, the latter would. As in paragraph 114, if kappa reproduces less rapidly at lower fission rates, all estimates must be correspondingly reduced. As concluded earlier (149), the effect of autogamy is not due to any genetic feature of the process. It now appears that it is due simply to the associated condition of arrested or retarded reproduction permitting an effective increase of kappa concentration. Thus, all the known conditions that transform sensitives with kappa into killers — inadequate food, old age and autogamy — have a common explanation: kappa increases relatively faster than the animals multiply and this results in raising the level of kappa concentration.

116. The interpretation to be given to the origin of pure sensitives from pure killers at macronuclear regeneration (paragraph 107) is not yet certain, but one possibility may be suggested. Macronuclear regeneration was induced by exposure to 38.5° C. for 3–5 hours. In view of recent knowledge (paragraph 108), this probably destroyed a considerable amount of kappa, although not enough to make the animals sensitives. Since sensitives arose only when the animals regenerated macronuclei from fragments of the old macronucleus, not when their macronuclei arose in the normal way, even after heat treatment, it may be supposed that regenerated macronuclei are less efficient than normal macronuclei in controlling increase of kappa. This assumption would account for con-

tinuing decrease in kappa concentration in those with regenerated macronuclei. At the next autogamy, however, normal macronuclei would be formed and these would control the maximal rate of kappa increase, thus checking its further decrease. Hence, cells that had become sensitive but still retained kappa could revert eventually (in old age or after autogamy) to killers, but those that had completely lost kappa would, of course, remain pure sensitives. This suggested interpretation for the macronuclear regeneration phenomena can readily be tested; if confirmed, it would provide a means of measuring the amounts of kappa destroyed by short exposures to 38.5° C. Present methods permit only calculations of the mean number of particles left after long exposures.

117. The fourth condition under which sensitives arise in clones of killers, *i.e.*, exposure to 38.5° C., has thus far been little studied (151b). By controlling the exposure time, the concentration of kappa can be reduced to any desired level and the process of return to the killer character can be followed in detail in the lines that still have some kappa. The results thus far confirm the interpretations given in the preceding paragraphs. They also demonstrate the existence of an intermediate character, resistant-nonkiller, when the concentration of kappa is slightly below that required for the killer character. The new techniques developed by Preer and Sonneborn open up a new field of possibilities for investigation which may be exploited increasingly in the near future.

F. SOME IMPLICATIONS OF THE NEWER KNOWLEDGE OF PARAMECIUM GENETICS

a. The Life Cycle

118. Sonneborn (151b) has suggested that the period of sexual immaturity following conjugation may be a period in which the concentration of a cytoplasmic factor determining mating type is slowly built up after its depletion during the process of conjugation; and that the phenomena of senescence may be, at least in part, due to the gradual reduction in concentration of cytoplasmic factors that are essential for life but unable to increase as fast as the animals. Pierson (113) and Gelber (45) found mortality at autogamy in *P. aurelia* to increase with time since the last previous fertilization. Such data might be used, like the fraction of irreversible sensitives in the work with kappa (paragraph 112), to compute the rate of increase and number of particles of such a hypothetical factor. Though it involves some difficulties that may prove disastrous, such a mode of approach to the classical problem of the Ciliate life cycle might be worth pursuing.

b. *Dauermodifikationen*

119. Sonneborn also suggests (151b) that Jollos' Dauermodifikationen (paragraphs 85–96) become intelligible in the light of the cytoplasmic concentration hypothesis. Dauermodifikationen may be produced by environmental conditions which reduce the concentration of a cytoplasmic factor, just as high temperature reduces the concentration of kappa (paragraph 117). Jollos' report that Dauermodifikationen might arise as a series of graded effects proportional to length of exposure to inducing agent is strictly comparable to the relation between length of exposure to high temperature and amount of kappa destroyed (the latter determining a graded series of phenotypic effects). Increase of concentration of such a cytoplasmic factor would result in loss of a Dauermodifikation and Jollos reported such loss as a consequence of old age, unfavorable conditions and fertilization, *i.e.*, under precisely the same conditions that retard or suppress fission and change sensitives with low concentrations of kappa into killers. Stepwise return to the original character is comparable to the relation between the rising concentrations of kappa and the character series: pure sensitives, sensitive with kappa, resistant, killer. The most puzzling feature of Jollos' observations, namely, that sometimes more than one fertilization is required to restore the original character, also has its parallel in the observed effects of autogamy on kappa concentration (paragraph 115): this situation occurs when the concentration of kappa is so low that the increase resulting from the temporary reduction of fission rate at one autogamy is insufficient to restore normal concentration. It would seem, therefore, that the new concentration hypothesis may well provide the key to the understanding and interpretation of Dauermodifikationen.

c. *The Properties of Cytoplasmic Factors*

120. From the moment of their discovery (145) in killer paramecia, the basic question about cytoplasmic factors has been their relation to genes. The first and foremost fact is the apparent 1:1 relation between gene loci and classes of cytoplasmic factors (149). In about half of the known "varieties" (or genetic species) of *P. aurelia*, namely, those that constitute the B group of varieties (154), there intervenes a cytoplasmic factor or class of related factors between the genes at each locus and their phenotypic manifestations. The model for the general relation is given by the one discussed extensively in this review, the killer character being dependent on the presence of kappa (in proper concentration), and kappa being dependent on the presence of gene $+^k$ (in the macronucleus). Every hereditary character thus far examined in varieties of group B depends likewise upon a cytoplasmic factor comparable to kappa. In most cases, the dependence of the cytoplasmic factor upon a gene has not been demon-

strated, as in the case of mating type determination. The two mating types of a variety seem to be determined by a pair of alternative cytoplasmic factors, though this case has not yet been reported in detail. No genic difference controlling the maintenance and increase of these cytoplasmic factors has yet been discoverable and perhaps none exists. If this be the situation, then it would be comparable in part to the difference between the sensitive stock 47 and the killer stock 51 of variety 4 where both are homozygous for the gene $+^k$ controlling kappa, though one contains and the other lacks kappa. In this case the difference in characters is due not to genic difference but to difference in presence or absence of a cytoplasmic factor, while, in the case of mating types, the difference in characters is due to a pair of alternative cytoplasmic factors, both presumably controllable by the same gene. In no case, however, is a character determined directly by a gene without the intermediacy of a cytoplasmic factor, except in the sense of a deficiency. That is, the sensitive character may be considered as determined directly by gene *k;* but it may merely be due to the absence of kappa in the presence of homozygous *k*. The possibility that there is an alternative cytoplasmic factor "sigma" for sensitivity in natural *kk* stocks has not yet been excluded; it is indeed suggested by certain observations (151b). Further, *k* can no more be considered as a direct determiner of sensitivity than its + allele, for both yield sensitives in the absence of kappa. In general, therefore, there appears to be a dual system of allelic determiners: one system is in the chromosomes, the other in the cytoplasm. The chromosomal alleles determine whether a given cytoplasmic factor can be maintained and multiplied. The cytoplasmic "alleles" determine alternative phenotypes. Each character, therefore, is determined by a particular gene-plasmagene combination, and there is a 1:1 relation between each gene locus and one or a series of "allelic" cytoplasmic factors.

121. This system of determination in the varieties of group B is in marked contrast to the system in the very closely related and morphologically indistinguishable varieties of group A (150, 154). In group A, cytoplasmic factors have not been found at all; the characters seem to be directly controlled by the genes without detectible intermediacy of cytoplasmic factors. In other words, when crosses are made between diverse stocks, the two members of each pair acquire the same genotype and phenotype independently of the occurrence or amount of cytoplasmic transfer between mates. As analogous traits (*e.g.*, mating types, antigens) are determined according to different systems in the two groups of varieties, it appears that the gene alone in group A varieties is the functional equivalent of gene plus cytoplasmic factor in group B varieties. Sonneborn therefore concluded (150) that the gene in group A is composed of two parts, one comparable to the gene of group B and the other comparable to

the cytoplasmic factor of group B. This view has two important implications. (1) The gene of group A probably liberates into the cytoplasm physiologically active factors comparable to the cytoplasmic factors of group B. They are not detectible by present methods in group A because they are always producible by the gene, and the two parts of the gene cannot be separated for experimental analysis. (2) The cytoplasmic factors of group B arose originally from their controlling genes and are to be considered as the free, physiologically active derivatives of genes.

122. The question now arises as to the extent to which the properties of cytoplasmic factors are like or unlike the properties of genes. Two basic similarities were immediately apparent: both control characters and both are self-reproducing under appropriate conditions. Further, both are transmitted from generation to generation, though one is transmitted through the nucleus, the other through the cytoplasm. Sonneborn has recently reported (151a) further important resemblances. The cytoplasmic factors for different characters are discrete and separable. Different kinds of cytoplasmic factors existing in the same clone are separately transmissible during conjugation: when little cytoplasm is exchanged, one kind of factor can be passed across to the mate while another kind is not. Hence, like genes, cytoplasmic factors may be separated and assembled into new combinations, though the mechanisms for achieving this differ in the two cases. Moreover, there is some evidence (151a) that cytoplasmic factors may mutate and then reproduce in the mutated condition.

123. The new work (paragraphs 105–116) shows, however, certain marked differences between genes and cytoplasmic factors. There is no necessary correlation between their rates of reproduction. In variety 4, kappa doubles itself at the same rate as the cells (and presumably, therefore, at the same rate as the genes) when the cells are dividing 6 times per day; but when the cells divide more slowly in old age or at periods of fertilization, the cytoplasmic factors multiply more rapidly than the cells and genes. In variety 2, Preer's studies show that kappa may multiply more slowly, more rapidly or at the same rate as the genes in dependence upon the rate of reproduction of the animals. Moreover, the maximal rate of reproduction differs for different cytoplasmic factors. The maximal rate for kappa in variety 4 is at least 6 duplications per day; for kappa in stock G of variety 2, it is less than two duplications per day, and for kappa in other stocks of variety 2 it is even lower. The reproduction of genes and of cytoplasmic factors are, therefore, neither synchronized nor necessarily equal in rate. This apparent discrepancy may of course be due in part to the lack of detailed information about possible gene reproduction during interkinesis. The comparison is based on the rate of nuclear division as if this were the rate of gene reproduction. If gene action consists of the

liberation of gene copies during interkinesis, the true rate of gene reproduction would be the sum of the rates of physiological and mitotic gene multiplication. Possibly the reproductive rates of cytoplasmic factors should be compared only with the rate of physiological reproduction of genes; but of the latter nothing is yet known.

124. Whether the number present per cell constitutes a difference between genes and cytoplasmic factors still remains unknown. In Paramecium, the macronucleus is compound (paragraph 55) and the number of component parts is known to be at least about 30, though it may well be higher. At equilibrium there may be as many $+^k$ genes as kappa particles (200–300) per cell. This can readily be tested by comparing the kappa number per cell in $+/+$ and $k/+$ cells, as well as in different stocks of killers. In summary, taking all the aspects of cytoplasmic factors now known into consideration, the resemblances between their properties and those of genes are certainly more impressive than the differences. If cytoplasmic factors are the free physiologically active parts of genes, the study of cytoplasmic factors may turn out to be a means of acquiring hitherto inaccessible information about the properties of genes.

d. Are Cytoplasmic Factors of Limited or Widespread Occurrence?

125. There remains the important question of whether the cytoplasmic factors of group B varieties of *P. aurelia* are unique and without general significance or whether they are more widespread and significant. The possibility that they are virus intruders has been raised. In view of their normal and regular participation in the genetic system in this material, for all characters investigated, the virus interpretation seems excluded. However, it may well turn out that one aspect of the situation will be found uniquely confined to ciliated Protozoa. Sonneborn (150) has pointed out how complete separation between reproductive and physiological components of genes could arise in organisms like the Ciliates, in which physiological and sexual functions are taken over by different nuclei in the same cell, while such a separation would be fatal for essential genes in organisms lacking such functional nuclear dimorphism. It would, therefore, be surprising to find in higher organisms genetic systems like the one in *P. aurelia*. One could scarcely hope to find more than occasional examples of this sort and then only for relatively non-essential characters. However, one might discover, if experimental means could be devised, that cytoplasmic factors continuously producible by genes are of widespread occurrence. The loss of ability to produce the cytoplasmic factors may be the unique, or almost unique, feature of the situation in *P. aurelia* and possibly some other Ciliates.

126. That the situation is not entirely unique is indicated by other

investigations. The analysis of CO_2 resistance in Drosophila by L'Héritier and co-workers (103, 104) presents in many respects a striking parallel to the results on *P. aurelia*. Similar results have also been reported (87) for the inheritance of mammary cancer in mice. The well-known work on the Plasmon of plants may also be interpreted in this way (146). These parallels are, perhaps, enough to show that the general pattern of the phenomena found in *P. aurelia* is not absolutely unique and may in some respects be of general significance.

e. Alternative Interpretations of the Work on the Killer Cytoplasmic Factors

127. Darlington (22) early proposed the assumption of two plasmagenes, one for killer and one for sensitive, with the former being suppressive of the latter in mixtures. The recessive gene *k* is further assumed to change killers into sensitives either by mutating the killer plasmagene into the sensitive plasmagene or by producing the sensitive plasmagene. The dominant allele + is assumed to have no detectible action. As already mentioned (paragraph 120), the existence of a cytoplasmic factor for sensitivity in *kk* stocks is a possibility not yet excluded and certain later observations (151b) seem to point in that direction. On the other hand, later work (150, 151b) showed that killers can be experimentally converted into pure sensitives in the absence both of *k* and of cytoplasm from a sensitive stock. Moreover, Darlington's assumed "mutafacient" action of *k* seems incredible in view of the speed with which the change from killer to sensitive occurs and the recent discovery of the number of kappa particles per killer cell (paragraph 112). Altenburg (151b), adhering to Darlington's assumptions of the possible roles of gene *k*, suggests that the normal allele (+) protects its possessor against the killing action of kappa but plays no role in the multiplication of kappa. This view is untenable, for animals with + and either no kappa or small amounts of kappa are sensitive, not resistant, to the killer action. Several critics (Spiegelman, Altenburg and, especially, Lindegren, 151b) have suggested or urged that kappa is a virus. Aside from the remarkable fact that sensitivity is due to *absence* or low concentration of kappa, while resistance depends on the presence of kappa, the fact that kappa is but one example of a normal and regular system of cytoplasmic factors seems to exclude the virus interpretation (151b). Emerson (32) attempted to interpret the relation of kappa to gene $+^k$ in terms of the template hypothesis of gene action, but he recognizes the difficulty of harmonizing some of the later work on kappa with this hypothesis. Muller (108) suggests that $+^k$ produces π, a precursor of kappa, and that π and kappa interact to produce more kappa in the way that pepsinogen and pepsin interact to produce pepsin. Spiegelman, after

suggesting earlier (159) that kappa may be multiplied very slowly in the absence of $+^k$, a suggestion impossible to reconcile with the repeated failure to detect kappa when $+^k$ is reintroduced, has recently (151b) suggested that $+^k$ produces a plasmagene which is self-reproducing but inactive with respect to the killer character and that kappa unites with this plasmagene to form a compound self-multiplying plasmagene which can mediate the production of an enzyme essential for the production of the killer substance. He further suggests that these two components of the compound plasmagene were originally produced as a single plasmagene by an allele of $+^k$ from which the latter arose by mutation. These later views of Spiegelman are in formal accord with Sonneborn's interpretations of the relation of genes to cytoplasmic factors in *P. aurelia* (paragraph 122).

G. EVOLUTION AND COMPETITION

128. The subjects of evolution and competition are beyond the scope of this review. Kimball (98) may be consulted for an excellent review discussing, among other subjects, the evolution of mating type varieties. Further comments will be found in Sonneborn and Dippell (154). Gause and his co-workers (39, 40, 41, 43, 44) have reported studies on competition; there is available also an English review (42) of this work.

III. Paramecium bursaria

1. Mating Types and the Breeding System

129. Paramecium bursaria has been extensively studied from a cytogenetic point of view. In certain respects it has been studied more fully than any other species of ciliated Protozoa and a number of important phenomena were first discovered in this species. The modern work on this species is almost entirely due to Jennings and T. T. Chen. Mating types were discovered (67, 68) in *P. bursaria* soon after their discovery in *P. aurelia* and immediately two new relations were found: first, Jennings found systems of multiple (instead of binary) interbreeding mating types; second, he found that different groups of mating types were sexually isolated. The further knowledge of the breeding system in *P. bursaria* is contained in a series of papers (15, 20, 67, 68, 69, 70, 82), the main results of which are summarized in Table II. There are now known 6 varieties which, with one exception presently to be mentioned, are sexually isolated from each other. One variety (II) contains 8 mating types (E to M) each of which mates with every other mating type of the variety, but not with itself. Three varieties (I, III and VI) contain four mating types each; in each of these varieties, any mating type mates with all the others of the same variety, but not with itself. One variety (IV) has but two mating

TABLE II

The System of Breeding Relations in P. bursaria

+ indicates the occurrence of conjugation, − indicates that no conjugation occurs, in mixtures of the two mating types represented on the corresponding row and file.

Variety		I				II								III				IV		V	VI			
	Mating Type	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
I	A	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	B	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	D	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
II	E					−	+	+	+	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−
	F					+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	G					+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	H					+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	J					+	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	K					+	+	+	+	+	−	+	+	−	−	−	−	+	−	−	−	−	−	−
	L					+	+	+	+	+	+	−	+	−	−	−	−	+	−	−	−	−	−	−
	M					+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−
III	N													−	+	+	+	−	−	−	−	−	−	−
	O													+	−	+	+	−	−	−	−	−	−	−
	P													+	+	−	+	−	−	−	−	−	−	−
	Q													+	+	+	−	−	−	−	−	−	−	−
IV	R																	−	+	−	−	−	−	−
	S																	+	−	−	−	−	−	−
V	T																			−	−	−	−	−
VI	U																				−	+	+	+
	V																				+	−	+	+
	W																				+	+	−	+
	X																				+	+	+	−

types that mate with each other in the same way as do the two mating types characteristic of all the varieties of *P. aurelia*. Finally, one group of clones obtained from Russia have not been found to mate with each other or with any of the other known mating types; these are considered to constitute one mating type (T) of a variety (V) of which the other mating types have not yet been found. Varieties I, II and III occur in the United States; varieties IV, V and VI come from Europe (IV and V from Russia, VI from England, Ireland and Czechoslovakia).

130. The one exception to complete sexual isolation of the varieties was the first case of intervarietal conjugation reported (82) in Ciliates. Type R of variety IV conjugates with four of the mating types (E, K, L and M) of variety II, but not with the other four types (F, G, H and J) of that variety. This has been interpreted independently by Chen (17) and others (154) to indicate homology, on the one hand, between type R of variety IV and the four types F, G, H and J of variety II, because they all mate with E, K, L and M; and between S (variety IV) and the four types E, K, L and M (variety II) because they all mate with type R. Further study may reveal a system of homologies between mating types of different varieties of *P. bursaria* comparable to the system now known in *P. aurelia*. Attempts to discover such homologies in *P. bursaria*, however, are greatly complicated by the existence of multiple mating type systems. As in *P. aurelia*, conjugation between different varieties is of little genetic value; in *P. bursaria*, all the hybrids die without completing conjugation (17). The process of conjugation, after proceeding normally until late prophase of the first maturation division, suddenly stops; the first meiotic division is never completed. Whole chromosomes and even parts of chromosomes become abnormal and clump, indicating the passage of substances from mate to mate that greatly alter chromosome behavior.

2. Cytological Basis of the Genetic System

131. Normally, *P. bursaria* contains one macronucleus and one relatively large micronucleus. The macronucleus is fairly constant in size and structure, but much variation has been reported concerning the micronuclear condition. Variations of from four micronuclei to none at all occur within a single stock (11, 13, 122, 160); the micronuclei may have normal structure or be "ghosts" with little or no staining capacity. Different stocks differ characteristically in the size, shape, stainability and arrangement of the chromatin in their micronuclei (9, 10, 11, 12, 13, 14). Chen's beautiful cytological studies have shown that there are also great chromosomal differences between stocks. In late prophase of the first meiotic division, the chromosome number of different stocks may vary from 80 up to several hundred (9, 12, 13). Chen believes his stocks consti-

tute a polyploid series which has arisen as a result of rare abnormalities in the process of conjugation. Diverse chromosome numbers are even found in different stocks of the same mating type (14). Still more remarkable are varietal and stock differences in the size and shape of the chromosomes (20). The chromosomes of varieties II and IV are thin and short; those of variety III are longer and larger; in variety VI, one stock has its chromosomes in the form of small spheres or short rods, while another stock has larger and longer chromosomes.

132. The main features of the process of conjugation had been worked out by a number of earlier investigators, especially by Hamburger (52); but the detailed study of timed stages made on a very extensive scale by Chen (8, 10, 11, 12, 13, 16, 17, 20, *etc.*) has corrected errors in the earlier work and has added many important details. The first maturation division, as is usual in Ciliates, is long and complicated; one product of this division disintegrates and the other passes rapidly through a second division. Again one product disintegrates and the survivor divides to produce the two gamete nuclei, which may or may not differ in size or shape. In structure they resemble resting vegetative micronuclei; stock differences in resting micronuclei are paralleled by differences in gamete nuclei. Normally, one gamete nucleus from each mate migrates into the other and there fuses with the stationary gamete nucleus to form a syncaryon. This has been demonstrated by Chen in many ways, especially by following the process in crosses between stocks whose gamete nuclei differ markedly in size, structure and chromosome number. The syncaryon in each mate divides and one product disintegrates; the survivor divides twice and two of the products form macronuclei, two form micronuclei. The mates separate just before or after the third post-zygotic nuclear division. Unlike the condition in *P. aurelia*, the old macronucleus never unravels into a skein or breaks down into fragments; it apparently just wastes away.

133. Chen reports (20) an interesting difference between varieties in the rate at which the nuclear processes of conjugation take place under the same conditions. The first maturation division takes about twice as long (20 hours) in variety II as it does in variety I, although the remainder of conjugation shows much less difference in rate. Variety IV conjugates at about the same rate as variety II. Differences in rate of conjugation could play a role in bringing about sexual isolation of varieties. In the same paper, Chen also reports on crosses between the two stocks of variety VI that differ in chromosome form and size (paragraph 131). The conjugants show much variation in the details of the nuclear processes, and about 50% of the exconjugants show abnormal numbers of nuclei. There is as yet no report on the viability of the later generations from these hybrids. This would be of interest in relation to the possible role of visible

chromosome differences in bringing about sexual isolation, as suggested by the visible differences between the chromosomes of variety III as compared with those of varieties II and IV (paragraph 131).

134. Cytoplasmic exchange during conjugation of *P. bursaria* has recently been reported by Harrison and Fowler (54). They find that antigens previously limited to one mate appear during the course of conjugation in the other mate in about 95% of the conjugant pairs, and that the newly acquired antigen persists thereafter during vegetative reproduction. They also have observed that the symbiotic Algae normally occurring in *P. bursaria* pass from one mate to the other during conjugation between an individual that contains and one that lacks these Algae. Harrison and Fowler point out that these observations were made under the abnormal condition of exposure to antiserum, which modifies the surfaces of the animals. The transfer of cytoplasm between mates may, therefore, be a consequence of these special conditions. On the other hand, Chen (17) observed that chromosome behavior is modified early in conjugation (at a stage prior to the one in which gamete nuclei should be exchanged) when different varieties are crossed (paragraph 130). This implies passage from mate to mate of materials that affect chromosome behavior. Further, these conjugants later often fuse into a single body with obvious cytoplasmic union, although gamete nuclei are neither exchanged nor formed. He also observed (8, 16) that contact of a third animal with a member of a conjugant pair induces it to undergo meiosis and fertilization. Further, Jennings (75) finds that conjugation between young and old clones usually kills both mates, abnormalities appearing during conjugation itself. On the other hand, the young clone shows normal conjugation and high survival rate when its members conjugate with young clones. The result of crossing young by old clones thus indicates transfer of substances or influences from the old to the young mate during conjugation. There are thus a number of reasons for believing that materials other than gamete nuclei pass from mate to mate during conjugation in this species, at least some of them passing over before the stage at which the gamete nuclei are exchanged.

135. No other special nuclear processes aside from conjugation are known to occur regularly and normally in *P. bursaria.* Erdmann (36) searched for endomixis and failed to find it in isolation cultures; but in mass cultures she found, occurring sporadically, nuclear processes believed to be endomixis. Woodruff (166) had another race of *P. bursaria* under observation for 7 years and was unable to find any indication of the occurrence of endomixis. Chen has intensively examined many stocks of this species over an even longer period and has never reported the occurrence of endomixis. Erdmann's finding of endomixis in this species, as in *P.*

aurelia, thus remains unconfirmed. On the other hand, Chen's investigations show that *P. bursaria* is capable of undergoing autogamy, though there is no cytological evidence to indicate that it occurs normally or often. He has found autogamy under three, possibly four, abnormal conditions. (1) When a third animal attaches itself abnormally to one member of a conjugating pair, usually to its posterior end, the third animal undergoes autogamy while the other two conjugate (8, 16). (2) When a normal stock "conjugates" with a stock that lacks a micronucleus, usually the amicronucleate member receives one reduced gamete nucleus from its normal mate, the other one remaining in the normal mate, and these "hemicaryons" in the two mates give rise to the vegetative nuclear complex in the usual way. Sometimes, however, neither gamete nucleus passes into the amicronucleate member, both remaining in the normal mate, fusing to form a syncaryon there and thus accomplishing autogamy for this member of the conjugant pair (11). (3) Sometimes animals fail to complete fission, forming double animals or chains of two. These are capable of conjugating, but commonly only the anterior animal of the chain conjugates. The posterior animal of the chain, if it has normal nuclei, may then proceed to undergo autogamy while the other half of the chain conjugates (18). (4) Chen also found (15) that addition of the fluid in which certain stocks have lived to cultures of certain other stocks can induce abnormalities and atypical pair formation between animals presumably of the same mating type. (These effects are similar to those of one of the killers in variety 2 of *P. aurelia*.) In addition to producing abnormalities and atypical conjugant pairs, the active fluid also induces in unmated animals nuclear reorganizations that may be of an autogamous nature, but the details are still unreported. In all of the situations in which the details are reported, the nuclear processes are identical with those of conjugation, except that the syncaryon is formed by the fusion of the two gamete nuclei that have arisen in one animal.

3. Genetics

136. The study of heredity in *P. bursaria* has proved to be more difficult than in *P. aurelia*, in large part because the organisms are incapable of mating for long periods after conjugation (weeks, months and, in some cases, years). Lesser, but still serious, difficulties are the high proportion of non-viable progeny often produced at conjugation, the widespread occurrence of polyploidy and the absence of regular and frequently occurring autogamy. In spite of these great difficulties, Jennings has accumulated with characteristic thoroughness a large body of data concerning heredity. He (67, 68, 69, 70, 74) finds many hereditary differences in mating reactivity, size, form, fission rate and vigor; but most attention

has been directed to the inheritance of mating type. Each mating type is strictly inherited during vegetative reproduction, with certain possible rare exceptions to be dealt with later, after an account of inheritance at conjugation.

137. The inheritance of mating type at conjugation seems to follow the same rules regardless of whether the clones crossed belong to the same stock or to different stocks (67, 69, 73). Most of the work has been done on the inheritance of the four mating types, A, B, C and D of variety I, with some attention also to inheritance of the types in variety II and variety IV (82). The usual results and the interpretations given to them may be briefly summarized. (1) In 97.5% of all conjugant pairs, the clones produced from the two mates in any one pair are alike in mating type (though, of course, the parents differed in this respect). This is interpreted to mean that the mating type is determined by the nuclei and that the third maturation division is normally equational, because, under these conditions, the two mates of a pair get identical syncaryons (paragraph 33). (2) The 2.5% of the pairs that give other results show either of two conditions: in some pairs, the clone from one exconjugant differs in mating type from the clone produced by the other exconjugant; in other pairs, the two caryonides produced from one exconjugant differ in mating type (as occurs commonly in two-type stocks of *P. aurelia*, paragraph 79). In these cases, Jennings suggests that the third maturation division might have been reductional or that other factors may be involved. In any event, these exceptions are so rare that they may be considered as in a separate category from the normal. (3) In about 80% of the conjugant pairs showing normal inheritance, the percentage varying considerably from cross to cross, both members of about half of the pairs yield clones with mating type the same as one of the parents and both members of the other half of the pairs yield clones with mating type the same as the other parent. In the remaining 20% of these conjugant pairs, both members yield clones of a mating type different from that of either parent. This percentage also varies, in a few crosses a majority of the pairs yielding this result. Jennings interprets these results to mean that commonly the gamete nuclei from one or the other mate determine the mating type, those from one mate determining the type in ½ the pairs and those from the other mate in the other half of the pairs, when the progeny are like one or the other parent; and that possibly new nuclearre combinations are formed in the few cases in which the progeny differ from both parents. (4) Crosses of most mating types (and perhaps crosses of any two mating types) can yield among the progeny all mating types of the variety. For example, the cross of type A×type B yields in different exconjugant pairs clones of mating types A, B, C and D. In certain crosses, however, one or

two mating types of the variety may be lacking among the progeny. Faced with this perplexing result, Jennings has thus far found it impossible to present a satisfactory chromosomal hypothesis to account for the inheritance of mating type at conjugation.

138. As mentioned earlier, there is a possibility that exceptions occur to the rule of clonal constancy in mating type. Very rarely, Jennings (67, 68, 69, 72) found conjugation taking place among the progeny of a single individual. He is inclined to believe that this is traceable to a prior nuclear reorganization (endomixis or autogamy), though in no case have such processes been found in these cultures. Kimball (98) suggests that this case may be comparable to the unstable caryonides he observed (93) in *P. aurelia*, where changes of mating type took place in the absence of nuclear reorganizations. The basis of the phenomenon is, therefore, not entirely clear, but the results are sharp and striking. Jennings showed that when conjugation occurs under such conditions it is due to the presence of two different mating types in the culture, although originally only one type was present. Moreover, the two types that appear in any culture are characteristic for that culture, reappearing again if another branch of the same original culture undergoes "self-differentiation," as the process is called. Still more remarkable is the fact that when a given new mating type has been produced by self-differentiation and this is now cultured alone, it may undergo a second self-differentiation. When it does, however, the new type it produces is always the same as the original type before the first self-differentiation. Thus, a culture of type E may differentiate into types E and F. If these two types are now isolated, and if they undergo further differentiation, E will produce again only E and F, F will produce only F and E. The differentiation is thus reversible, and two, and only two, types are producible by self-differentiations within a single original clone. However, different clones of the same mating type may yield different results when they self-differentiate, the new type being any one of the other types in that variety: one clone of type A may differentiate always into A and B, another clone of type A may differentiate always into A and C, a third clone may differentiate always into A and D. These relations are all the more remarkable in comparison with the results obtained when two such differentiated types from a single parental clone are allowed to conjugate with each other: at conjugation their progeny are not restricted to these two types, but can produce *all* the mating types of the variety to which they belong. If the two types A and D arise from a clone of type D, crosses of these types (A×D) may yield A, B, C and D among the progeny. Kimball (98) suggests that the two types into which each clone can self-differentiate constitute a clonal character which, rather than the single mating type of the clone itself, might profitably be used as the character

followed in inheritance through conjugation. An interesting feature of self-differentiation is that the resulting two mating types are often associated with differences in other characters, such as size, shape, fission rate and degree of sexual reactivity, but this is by no means an invariable occurrence.

139. Jennings has devoted a great amount of study (69, 72, 75, 76, 77) to other changes that regularly take place within all normal clones. These are the progressive changes which are commonly called "aging." Beginning immediately after conjugation, clones are immature (*i.e.*, unable to conjugate) for variable periods of from a few weeks to years. Then by degrees they become capable of giving the mating reaction and conjugating. In variety I, there is some evidence that at first they acquire a more generalized mating type, capable of mating with only two of the four mating types, either A and B or C and D; later they become specified as a single type, acquiring the capacity to mate with a third type. During the long period of maturity, the organisms show progressively increasing incapacity to survive conjugation until finally none can do so. They are now old and doomed to die sooner or later whether they mate or not. (Comparable relations exist in *P. aurelia* when the organisms are prevented for long periods both from conjugating and from undergoing autogamy, paragraph 97.) In addition to the effect of aging on inability to survive conjugation, Jennings also found that repeated inbreeding rapidly increases the mortality at conjugation, although the mating of two types that have been produced by self-differentiation within a clone yields less drastic effects than when sibs are crossed.

140. Certain general comments may be made on the general status of the genetics of *P. bursaria.* As pointed out by Kimball (98), the common occurrence of polyploidy in *P. bursaria* may well be a factor that has served to obscure the significance of the genetic results, and that has thus far made it impossible to discern the operation of any single chromosomal or genic difference in the determination of mating type. It may further be suggested that a prime necessity for further analysis is to produce experimentally simple diploids to be used as material for genetic study. This might be done by utilizing certain observations made by Chen (10, 11, 13). When a polyploid mates with an amicronucleate animal, normally the two reduced gamete nuclei formed in the one mate serve to reconstruct the new nuclei of the exconjugants, one such nucleus serving each mate. The nuclei of the exconjugants thus contain ½ the number of chromosomes with which the parent polyploid clone was equipped. If the new clones do not double their chromosome number (and observations on other species indicate they will not), they can then be mated again to an amicronucleate clone, achieving another halving of the chromosome number. This can be

done repeatedly until the diploid and haploid conditions have been attained. The same could be done starting with different original polyploids, differing in mating type, and thus one could build up haploid or diploid stocks of the diverse mating types. These diploids could then be put into absolutely homozygous condition by utilizing one or more of the methods discovered by Chen (paragraph 135) for the induction of autogamy. With such homozygous diploid material, the discovery of any genic or chromosomal basis for mating types should be readily forthcoming.

141. There appears to be a possibility, however, that the result of such analysis would only serve to show that differences of mating type are not due to differences in the chromosomes and genes, though Jennings rejects this possibility. His rejection is partly based on the assumption that no cytoplasmic exchange occurs during conjugation. Evidences against this assumption were reviewed in paragraph 134. If cytoplasm is indeed exchanged normally or often at conjugation, the interpretation to be given to the genetic results may be quite different from the nuclear one Jennings puts forth. The fact that all progeny of any one pair of conjugants usually agree in mating type might then as readily imply cytoplasmic as chromosomal determination. In support of this alternative, the inheritance of mating type in variety 4 of *P. aurelia* may be cited. The mating types of this variety are determined (paragraphs 42, 59) by cytoplasmic factors and cytoplasm is usually not exchanged at conjugation; but, when much cytoplasm is exchanged, the type of inheritance is essentially the same as in *P. bursaria.* Both conjugants then usually produce clones of predominantly the same mating type, sometimes like the type of one parent, sometimes like the type of the other parent. It would, therefore, seem that cytoplasmic determination of mating type is by no means excluded in *P. bursaria.* One further possibility may be mentioned. The phenomena of self-differentiation (paragraph 138) bear at least a superficial resemblance to the phenomena of mating type determination in the two-type stocks of *P. aurelia* (paragraphs 79–84), where there exist two alternatives in any one clone and these are redetermined at random at autogamy. The situation is obviously more complicated in *P. bursaria,* in which there may be diverse two-type alternatives within the same variety. In summary, it may be said that *P. bursaria,* thanks to the prodigious labors of Jennings and Chen, is now in condition in which it appears possible, by taking advantage of their many valuable discoveries, to penetrate deeply into the genetic mechanisms involved. The data they have already obtained indicate that discoveries of great and general interest may be expected to emerge from the further investigation of this organism.

IV. Paramecium caudatum

142. Among the ciliated Protozoa, *Paramecium caudatum* has long been the favorite species for experimental work and the literature on it is enormous. An adequate review of the cytology and genetics of this species would require a space as great as that here devoted to *P. aurelia.* Within the limits of this review, only one of the two species could be treated in detail. The decision to omit *P. caudatum* was based on the fact that very little work has been done on it along modern lines, using the methods of cross-breeding made possible by the knowledge of mating types, and on the fact that the earlier work on this species shows essentially the same general phenomena as discussed in full in the section on *P. aurelia.* In the case of *P. aurelia,* the interpretation of the earlier work depends in large measure on recent work. It would, therefore, seem wise to defer a detailed review of the genetics of *P. caudatum* until such time as work along modern lines has progressed to the point where it can be used to assist in the understanding of the earlier work. We shall, therefore, limit the present account to a brief review of the status of the recent work.

143. Mating types have been found in *P. caudatum* by Gilman (49, 50), Giese and Arkoosh (48), and Y. T. Chen (21). The breeding system is essentially the same as in *P. aurelia;* there are a number of sexually isolated varieties, each containing two interbreeding mating types. Gilman found, in this country, 4 or 5 varieties and Chen found, in China, 4 varieties. Although the American and Chinese varieties have not been tested against each other, the results on *P. bursaria* with European and American varieties (paragraph 129) make it probable that the Chinese varieties of *P. caudatum* are different from those found in this country.

144. In view of the small amount of genetic material to be discussed, it does not seem worth while to go into detail at present concerning the possible cytological bases for them. It need only be stated that the nuclear processes have been studied by many investigators: conjugation by Maupas (106), Calkins and Cull (6), Dehorne (26), Muller (109), Penn (112); endomixis by Erdmann and Woodruff (37), Fermor-Adrianowa (38), Chejfec (7); cytogamy by Wichterman (163); and various other aspects of nuclear behavior by Klitzke (101, 102), Ilowaisky (59), King and Beams (99, 100) and Diller (30, 31). For present purposes it is important only to note that (1) conjugation follows essentially the pattern already presented for the other species, with minor differences in detail; (2) sometimes the conjugants fail to exchange gamete nuclei, each mate undergoing autogamy (cytogamy, Wichterman, 163); and (3) periodic nuclear reorganization occurs in the absence of mating. The latter has been described as endomixis, but, in view of the doubtful status of endomixis at present, this requires reinvestigation to ascertain whether the process might not be one of autogamy

instead. Great variations have been reported in chromosome number in this species; it seems likely that a polyploid series may exist.

145. Modern genetic work is limited to a few aspects of the inheritance of the mating types. The main facts are as follows. (1) Many clones remain pure for one mating type for long periods, but others undergo selfing at intervals of but a few weeks (48, 49, 50). (2) In Gilman's varieties 1 and 2, the purity of cultures is correlated with their mating types. Type I of variety 1 and type IV of variety 2 reproduce true to type for very long periods (many months or permanently); but the other mating type in each of these varieties (types II and III) reproduces true to type for only a few weeks. (3) Gilman (50) looked for endomixis or autogamy as a possible cause of the onset of selfing but was unable to establish such a correlation. He showed that at least some of the selfing was probably not due to the differentiation of a culture into two mating types as a consequence of nuclear reorganizations, for he was unable to isolate two diverse types from some of the selfing cultures. In other cases, two types could be isolated. In these cases, the observations did not preclude the prior occurrence of nuclear reorganization. On the whole, these observations seem similar to some of those known in *P. aurelia.* The cases in which selfing is not correlated with the presence of two hereditarily distinct mating types are comparable to Kimball's (93) cases of unstable caryonides, in which the diverse types arise during strictly vegetative reproduction. Further, in the *P. aurelia* varieties of group B (paragraph 102), such unstable caryonides are common, and one of the two mating types in each of these varieties remains pure for long periods, while the other is much more apt to yield a few clones with changed mating type when autogamy occurs. Possibly further investigation of *P. caudatum* will bring out even more clearly the parallel to the conditions in *P. aurelia,* particularly in the varieties of group B.

146. The most remarkable observation yet reported on *P. caudatum* is one by Y. T. Chen (21). Among a group of 78 pairs of conjugants obtained by crossing the mating types I and II of his variety 1 (probably different from Gilman's), 3 pairs yielded clones which could not mate either with the parental types I and II or with any of the other 4 mating types (of his varieties 2 and 3) that had been collected in nature. The other 75 pairs gave rise to clones of types I and II. The three exceptional pairs contained two new mating types (VII and VIII) that formed a fourth separate, sexually isolated variety of their own. According to Chen, therefore, the cross of two mating types of one variety can produce two mating types of a distinct variety. This observation is so unique and unparalleled in studies on Ciliates, that it seems desirable to wait for confirmation

before enlarging on its obvious implications with reference to evolution in these creatures.

V. Other Species of Paramecium

147. Genetic work along modern lines in other species of Paramecium is almost completely lacking. Mating types have been found in *P. multimicronucleatum* (46, 47, 48), in *P. trichium* (130), and in *P. calkinsi* (4, 130). These species show either two mating types per variety (as in *P. aurelia*) or multiple types (as in *P. bursaria*). Genetic work has been thus far limited to establishing the inheritance of mating type during reproduction within a clone and to demonstrating a difference in respiratory rate between two mating types in *P. calkinsi* (4).

VI. *Euplotes patella*

1. Introduction

148. The only genus of ciliated Protozoa other than Paramecium which has been subjected to cross-breeding analyses is the genus Euplotes. Fortunately, for purposes of comparative genetics, these two genera are very diverse; they probably differ as much as do a bat and a gorilla. Although mating types have been found (98) in at least three species of Euplotes, the only species employed in the modern genetic work is *E. patella* (Fig. 11a) and all of this work has been reported by Kimball and his student, Powers, in four important papers (94, 95, 96, 116). Prior to this modern work, much valuable work was done on the cytology, genetics and general biology of a species called by the investigators, *E. patella;* but Pierson (114) has shown that the organism used in those studies was really *E. eurystomus*, a closely related species, but one on which modern genetic studies have not yet been made. The following account will therefore be limited almost entirely to *E. patella*, on which no earlier cytogenetic work is recorded.

2. Cytological Basis of the Genetic System

149. E. patella (Fig. 11a) has one macronucleus and one micronucleus. The macronucleus is of special interest: it is C-shaped and very long. At the time of fission, a differentiated region, the reorganization band, known also by various other names, appears at each end of the macronucleus. These 2 bands are said to move gradually away from the ends of the macronucleus until they meet in the middle. Thereupon, the macronucleus shortens and thickens into an ovoid form, later elongating and dividing into two products, one of which passes to each daughter animal and reacquires the typical C-form. These remarkable transformations of the

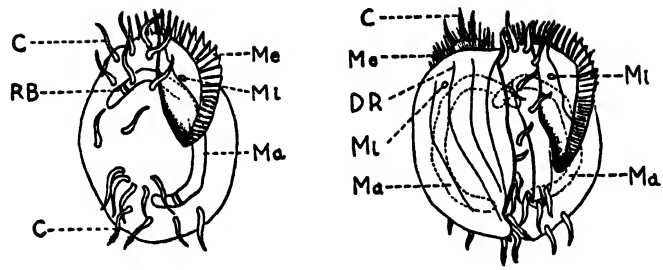

FIG. 11

E. patella

a — Ventral view of a normal individual. b —A double individual (based on Fig. 1 of Kimball, 95). The two members of the double animal face in opposite directions so that, in the figure, one sees the dorsal surface of the left member, the ventral surface of the right member. C — cirri. DR — dorsal ridges. Ma — macronucleus. Me — membranelles. Mi — micronucleus. RB — reorganization band.

macronucleus (found in many other related organisms, also) have received a number of interpretations by protozoologists, but their meaning is still not known with certainty. Without wishing to make a dark problem darker, one may at least raise the question as to whether the "progression" of the reorganization band may not represent a wave of mitoses passing through successive component subnuclei along the length of the macronucleus. The fission of Euplotes, as is characteristic of the group of Ciliates to which it belongs, also involves noteworthy processes with reference to the compound ciliary organelles, the cirri (Fig. 11). At each fission, all the cirri are lost and two new complete sets of rudimentary cirri arise in a definite order and location and then migrate to their proper positions in the two daughter animals. The reformation of new organelles at fission is thus even more striking in Euplotes than in Paramecium.

150. Taylor and Farber (161) removed the micronucleus from *E. eurystomus* and found this proved fatal to the organisms. That the micronucleus plays some direct part in vegetative processes in *E. patella* is also indicated by Kimball's observations (95). Double animals (Fig. 11b) have at their origin a macronucleus and micronucleus on each side (*i.e.*, in each of the two component animals); but the micronucleus on one side is sometimes lacking among some of their vegetative descendants. One micronucleus appears to be sufficient for a double animal. As in *P. aurelia* (Fig. 6b), the doubles occasionally produce one single animal from each side, and one of the two is, of course, amicronucleate when the parent double lacked the micronucleus on one side. These amicronucleate animals usually die, but some live and reproduce slowly. Kimball maintained 4 amicronucleate lines for considerable periods, one for 341 days (more than

100 fissions?). Moreover, amicronucleate animals always have a mating type and are capable of mating, even with other amicronucleate animals. These observations show that different lines of descent in the same species differ with respect to the effect of the amicronucleate condition: in some lines, the micronucleus seems to be indispensable, in others not, but in all its loss results at least in reduced reproductive rate, though apparently not in any impairment of the mating type or ability to mate. Differences from line to line in apparent effect of the micronucleus are also reported by Chen (19, 20) in *P. bursaria;* and reduced fission rate in amicronucleate lines of *P. aurelia* has been observed (paragraph 76). Altogether, the observations on *E. patella,* more uniformly than those on Paramecium, indicate some effect of the micronucleus during vegetative life, but the variability in the magnitude of this effect is difficult to understand.

151. The process of conjugation has been reported by Turner (162) for *E. eurystomus* and, according to Kimball (95), the description applies in most respects to *E. patella.* The general pattern of the nuclear processes is the same as in Paramecium (Fig. 2), with the following differences in detail. (1) The micronucleus undergoes one preliminary division before the maturation divisions commence. (2) Two products of the second maturation division, instead of only one, undergo the third maturation division (compare Diller's observations on *P. aurelia,* paragraph 26), but only two of the resulting four nuclei function as gamete nuclei. (There is some genetic evidence (96) that these two gamete nuclei are normally sister nuclei, because the two exconjugants of a pair usually produce clones of identical genotype. This evidence needs to be extended.) (3) Of the four nuclei produced by two successive divisions of the fertilization nucleus, two degenerate, one forms a micronucleus, and one develops into a macronucleus. In *E. patella,* the clone and caryonide are thus identical. (4) The old macronucleus does not develop a skein, but directly breaks up into several pieces. (5) Turner maintains that one of these pieces of the old macronucleus frequently or regularly fuses with and becomes a part of the developing new macronucleus. As will appear, this is an event of much genetic significance.

152. Three variations of the normal process of conjugation seem to occur under certain conditions. (1) From some of the genetic data (96, 116), it seems likely that cytogamy occasionally takes place: the conjugants fail to exchange gamete nuclei, each one undergoing self-fertilization or autogamy. (2) When double animals that have only one micronucleus conjugate on the amicronucleate side of the body with a normal animal, the unmated side of the animal (containing a micronucleus) simultaneously undergoes autogamy (95). (3) In the same cases, the amicronucleate half of the double reconstitutes its micronuclei and macronuclei from a hemicaryon;

and similar functional hemicaryons are observed in other special matings (95, 115). The hemicaryon is smaller than a normal syncaryon and should have only half as many chromosomes. When individuals with nuclei descended from a hemicaryon conjugated, Kimball found at late prophase of the first maturation division about 20 chromosomes instead of the usual number, approximately 40.

153. These observations on chromosome number at late prophase of the first meiotic division are important and remarkable. They indicate, it seems to the reviewer, one of two alternatives. Either synapsis has not yet taken place or synapsis has already taken place and the tetrads have separated into dyads. As some observers fail to find a distinct metaphase at the first maturation division in some Ciliates, the latter may be the correct alternative. In either case, it would appear that chromosome counts at this so-called late prophase give the diploid number. It would be possible to infer that the chromosomes at this stage were synapsed in tetrad condition, if the 40 chromosome number is polyploid; but the genetic evidence is strongly against this interpretation. This problem needs further investigation both in Euplotes and in Paramecium.

154. Besides conjugation and its variations (cytogamy and autogamy), no other special nuclear processes have been found in *E. patella* by Kimball (94). Search for processes like endomixis or autogamy in unmated animals gave only negative results. Kimball (95) also reports observations indicating that macronuclear regeneration does not occur in this species. When two amicronucleate animals mate with each other, there is no possibility of forming new macronuclei in the usual way, from micronuclei. Under such conditions, the old macronucleus fragments as usual, but the fragments do not get smaller and darker as they do normally at conjugation. Instead they swell up, become vacuolated and stain more lightly. Yet they never form new macronuclei and the organisms all die. So far as it goes, this indicates perhaps an incapacity for macronuclear regeneration, but, as already pointed out, amicronucleate animals are always weak and usually non-viable anyway. Perhaps, before completely rejecting the possibility of macronuclear regeneration, observations should be made, if possible, on the fate of macronuclear fragments in animals that contain micronuclei which have failed to give rise to new macronuclei.

3. Mating Types and the Breeding System

155. Soon after the discovery of mating types in Paramecium, Kimball (94) reported their occurrence in *E. patella.* Although at least two non-interbreeding varieties have been found in this species, only one of them has been studied in detail and these are all descended from two wild individuals which gave rise to cultures of the two mating types I and

II and, after intercrossing, to four other mating types, III, IV, V and VI. As an adequate search has not yet been made, it is possible that other mating types belonging to this variety may be found in nature. When cultures of the six different mating types are mixed together, two at a time, in all possible combinations, conjugation takes place in all the mixtures. That is, conjugation occurs when any type is mixed with any one of the other five, but not, as a rule, when two of the same type are mixed together. The breeding system thus seems to be like the one in *P. bursaria*, but, as will appear at once, there are striking differences, some of them fundamental.

156. Immediate agglutinative mating reactions, so characteristic of Paramecium, are totally lacking in Euplotes. No reaction appears for at least 1½ hours, and usually for a longer period, after two types are mixed together, and then no clumps, but only pairs, are formed. More important, the conjugating pairs in certain mixtures include some that consist of two individuals of the same clone, presumably of the same mating type: pairs, separated before they conjugate, yield clones of the same mating type. This was clearly demonstrated by Kimball by mixing a culture of double animals (Fig. 11b) of one mating type with a culture of normal single

TABLE III

Induction of Selfing in E. patella

Results of mixing animal-free fluid from cultures of each mating type with animals of each mating type. The plus sign indicates that selfing normally occurs, the minus sign, that it normally does not occur, in mixtures of fluid from the source indicated on the corresponding row, with animals of a type indicated in the corresponding column. The numbers 1, 2 and 3 represent the three conjugation-inducing substances assumed to be produced by the diverse mating types and to occur in the fluid in which they live.

			Cultures Observed for Selfing					
	Mating Type		IV	VI	III	I	II	V
		Substances Present	1	2	3	1, 2	1, 3	2, 3
Sources	IV	1	−	+	+	−	−	+
of	VI	2	+	−	+	−	+	−
Fluid	III	3	+	+	−	+	−	−
	I	1, 2	+	+	+	−	+	+
	II	1, 3	+	+	+	+	−	+
	V	2, 3	+	+	+	+	+	−

animals (Fig. 11a) of another mating type. The pairs that form in some such mixtures include matings of doubles with doubles, singles with singles and doubles with singles. Following this clue, Kimball (94, 96) discovered that animal-free fluid in which cultures of certain types have lived will induce animals of certain other mating types to conjugate among themselves. The results form a definite system, as shown in Table III. Fluid from cultures of each of the three mating types, IV, VI and III, induces animals of three other mating types to self, each being active on a different set of three types. Kimball postulates that these effects are due to the presence in these fluids of three different conjugation-inducing substances, each substance inducing selfing only on those mating types which do not produce the same substance. Thus, if type IV be assumed to produce substance 1, then it follows that this substance is also produced by types I, II and IV (because these are not induced to self by the fluid from type IV cultures); but it is not produced by types III, V and VI (because these types are induced to self by fluid from type IV cultures). In like manner, type VI may be assumed to produce substance 2 and it then follows similarly that this substance is produced by types I, V and VI, but not by types II, III and IV. And type III may be assumed to produce substance 3, from which it follows that this substance is also produced by types II, III and V, but not by types I, IV and VI. From the statements already made, the substances produced by all the types are definitely assigned: the types IV, VI and III must produce only one substance each, substances 1, 2 and 3, respectively. The other three types must each produce two substances: type I produces 1 and 2, type II produces 1 and 3, and type V produces 2 and 3. All the results with fluids from types I, II and V may then be predicted and the predictions are confirmed by the observations summarized in Table III.

157. Further support for this interpretation comes from two sources. Kimball (96) finds a general confirmation in observations on the amount of conjugation that takes place in mixtures of animals of two mating types: small proportions of animals conjugate when types are mixed of which one or the other is not induced to self by the fluid produced by the other; larger proportions conjugate when each type produces a fluid that makes the other type self. For example, smaller proportions of animals conjugate in mixtures between a type that produces only one substance and a type that produces both this substance and another, because the type that produces both substances is not induced to self. Powers (116) provides support for the hypothesis of conjugation-inducing substances in studies on the kinds of associations in conjugating pairs when mixtures are made between double animals of one type and single animals of another type; but he also reports a number of exceptions. For example, contrary to the

hypothesis, doubles of type IV (which produces only substance 1) induced selfing among singles of type I (which produces substances 1 and 2) and among singles of type II (which produces substances 1 and 3); and a few other exceptions of this sort are reported. Kimball likewise has found exceptions: selfing sometimes occurs in mixtures of two cultures of the same mating type and this may take place both in cultures of mating types that produce only one substance and in cultures of mating types that produce two substances. Consequently, both Kimball and Powers suggest that factors other than the postulated conjugation-inducing substances may also be involved in bringing about conjugation. Kimball has excluded, at least in some instances, the possibility that persistent changes of mating type occur in the exceptional selfing cultures, for if two animals from such a culture are separated and isolated when preparing to conjugate, they both give rise to cultures of the same mating type.

158. Although the fluids from the cultures are active in inducing selfing, according to the system shown in Table III, the relation of this induced selfing to cross-conjugation between the two mating types in a mixture is by no means clear. Mating reactions and conjugation are said to occur when animals of any type are mixed with animals of any other type; but this does not necessarily mean that the two types conjugate with each other, for the mixture is said to react if conjugant pairs of *any* kind are formed in it, even if they are exclusively selfing pairs from one of the two cultures that were mixed. One of the few explicit comments on this is the following quotation from Powers (116, p. 183). "On the basis of Kimball's observations on the importance of the nature of the fluid in relation to conjugation in Euplotes, one does not expect that in a mixture of any two different mating types there always should be formed pairs between the animals of different mating type. Indeed, the evidence indicates that, in certain mixtures, at times, in contrast to the situation described in various species of Paramecium, only one of the two kinds of organisms present conjugates at all." This idea is not further elaborated, nor are data anywhere given as to the system of combinations that yield this result. This uncertainty serves to emphasize that the term "mating type" is used in Euplotes in a different sense than in Paramecium. In Euplotes, two clones are said to be of different mating type if conjugation regularly occurs when they are mixed together, regardless of whether they interbreed or merely self.

159. In order to try to understand the cross-breeding system in *E. patella*, I have searched the data of Kimball and Powers for clues and find considerable evidence to support the following idea, which, while it may have been intended as a part of Kimball's hypothesis, was not explicitly stated. Suppose cross-breeding between two mating types normally takes

TABLE IV

The Assumed System of Cross-Breeding in E. patella

It is assumed that two mating types normally interbreed only when each produces a conjugation-inducing substance that the other does not produce. The plus sign indicates that, on this assumption, cross-breeding would occur in the mixture of the mating types on the corresponding row and column; the minus sign, that it normally would not.

Conjugation-Inducing Substances Produced	Mating Type	2; 3 V	3 III	1; 3 II	1 IV	1; 2 I	2 VI
2; 3	V	−	−	+	+	+	−
3	III		−	−	+	+	+
1; 3	II			−	−	+	+
1	IV				−	−	+
1; 2	I					−	−
2	VI						−

place only when each one induces the other one to self; *i.e.*, when each produces a conjugation-inducing substance that the other one does not produce. If this were the case, then the system of cross-breeding, unlike the system of unilateral induced selfing shown in Table III, would be as set forth in Table IV. Of the 15 possible mixtures between diverse mating types, 9 should interbreed, 6 should not. The papers of Kimball and Powers show that 5 of the 9 crosses, that are possible on this interpretation, have been made: I×II and IV×VI are reported by Kimball (96); I×III, II×VI, and III×IV are reported by Powers (116). There is also nothing in the published material to indicate that the other 4 crosses cannot be made. Of the 6 combinations of types that, on this interpretation, should not interbreed, Powers (116, Table 1, rows 7, 8 and 9) has data indicating that 4 of them actually do not interbreed (I×IV, II×III, II×IV, III×V): no conjugating pairs consisting of a double and a single animal appeared in mixtures involving these type combinations when one type consisted of double animals, the other of single animals. On the 5th combination (V×VI), I can find no information. The 6th combination constitutes an exception to my interpretation: Powers reports (116, p. 189) one cross of doubles of type I to singles of type VI. How serious this exception may be, is difficult for one who has not handled the material to decide. As mentioned above, there are unexplained exceptions also to the system of fluid-induced

selfing: sometimes cultures just self for unknown reasons. If the two types (I and VI) were both selfing, for similar unknown reasons, then crosses might well have taken place. This problem can be solved only by investigations directed expressly toward the point at issue. Meanwhile, there is sufficient support for the interpretation here proposed to accept it tentatively. To complete the evidence, the combination of types I and VI needs to be reinvestigated and the results of the critical combination of types V and VI need to be discovered. If the latter should prove to cross, the interpretation here proposed would be invalidated; but this would lead to another equally interesting possibility, for then Table IV would simulate the tables of relative sexuality reported by Hartmann and Moewus for Chlamydomonas and other Algae.

4. Genetics

160. Genetic studies on *E. patella* have centered about the inheritance of the six mating types and certain problems concerning the action of the genes determining them. One other pair of characters (alternative modes of swimming) has been analyzed genetically, but this shows no points of special interest and has been only briefly reported (97), so we pass at once to the mating types. As pointed out above, the uncertainty as to which animals are conjugating when two mating types are mixed together was overcome by employing one mating type of double animals and one mating type of normal single animals in the mixtures. The validity of this technique was established in great detail (95). The main facts on the inheritance of the mating types are presented in one paper (96).

161. The essential features of the system of determination and inheritance of the six mating types are brought out clearly in results obtained from selfing among individuals of one mating type, induced by means of fluid (with or without animals) from cultures of certain other mating types (Table III). Two of the mating types, IV and VI, yield, after selfing, only clones of the same mating type as the parent: IV×IV produces all IV; VI×VI produces all VI. On the other hand, when type I selfs, it produces three types of offspring in a 1:2:1 ratio: 1 IV: 2 I: 1 VI. Hence, mating type I is heterozygous for a pair of mating type genes, mt^1 and mt^2, which are present in homozygous condition in types IV and VI. The gene homozygous in type IV is arbitrarily designated mt^1; therefore type VI is homozygous for mt^2. In like manner, when mating type II selfs, it also produces three types of offspring in a 1:2:1 ratio: 1 IV: 2 II: 1 III. Hence, type II is also heterozygous for a pair of mating type genes that are homozygous in types IV and III. As type IV has already been shown to be homozygous for mt^1, this must be one of the genes in type II. The other cannot be mt^2; it must be a third allele, mt^3, which is

homozygous in type III. The constitution of the remaining type V is shown by the results of allowing it to self; it also yields three types of offspring in the 1:2:1 ratio: 1 VI: 2 V: 1 III. As VI is homozygous for mt^2 and III is homozygous for mt^3, V must contain mt^2 and mt^3.

162. This genic analysis is confirmed by two other matings. The cross of type IV (mt^1mt^1) by type VI (mt^2mt^2) should yield only one genotype, mt^1mt^2, and this must be type I. The cross of type I (mt^1mt^2) by type II (mt^1mt^3) should yield four types: IV (mt^1mt^1), II (mt^1mt^3), I (mt^1mt^2) and V (mt^2mt^3). These are in fact the results obtained. The expected kinds of offspring and no others were obtained in all crosses, except for certain minor discrepancies readily accounted for and one unexplained pair in one cross. The ratios also fit expectations reasonably well in nearly all crosses. In spite of the fact that only 7 of the 21 combinations of types have been crossed, the results appear sufficient to establish the correctness of the genic analysis. (If the suggestion made in paragraph 159 is correct, there are only 15 instead of 21 possible combinations of types, including the six selfings, that can be made to conjugate.)

163. Kimball's analysis of the genes determining the six mating types is of interest in several connections. It was the second demonstration of genic inheritance in ciliated Protozoa and the first demonstration of multiple alleles. Its great interest lies, however, in the relation between these alleles and the three conjugation-inducing substances postulated by Kimball (paragraph 156, Table III). There is a 1:1 correspondence between these three alleles and the three substances, as follows:

Mating Type	Genotype	Substances Produced
I	mt^1mt^2	1 and 2
II	mt^1mt^3	1 and 3
III	mt^3mt^3	3 only
IV	mt^1mt^1	1 only
V	mt^2mt^3	2 and 3
VI	mt^2mt^2	2 only

Kimball therefore concludes that each mating type allele determines the production of a corresponding conjugation-inducing substance and a correlated "immunity," as it were, to this substance, and that each allele acts independently, as in the well-known cases of genes controlling antigens and self-sterility. It is of interest that, during the period of immaturity (about 1 month), not only are the clones unable to conjugate, but no conjugation-inducing substances can be detected in the fluid in which they live (98). There is thus a correlation in time between ability to conjugate and ability to induce selfing in other mating types. Kimball (98) discusses the question of whether the same substance is responsible for both actions

and concludes that a decision is not yet possible with the evidence now available.

164. The further pursuit of the problem of gene action has been directed mainly toward the study of gene interaction in double animals (95, 116). These doubles, arising probably by incomplete fission followed by shifting of position until the two animals have a parabiotic orientation (Fig. 11b), have no membrane between the two sides: the cytoplasm is continuous from one component animal to the other. There is a macronucleus in each component and a micronucleus in one or both. Kimball observed (95) that, when a double animal produced a single animal from each component (in the same way as in the case of double animals of *P. aurelia*, Fig. 6b), the mating types of these single animals were sometimes different from the mating type of the parent double animal. This opened up a field of investigation which was explored in detail by Powers (116).

165. Powers found, in brief, that the phenotype of a double animal with separate nuclei was the same as if the kinds of alleles present in these nuclei had been together in one nucleus. For example, a clone of double animals was of mating type I, which is determined by the gene combination mt^1mt^2, and the two kinds of single animals produced from these doubles were of mating types IV (mt^1mt^1) and VI (mt^2mt^2). The types of the singles show that the nuclei on one side of the double contained only the gene mt^1, while the nuclei on the other side of the double contained only the gene mt^2, yet the double had the same mating type (I) as single animals with the genes mt^1 and mt^2 in one nucleus. In other cases, doubles of type I produced singles of types I and VI, indicating that the nucleus on one side had genes mt^1mt^2, while the nucleus on the other side had only gene mt^2 (possibly haploid). This case not only agrees in principle with the first, but also shows that there is no evidence of a dosage effect: doubling the dose of mt^2 in proportion to mt^1 gave the same phenotype obtained when the doses of the alleles were equal. Similar analyses were made of type II (mt^1mt^3) doubles that produced singles of types III (mt^3mt^3) and IV (mt^1mt^1); of others that produced singles of types II (mt^1mt^3) and III (mt^3mt^3); and of type V doubles (mt^2mt^3) that produced singles of types III (mt^3mt^3) and V (mt^2mt^3). All these observations agree in principle: the genes in separate nuclei but a common cytoplasm produce the same effect as if in the same nucleus, and no evidence of a dosage effect appears.

166. The fact that the singles produced from the doubles showed no effect of the common cytoplasm of the double, but had their phenotype determined solely by their nuclear constitution, eliminates the cytoplasm as an independent determiner of mating type. The facts that amicronucleate animals maintained their mating type and that loss of one micro-

nucleus had no effect on the mating type of doubles further shows that the micronucleus has no direct effect on mating type. This leaves only the macronucleus as the direct determiner of mating type, as in *P. aurelia* (paragraph 81). Powers points out in this connection a remarkable situation. If Turner's observation (paragraph 151) is correct concerning the incorporation of a fragment of the old macronucleus in the new macronucleus at conjugation, how can the determination of the mating type by the macronucleus be reconciled with the observation that it is the constitution of the new macronucleus alone that determines the mating type? It must be assumed that the action of the fragment of the old macronucleus has in some way been nullified.

VII. Concluding Comments

167. To the reviewer, the main impression made by this survey of the recent advances in the genetics of ciliated Protozoa is that this material provides great opportunities for investigation of the nature of the gene and of gene action. To be sure, these opportunities are just beginning to be seized and the material holds more of promise than of solid accomplishment. But it is already apparent that the macronucleus and the cytoplasm must be the focal points of the attack on these problems in material of this kind. If this be true, then anyone acquainted with the wide range of variations among the ciliated Protozoa with respect to the organization and behavior of the macronucleus and the cytoplasm, especially during mating, cannot fail to be impressed by the possibilities that await a full genetic exploration of the available materials. The two genera, Paramecium and Euplotes, that have thus far been investigated, do not begin to give an idea of the untapped resources that remain unexplored. A few examples may be cited to bring home the point. In genera like Metopus and Vorticella, there is regular cytoplasmic fusion at conjugation. In Dallasia, two sexual processes occur: copulation, involving complete fusion of gametes; and conjugation, involving only exchange of nuclei. In some species of Chilodonella, the macronuclei as well as the micronuclei are exchanged at conjugation. Loxodes has a small, simple macronucleus that divides after fission. Stentor has its macronucleus in the form of a string of beads, each bead being sufficient for regeneration and maintenance of life. Dileptus has its macronucleus in a permanently fragmented form, each piece being of the same order of magnitude as the micronuclei. Colpoda regularly extrudes a piece of the macronucleus at every fission. If these samples of the conditions to be found in different species of Ciliates be considered in relation to the problems that have already emerged in the recent investigations recounted in the body of this review, one can scarcely avoid concluding that here indeed is an endless source of favorable material

awaiting enterprising students intent on attacking the problems of the nature of the gene and gene action.

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Mutations in Wild Populations in Drosophila

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Contents

I. Introduction

Most biologists know that many mutations have been discovered in flies of the genus Drosophila. Few of them have any idea of the order of magnitude of the number of these mutations. The latest editions of several modern texts on General Biology state this number as somewhere between 500 to 1000. Actually the number of natural and induced muta-

tions found in this genus would be much closer to 100,000 and probably in excess of this figure. There are individual investigators who have discovered hundreds of natural visible mutants in Drosophila, and others who have certainly found well over 1000 natural and induced lethals.

Even fewer biologists realize that, at certain seasons, within a few hundred yards of almost any biology laboratory in the country there occur wild or semi-wild populations of Drosophila carrying many more than the 1000 mutants reported in the textbooks. These and other Drosophila populations provide a relatively untapped source of genetic variability, ready to be extracted and fashioned into the tools of the laboratory geneticist. Actually, certain kinds of mutants can be secured more easily from wild populations than by radiation or other methods of induction.

However, the main interest in the genetic variability of populations relates to its evolutionary significance. The writer hopes that through this review some teachers and particularly students may become interested in the possibilities of Drosophila population work as an approach to the study of the dynamics of evolution.

It would be relatively easy to present in summary form a chronological resume of the several investigations of genetic variability in wild populations of Drosophila. Rather, in the hope that the present review may prove useful in future research the author has chosen to discuss the different methods of population analysis, pointing out their limitations in connection with the nature of the information sought, the genetic tools available for study of a species and the results obtained for the labor involved. This is followed by a summary of the work which has been done on each species thus far studied.

II. Kinds of Mutations

The term mutation requires definition. In the broad sense it may include any change in the genetic consititution of an individual, or part of an individual, not due simply to recombinations of pre-existing genotypes. Thus addition or subtraction of whole chromosome sets, polyploidy or haploidy; addition and subtraction of single chromosomes, polysomics and monosomics; additions and subtractions of parts of chromosomes, duplications and deficiencies; and rearrangements of the chromatin within or between chromosomes, inversions and translocations, may be considered mutations. In contrast to the gross chromosomal changes mentioned there is a large and diversified class of mutations, the so-called point mutations, which have to do with inherited changes occurring in a narrowly delimited area within a chromosome. There is much evidence that many of these point mutations are actually minute chromosomal aberrations.

Our review will deal with the localized mutations, although these in

some cases may be associated with inversions and other gross chromosomal rearrangements — with mutation, therefore, in the narrow sense of the term. We make the assumption, furthermore, that the genetic variants found in wild populations are due to mutations which have occurred at some undetermined time in the past history of the population or of the genetic lines which have contributed to its structure. This review will deal primarily with the genetic variability present in given populations at the time of analysis and not with the rate or time at which such mutations have occurred. Conceivably, a population might be genetically stable in regard to the origin of new mutations and yet full of genetic variability. However, the admittedly inadequate studies of Drosophila populations, especially those carried on for several years, indicate that new mutations are arising and that the genetic variability of populations is due to inherited changes in the immediate as well as the more remote past.

Among the point mutations several categories may be recognized, though there is considerable overlapping in such classification due, in part, to changes in phenotypic expression under differing environmental and genetic modifiers. Such mutations may be divided into dominants and recessives, with many cases showing incomplete and variable dominance dependent upon the opposing allele. Penetrance may vary from 100% under all known genetic and environmental conditions to 0% under most conditions. Expressivity may be constant or variable depending largely upon the phenotypic nature of the character; so-called quantitative characters showing variable expression and qualitative characters little variation. Gene differences leading to all-or-none reactions will result in relatively constant characters such as many eye and body colors. Many bristle, venation, wing margin and eye shape mutants will show variable expression.

Mutations present in wild populations may also be classified in regard to their effect on the viability and longevity of the individual. Owing to the facts that mutation in Drosophila has largely been studied in the adult or imago stage and that eclosion represents a crisis in the life of the individual, by far the commonest classification of mutants has been into lethals and visibles. Lethals have somewhat arbitrarily been considered as those mutants which kill the individual in egg, larval or pupal stage. Some of these mutants may show visible characters aside from the lethal effect. Obviously all such mutants are recessive lethals, although some may have dominant visible effects. There is no reason to suppose that dominant lethals do not occur in wild populations just as they have been demonstrated in laboratory experiments. Conversely, many visibles have a lethal effect early in the normal life span of the adult fly and, in many other cases, mutants kill off many but not all of the individuals before eclosion. Many of these semi-lethals when they emerge show marked

abnormalities; others apparently normal in appearance and viability, are recognized only by the reduction in total numbers. For some of these semi-lethals the expression both in numbers of individuals and in the viability of survivors may vary widely with different environmental conditions. Under some conditions they may appear as true lethals, under others they may approach normal viability. Among visibles some mutations may be easily and accurately classified by anyone with the naked eye; others may be recognized and classified only by the trained observer with optimum magnification and illumination. In spite of the difficulties in the recognition and classification of many mutants, the border-line cases with low penetrance and variable expression, and the fluctuations due to genetic and environmental backgrounds, a large proportion of both lethal and visible mutants may be classified accurately and dealt with objectively.

In laboratory stocks of Drosophila visible mutations have been widely used in studies of linkage, chromosome mapping, relations of multiple alleles, factor interaction and many problems in physiological genetics. For studies on mutation rate and the induction of mutation by extrinsic factors, such as X-rays, temperature shocks and chemicals, lethal mutations have been used. In mutation rate studies more lethals could be found for the labor expended (a) because from 5 to 10 times as many lethals as visibles occurred and (b) with the use of certain genetic tool stocks each culture could be checked more quickly and objectively for the presence of a lethal than for a visible.

It is not, therefore, surprising that the same tools, which were first used in mutation rate studies in the laboratory by Muller (36), were later applied to the analysis of wild populations for the presence of lethals and with marked success, as reported in many of the studies below. The store of genetic variability in most wild Drosophila populations is, however, so rich in both lethals and visibles that either or both types of mutations may be sought with profitable results for the work involved. The nature of the information sought in regard to the genetic variability of a given Drosophila population should determine the tools and methods to be used. The beautifully exact methods used for the discovery of hidden recessive lethals are not those best adapted to reveal recessive visibles most efficiently. Where the total genetic variability of a population is to be adequately sampled a combination of methods will be needed. We shall outline below the several methods of population analysis and the type of information to be gained from each method.

III. Methods of Analysis for Visibles

1. *Direct Observation of Flies from a Population*

Where large samples of flies from a population can be secured by various trapping methods the individuals may be etherized and examined individually for aberrant phenotypes. Visible dominants in heterozygous or homozygous condition, sex-linked recessives in males, and homozygous recessives in both sexes may be found. The chance of finding mutant phenotypes is considerably increased where collections of pupae are made and the adults reared out, as most mutants are less viable than the wild-type. The aberrant individuals found, however, must be tested by breeding before they can be considered genetic variants, as phenocopies of many mutants may appear in wild populations under the rigors of natural conditions. The method yields valuable information on certain genetic factors which are species-specific in their incidence or variability, as light body color in *D. repleta*, bobbed in *hydei*, extra veins and bristles in *immigrans*, and extra bristles and trident pattern in *melanogaster*. In individual populations it may show the high incidence of certain recessive visibles. Valuable autosomal dominants and sex-linked recessives may be found. The discovery of homozygous recessives which are not found in large numbers in other populations of the same species indicates an adaptive relation between the mutant and the specific environment or more likely a population pattern with sharp bottle-necks at certain seasons of the year, followed by rapid expansion at other times. For some species and for some populations in other species, and particularly where large numbers of pupae can be collected easily, this method of analysis gives much valuable information. For other species and populations, even though large numbers of individuals are examined, little of interest will be found. In any case, the method gives little idea of the rich hidden store of recessive mutants present in heterozygous condition.

2. *Direct Breeding Test for Specific Visibles*

Male flies from a wild population or virgin females reared from wild pupae may be mated individually to flies homozygous for one or more recessive mutants and tested in this way for mutants at specific loci. This may seem like looking for the proverbial needle in the haystack. However, if flies from a stock containing six mutants at loci known to have mutated repeatedly in a species, for instance black, purple, vestigial, plexus, peach, spineless in *melanogaster* were to be crossed individually to 1000 wild individuals from a large population or several populations, the author would not find it surprising if one or more alleles of the tested mutants were found; in fact it would be more surprising if none turned up.

The method is particularly applicable, however, where a mutant has been found in a population by other methods and it is of interest to know its incidence from season to season. As approximately half the offspring from a heterozygous wild individual will show the mutant in the test culture such cultures can be checked very rapidly and for many mutants even without etherization. The method is applicable to the study of variations in gene frequencies at one or more specific loci in a population and, with improved culture techniques, will prove a valuable tool in studies of population structure.

3. *Inbreeding by* F_1 *Mass Cultures*

Wild flies from a population may be mated in pairs or offspring may be reared from wild females already impregnated before capture. In the latter case experimental studies on *D. melanogaster* indicate that the offspring from a given batch of eggs come from one impregnation. As this point has not been checked for other species a better procedure would be to separate males from females in a collected sample and then after several days mate them in pairs in fresh culture vials. The ensuing offspring will generally, if not always, come from the pair mating. In case both parents are heterozygous for a given recessive this will be expected to appear in about one-fourth of the offspring if the mutant approaches normal viability and no other disturbing factors are present.

If only one parent carries the recessive factor, the situation which will hold in the vast majority of cases, then the mutant character will first be observed in the F_2 generation by inbreeding F_1 individuals. The F_2 may be reared from one or more mass matings of F_1 individuals. While some mutants will be recovered in this way the method is not recommended unless the object is to recover only a few of the most viable types. Theoretically, if a large number of F_1 parents are used, half of them should carry the recessive factor and, assuming random mating, one-sixteenth of the F_2 flies should show the mutant character. Actually, because random mating in many cases does not occur and particularly because of crowding in mass cultures, even highly viable mutants seldom appear in the expected proportions and many fairly viable ones appear only in a few flies or the mutant is entirely swamped by larval competition. While the method of mass F_1 cultures can be used for a rough comparison of the genetic variability of two or more populations, it gives a poor idea of the total number of visibles present.

4. *Inbreeding by* F_1 *Pair-Matings*

A far more accurate and productive procedure is to make up one to many pair-matings of the F_1 flies and examine the F_2 for mutant types.

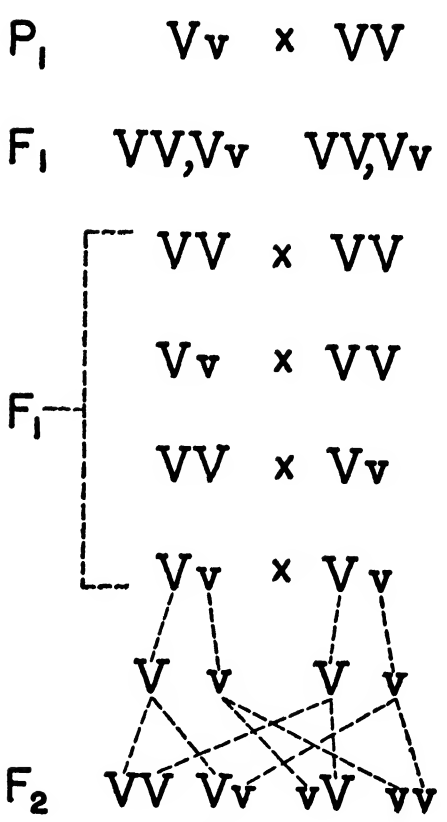

FIG. 1

Breeding Procedure for the Extraction of Visibles from Wild Populations. If one of a pair of wild flies carries a visible (*v*) then, on the average, in one-fourth of the F_1 pair-matings both parents will carry the visible and one-fourth of their offspring will be expected to show the character.

The number of F_1 pair-matings to be reared from each P_1 pair will depend upon the type of information sought and the ease with which samples of the populations being studied can be collected. The method is outlined in Fig. 1. Since one-half of the F_1 flies will, on the average, carry the mutant factor for which one of the P_1 parents was heterozygous the chance that any one mating will be of the favorable type with both F_1 parents heterozygous will be one-fourth. Table I presents the chances of at least one of the F_1 pair-matings being of the favorable type for 1–10 matings. If one is interested primarily in the total numbers of mutants carried by individual pairs of wild flies, then, obviously, a large number of F_1 pair-matings should be made up. With 10 F_1 pairs for each P_1 mating, it will be seen

by referring to the table that 94% of the visibles present in the sample tested should be found. In a favorable pair-mating one-fourth of the flies would be expected to show the character. Since, with good culture conditions, from 100 to 200 flies per culture will be reared, in testing most species 25 to 50 flies showing the mutant will be expected. The better culture conditions from less larval crowding than in mass cultures will allow a larger proportion of the expected mutant type to come through. Thus even many semi-lethal types will be represented by several flies in a culture. In case the sample from a population is small, large numbers of pair-matings will also be reared from each pair of P_1 flies in order to gain as much information as possible.

TABLE I

Per Cent of Autosomal Recessive Mutants Recovered from a Population Sample by Rearing 1 to 10 F_1 Pair-Matings of the Offspring of Each P_1 Pair and the Per Cent of Autosomals Recovered Per Culture Reared.

Cultures Reared			Per Cent Mutants Recovered	Per Cent Mutants Recovered Per Culture Reared
P_1	F_1	Total		
1	1	2	25	12.5
1	2	3	44	14.6
1	3	4	58	14.5
1	4	5	68	13.6
1	5	6	76	12.6
1	6	7	82	11.7
1	7	8	87	10.9
1	8	9	90	10.0
1	9	10	92	9.2
1	10	11	94	8.5

If, however, the maximum information concerning the visible mutants carried in a population is desired and large samples can be collected easily, then three F_1 cultures per original pair-mating should be reared. While from Table I it can be seen that the rearing of two pair-matings gives a little more information per culture reared (the 1 P_1 culture and the 2 F_1 cultures) the work of collecting the sample of wild flies must be considered. When this factor is added, the maximum information for the work expended comes from rearing three F_1 pair-matings. The breeding procedure which samples the mutants from a collection (with 3 pair-matings 58% of the mutants present should be recovered) rather than attempting to breed out all or almost all the mutants in the collection is recommended, for it then allows for the analysis of a correspondingly larger sample of the wild population and gives a truer picture of the genetic variability of the

population for the time and work expended. This is the method which should be used when populations of a species on which little genetic work has been done are being investigated. Whenever large samples of flies can be secured it will probably yield a supply of mutant stocks for autosomal linkage studies.

5. *Inbreeding by F_1 Pair-Matings Combined with Linkage Tests*

This method can be used only after some genetic work has been done on a species. It consists in mating wild males or virgin females reared from wild pupae individually to flies from a multiple mutant stock of the species bearing a good recessive marker in each of the long autosomes. F_1 pair-matings are then made up exactly as in method 4. Owing to the fact that no crossing-over occurs in the Drosophila male, any recessive mutant present in the fly being tested will segregate in the F_2 flies from the particular recessive marker belonging to the linkage group of that mutant. Moreover, the mutant will appear in double recessive combinations with all other marker genes. Failure to segregate from any marker indicates presence on the dot chromosome for which no marker is provided. Such mutants will be found rarely, owing to the small size of the dot. The determination of the linkage group to which a mutant belongs at the time it is discovered and with the rearing of only two generations of flies has particular value in case of many sterile or low viability mutants which may then be discarded. Segregation with markers also serves to distinguish between phenocopies and weak dominants on one hand and recessives on the other. The added information for many studies more than offsets the disadvantage that the method has of cutting in half the total number of mutants recovered per culture in contrast to method 4. It should be emphasized that markers should be chosen which are readily identifiable in double recessives and which will have little chance of being epistatic to new mutant forms.

6. *Locus Determination of Mutants in the F_3 Generation*

By an extension of method 5 it is possible in one generation, after the visible recessive is found, to establish its approximate position in the autosome in which it is located. This method is applicable not only to recessive mutants of good fertility but also to those which are completely sterile in both sexes. The marker genes for the multiple mutant tester stock should, if possible, each lie near one end of their respective chromosomes. The method may best be followed by referring to Fig. 2. Only the chromosome pair involving the new mutant to be discovered and located is figured. Let us assume that the new mutant is sterile in both sexes but of normal viability. (*m*) refers to the marker gene with normal viability

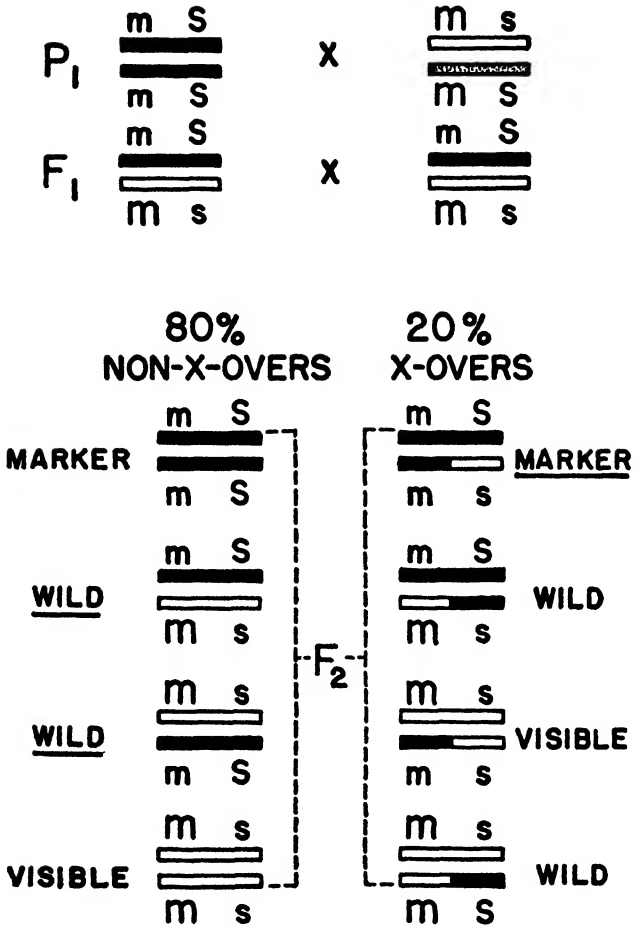

FIG. 2

Experimental Procedure for Determining the Approximate Locus of a Recessive Autosomal Visible in 3 Generations (1 Generation after Discovery). If a stock female homozygous for a visible recessive marker (*m*) in a pair of autosomes is mated to a wild male which happens to carry a recessive visible (*s*) in one of the homologous autosomes, then the linkage of (*s*) to (*m*) is determined by segregation of visible from marker phenotypes in the F_2 from an F_1 pair-mating in which both parents carry the visible, as there is no crossing-over in the male. If the new visible and the marker happen to give 20% crossing-over, then a mating of F_2 marker male to F_2 wild-type female will have a chance of .16 of being favorable for determining cross-over value between (*m*) and (*s*) in the F_2. Favorable marker male and wild-type females are underscored. See Table II for chance of favorable mating with other linkage values.

and fertility and (M) to its dominant allele in the wild chromosomes. (s) refers to the sterile visible mutant gene present in heterozygous form in the wild fly being tested and (S) to its dominant allele present in the wild fly in one chromosome and in both chromosomes of the marker stock. Let us further assume that this gene lies about 20 units away from the marker locus, that the two loci will give about 20% of crossing-over. From the F_2 flies whose genotypes are shown in the figure, homozygous marker males are mated to wild-type females in pairs. Ten such pair-matings are made up. The favorable type of pair-mating for determining linkage data will be one in which the marker male carries a cross-over chromosome and is of constitution $\frac{m\ S}{m\ s}$ and the wild-type female does not carry a cross-over chromosome and is of constitution $\frac{m\ S}{M\ s}$. The chance of any one pair-mating being of this favorable type is obviously $.2 \times .8 = .16$. Thus on the average 1.6 pair-matings out of the 10 will be of the favorable type where the linkage is as stated above.

The phenotypes and genotypes of the F_2 flies emerging from such a favorable cross are shown in Fig. 3. From 200 flies, on the assumption of normal viability made, 50 flies will appear showing the mutant character. Of these the ones also showing the marker character are cross-overs. If none of the 10 pair-matings shows the mutant type then a still larger lot of pair-matings of F_3 flies of which the males are marker and the females are wild-type may be made up. The failure of any mutant flies to appear among any of the F_4 cultures will show very close linkage of the new mutant to the marker gene.

In Table II the average number of favorable matings for cross-over counts out of 10 pair-matings is shown for linkage values running from 5% cross-overs up to 50% cross-overs. Obviously, if the mutant recovered in the F_2 is a valuable one, elaborate linkage tests using more genetic markers may be run. The method makes possible the approximate localization of many new mutants with a minimum of effort. 10 pair-matings are suggested on the basis of the average length of Drosophila autosomes already mapped. If linkage data are of relatively little importance in a given study, fewer matings could be made and, if of relatively great importance, more matings. Where the interest lay in the mapping of a particular chromosome multiple marker stocks for that chromosome could be used and only new mutants in that linkage group worked with.

For most population studies, and particularly in the many Drosophila species in which the autosomes each contain only one euchromatic limb, the identification of a mutant gene with a particular linkage group will be as far as localization studies need be carried. Such data will be of more

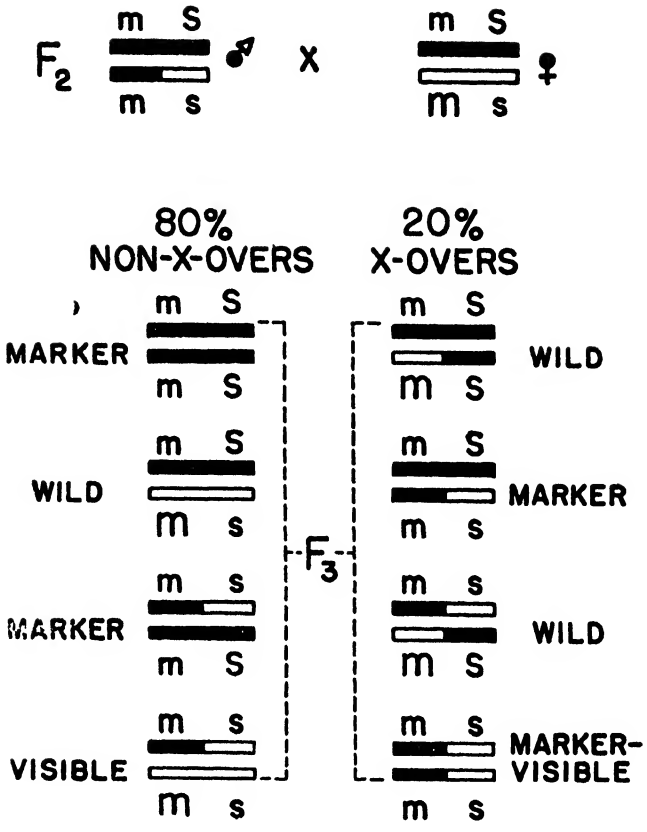

FIG. 3

The F_3 Generation from an F_2 Mating Favorable for Determining Linkage of a Marker (*m*) and a Newly-Found Visible (*s*). (See Fig. 2.) A tabulation of the visible and marker-visible phenotypes, disregarding all other flies in the culture, will give the approximate cross-over value between (*m*) and (*s*). The method is applicable to newly-found visibles, whether sterile or fertile. In a culture of 200 flies the Mendelian expectation will be 50 visibles. Even where fewer are found a rough locus determination for (*s*) can be made.

importance than exact locus determination for comparative studies, as there is a considerable body of evidence to the effect that chromosome elements or limbs have retained their gene complements during speciation but that the genes within a limb or element have shifted their position through intra-element inversions (see Sturtevant and Novitski 50).

In closing this section on the methods of analysis particularly applicable to the discovery of visibles present in wild populations, mention should be made of the somewhat elaborate formulae published by Gordon (27) for

the chance of recovery of a given mutant. It would seem to the reviewer that the application of such formulae to the analysis of data is less important than developing methods which may give fairly accurate comparative pictures of certain classes of visibles in different populations and in the same population at different seasons. A number of the points covered in the formulae are taken care of by the use of marker genes outlined in method 5. Certainly some workers are better adapted than others to recognize visibles but the pair-mating method in which many flies showing the visible character appear among 100 to 200 flies in a culture, greatly decreases the chance of a visible being overlooked. In any case there are large classes of mutants which can only be revealed by the use of special genetic or environmental sensitizers. Other mutants may be overlooked because their phenotypic effects are small. But a careful analysis of data from experiments where 7 F_1 pair-matings per P_1 culture have been reared and the F_2 flies examined indicates that the subjective error is much lower than many students of Drosophila have assumed. (See population analysis of *D. immigrans* below.)

TABLE II

Average Number of Favorable Matings for Linkage Tests in the F_2 out of 10 F_2 Pair-Matings of Marker Male ×Wild-Type Female for Cross-Over Values from 5 Per Cent to 50 Per Cent. (See text for full explanation.)

Per Cent Crossing-Over	Average Number of Favorable Matings out of 10
5	.5
10	.9
20	1.6
30	2.1
40	2.4
50	2.5

IV. Methods of Analysis for Lethals

We turn now to methods particularly applicable to the finding of lethal mutations in wild populations. While these methods can also be used in recovering visibles, most of the methods described above for visibles are poorly adapted for the demonstration of lethals. While lethals are generally more abundant than visibles, with the exception of species-specific variables often present in very large numbers, the demonstration of their presence in autosomes requires the use of special genetic tools.

7. *Recovery of Sex-linked Lethals by Direct Counts*

As a sex-linked recessive lethal results in the elimination of half the sons of any female which carries it, pair-matings from wild populations may be made and the offspring counted. A 1–2 sex ratio indicates that the female was heterozygous for a sex-linked lethal. Such a procedure is, however, time consuming and, as sex-linked lethals are relatively rare, owing partly to a low mutation rate but more largely to the high negative selection against them in a population, few mutants of this type will be demonstrated in most populations, even where they are full of autosomal lethals.

8. *Recovery of Autosomal Lethals from One Chromosome*

To demonstrate the presence of an autosomal lethal which has no other effect than the elimination in approximately equal numbers of homozygous males and females it is necessary to use a breeding technique whereby the absence of this class of flies from a culture can be recognized, in other words to have all flies not of this class marked phenotypically so

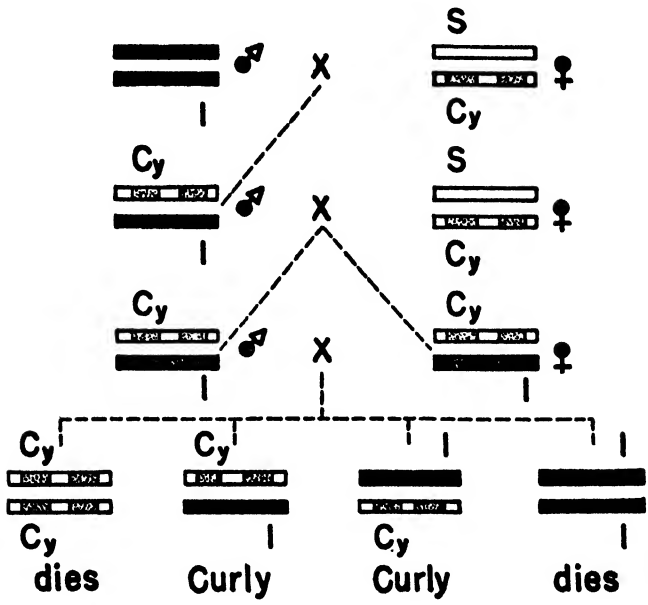

FIG. 4

Experimental Procedure for Detection of a Lethal in a Second Chromosome of a Wild *D. melanogaster* male. Wild chromosomes black; tester chromosomes white; inverted sections of latter stippled. Presence of a lethal (*l*) in tested wild chromosome shown by appearance of nothing but Curly flies in the final generation of the test. (See text for full explanation.)

that the absence of the lethal class is conspicuous when the flies from a culture are examined. One method in use for identifying lethals in the second chromosome of *D. melanogaster* is outlined in Fig. 4. It is assumed, for the sake of clarity in the explanation, that the wild male tested carries a recessive lethal marked (*l*) in one second chromosome. The test is limited to one second chromosome for each male. Reference to the figure will show that the wild male is first crossed to a tester stock female carrying a dominant gene, Star (*S*), in one second chromosome, and in the other second chromosome the dominant gene, Curly (*Cy*), and extensive inversions in both limbs of the chromosome which eliminate almost all cross-overs. From the F_1 generation a Curly male is selected, carrying one Curly chromosome and one second chromosome to be tested from the wild male. This male is crossed to a *S/Cy* female from stock. In the next generation Curly flies are mated together. These flies each carry intact the wild chromosome to be tested since crossing-over did not occur in their father and all homozygous Curly flies die. In their offspring two classes of flies will be expected, Curly and non-Curly, the latter being homozygous for the wild chromosome being tested. The presence of the lethal in this wild chromosome will result in the elimination of the non-Curly class. The presence of the inversions in the Curly chromosome eliminates crossing-over between the lethal and Curly. If the wild chromosome carries no lethal then one-third of the flies will be expected to be non-Curly or wild-type. If a few wild-type flies appear this indicates a semi-lethal killing off most, but not all, of the homozygotes. Such semi-lethals may also show visible characters. If the wild chromosome carries a visible but no lethal then all the non-Curly flies will show the visible character.

Some such method as this is necessary for the demonstration of autosomal lethals. It is distinctly inferior to the inbreeding methods outlined above for giving the most information on the presence of visibles for the following reasons. If a chromosome bears both a visible and a lethal then the visible will not be seen at all because of the elimination of crossing-over and the killing off of all flies homozygous for the visible. On the other hand with the inbreeding method which does not eliminate crossing-over, except in cases of very close linkage, some visibles will appear among the F_2 flies even when the visible gene is linked to a lethal. If, as has been demonstrated for some populations, 30% or more of the autosomes carry lethals, then 30% or more of the visibles present will never be seen if the mutations are distributed to the chromosomes at random. If, on the other hand, there is a tendency for mutations to be associated by non-random distribution, an even higher percentage of the visibles will be covered up. The number of lethals found will also be less than the true number if, in some cases, more than one lethal is present in

a chromosome. Furthermore, the additive effect of two semi-lethal or deleterious mutations in a chromosome where crossing-over is eliminated may be misinterpreted as due to a single lethal. All of these objections may be overcome by further breeding involving the chromosome in question but such tests are time consuming. Actually, the method gives an exact analysis of the viability potential of the gene complex within a whole chromosome. The main disadvantage of the method for the investigation of visibles, however, is that it samples too little material for the time and effort expended. By the inbreeding method one or two whole sets of autosomes are tested simultaneously in two generations, while only one chromosome is tested in three generations by the method of extracting a whole chromosome intact. The inbreeding method gives comparable information simultaneously on all the autosomes, and not on one or two autosome pairs. If it is argued that the use of refined genetic tools which extract chromosomes entire from a population is preferable to the apparently crude method of inbreeding with three F_1 pair-matings and calculating the total number of visibles present from the percentage recovered, it must be remembered that the whole procedure of analyzing populations is based on statistical sampling and not on an exact determination of all the variability present.

9. *Recovery of Autosomal Lethals from Two Chromosomes*

It is possible to use genetic tool stocks in which two wild autosomes, for instance, a second and a third chromosome of *D. melanogaster*, are extracted from a wild fly simultaneously and made homozygous in testing for lethals or visibles. But for species with more than two long autosome pairs the method would become cumbersome and impracticable for testing more than two autosomes at once, even providing the proper tool stocks could be synthesized. Special stocks can also be used in finding sex-linked lethals. Their use avoids the labor of counting individuals. Such stocks have been used extensively in mutation rate studies.

In concluding this section on methods of population analysis it should be emphasized that, whatever method is used, the genetic variability disclosed will represent only a fraction of that present. Thus, with the use of special environmental and/or genetic tools it is possible to demonstrate mutations which would escape detection by any of the methods outlined. The method of analysis to be used in a given study should be chosen with the following points in mind: (a) the size and availability of samples collected; (b) the type of information desired; (c) the maximum amount of comparable data for the work expended; (d) the possible value of material or facts found in the investigation of other problems.

V. Studies on Mutations in Wild Drosophila Populations

The author regrets that a part of the literature has not been available for this review. This is particularly true for a number of valuable studies carried on by Russian investigators and published in that language or in journals to which he has not had access. The reader should, therefore, consider this report as covering a large sample of the work which has been done and not as comprehensive. To those investigators who have done population studies on Drosophila and whose work is not mentioned here the author offers apologies. The report is divided into sections each dealing with work on a different species.

VI. Populations of the Subgenus Sophophora

10. Drosophila melanogaster *Populations*

As *D. melanogaster* has been used so extensively as a laboratory animal for genetic studies, it is not surprising that a larger number of investigators have undertaken the analysis of populations of this species than of any other. Even before Dr. T. H. Morgan and his students began their work on the genetics of this fly, Delcourt (8) had examined 8000 wild specimens for wing-vein abnormalities but found none. Instead of etherizing the flies he examined them under a binocular microscope in a constricted glass tube and a hollow, tapering glass prism. Perhaps his failure to find any abnormalities was partly owing to this crude method of examining flies. However, he did find a wing-vein mutant in *D. confusa* by this method. Lutz (30) collected *D. melanogaster* in New York City and 0.34% of them showed slight wing-vein abnormalities, which proved, on breeding, to be incomplete recessives subject to the action of genetic modifiers.

Chetverikov (5) seems to have been the first Drosophila student to have realized the extent of genetic variability in wild Drosophila populations and the underlying problems involved in the distribution of these variants. As his paper in Russian is not available, we quote this reference from Dobzhansky (10). "In a remarkable paper published in 1926, Chetverikov has outlined a program of studies on the genetics of natural populations of Drosophila. Owing to the well-known fact that most mutations are recessive and deleterious for viability, they may exist in natural populations without manifesting themselves except on rare occasions. Hence, a mere inspection of wild individuals is inadequate to detect mutations; genetic methods must be devised to reveal the concealed genetic variability. Only a quantitative study of this variability, taking into consideration factors of geography, ecology, subdivision of populations in semi-independent colonies, *etc.*, can furnish a true picture of population structure." In two brief notes Chetverikov (6; 7) has published the results

of his analysis by inbreeding of 239 wild *D. melanogaster* females from Gelendzik in the Caucasus. Thirty-two different visibles were found and two of these, "extra bristles" and a wing-vein mutant, "ramuli," were found respectively in the offspring of nearly 50% and 40% of the females.

H. A. and N. W. Timofeeff-Ressovsky (51) have published an extended and often quoted account of the analysis of a *D. melanogaster* population from garbage pails in a Berlin courtyard. They caught 78 females already impregnated, reared the progeny of each separately and then made from 3 to 13 pair-matings of the F_1 flies from each female, with an average number of 9 pair-matings. From this very extensive analysis of these 78 females they recovered only 10 different mutant types. Beaded, a weak autosomal dominant, was recovered from 12 females; venae plexoides, a very weak autosomal dominant, from 11 females; abnormal abdomen, weak sex-linked dominant, recovered 6 times; Venae abnormeis, variably expressed autosomal dominant, 5 times; sex-linked lethal[2] 3 times; alae divergentes, weak autosomal recessive, twice; and sex-linked lethal[1], the autosomal recessive venae incompletae, and the weak autosomal recessive venae bifurcatae each once. The finding of so few different mutants and the high incidence of several of those found indicates that the population had gone through a breeding bottle-neck, with reduction in numbers, from which it was expanding. The recovery of one sex-linked dominant and one sex-linked recessive visible is not surprising as they were both weakly expressed characters with many normal overlaps not, therefore, subject to rigid selection, particularly in an expanding population. Two sex-linked lethals out of 156 flies, females and males to which they had mated, is more than would be expected in normal populations, but the fact that one of these lethals was recovered three times indicates an expanding population with abundant food supply, a situation where selection against lethals would be low. The most remarkable thing about the population is not the high number of mutants recovered but rather the opposite. One female and her mate carried 5 mutants, two carried 3 mutants, and twelve carried 2 mutants. The authors considered this evidence for multiple impregnation. However, an analysis of the number of F_2 cultures in which the mutants were picked up out of the total F_2 cultures reared indicates that in most cases the offspring of a female came from a single impregnation. In the light of the genetic variability now known to occur in many wild populations it is not surprising to find one fly carrying two or more mutants, particularly when they have low penetrance and weak expression as did most of those found in this population. This particular population study has been discussed somewhat fully as it has so often been cited as out of line with the findings of other investigators. One extremely rare event was recorded, the finding of one female carrying a lethal in each of her X-

chromosomes, and one of these lethals associated with the gene, Abnormal abdomen. Her composition, $\frac{Ab\ l^1}{l^2}$, indicates that she had received l^2, already present in the population, from her mother, and l^1, a new lethal arising in the *Ab*-bearing sperm cell, from her father.

Gordon (27) has published an interesting account of the results of inbreeding and extraction of mutants from 13 *D. melanogaster* females and 12 *D. subobscura* females collected in the summer of 1933 from the grounds of the Biological Field Station at Slough, England, and from 9 *D. melanogaster* females and 17 *D. subobscura* females from the same place in the summer of 1934. From the *D. melanogaster* collections he bred out in the F_2 cultures 15 recessives of which one was a sex-linked visible. As only 4 F_2 cultures per female were reared, only about 70% of the mutants present in the flies would be recovered. On this basis about 21 should have been present and from F_3 matings 4 more mutants or a total of 19 were recovered. Gordon considers his findings out of line with those of the Timofeeffs, as the latter reported a sex-linked dominant and autosomal dominants. Actually one of the mutants recovered twice by Gordon, rough eye, was a weak dominant. The main difference between the two populations was a larger number of different mutants and fewer recurrences of the same mutant for the size of the sample analyzed in Gordon's study. This would indicate that the English material came from a more diffuse and less inbred population.

The chief value in Gordon's work is not the small body of data presented but his extended discussion of the factors involved in the deviations from Mendelian expectation generally encountered when a mutant is found in an F_2 culture. He proposes a term, coefficient of realization, for the ratio between the proportion of flies scored as mutant phenotypes in a culture and the Mendelian ratio expected. Factors pointed out as determining the coefficient of realization are linkage of a visible to a lethal or presence of a lethal in the homologous chromosome, penetrance and expressivity influenced by genetic and environmental modifiers, dominance relations and the subjective ability of the investigator. Gordon's paper should be read carefully by anyone planning to analyze populations for visibles by the inbreeding method. His analysis of the relation between the number of F_2 cultures in which a given mutant is found and the problem of polyandry indicates that the F_1 flies from his females impregnated in nature came almost invariably from a single impregnation, a fact in line with experimental studies on this subject made by Nonidez (39), Nachtsheim (38) and Dubinin (21) on *D. melanogaster*.

Recognizing the validity of the factors involved in the coefficient of realization, the author of this review believes that more valuable data

will be accumulated in most studies on population analysis of visibles by omitting the laborious individual counts of F_2 flies and settling on some standard, for instance the appearance of three flies showing the visible character in a culture as indicating the presence of a mutant. Where marker genes are used in linkage tests of the mutants at the time of discovery (see method 5 above) many problems dealing with dominance relations and the identification of variants as true mutants will be solved.

11. *A Study of Russian Populations of* D. melanogaster *by Method 1*

By far the most ambitious study of variation in flies from wild populations by direct examination of collected samples has been made by Dr. N. P. Dubinin and collaborators on flies collected in 1933, 1934 and 1935 and reported by Dubinin, Romashov, Heptner and Demidova (24). The flies were collected each year in September from fruit stores and vinegar factories. The collections came from 11 widely separated regions and, in all, contained 129,582 individuals. The three yearly collections from Gelendzhik totaled 31,725 individuals. The variants found are shown in Table III as a sample of the type and proportion of changes recorded. It will be noted that "trident" is a species-specific variant found in large numbers in the three Gelendzhik collections and incidentally in all others. The variants found from year to year differ in percentage, although in many cases this might be due to sampling errors. There seems, however, to be a real change in total percentage of variants probably not due either to subjective or sampling errors. In all 2700 variants, excluding "trident," were found in the 129,582 flies. Thus 2.08% of the flies were characterized by some morphological change of observable magnitude. The proportions of variants in different collections ranged from a high of 6.84% to a low of 0.49%.

A genetic analysis of all variants found in the 1933 collections was carried through. In Table IV a summary of these findings is given. The column marked phenocopies contains those cases in which, tested through at least two generations, the variant type did not reappear. Some of these cases may have been somatic mosaics, though most of them were probably changes produced by temperature, moisture conditions or other factors in the environment. Such phenocopies are seldom met with in laboratory cultures reared under optimum conditions, but many have been produced in large numbers by temperature shocks and other factors (see Goldschmidt 26). Among those variants which proved to be inherited, all were found more than once and several appeared many times and in different geographical areas. There were 5 different dominants and 9 different recessives. An analysis of wild chromosomes from many of the flies collected showed that for some of the recessives, for instance, divergent wings, the number

TABLE III

Visible Variant Types Found by Inspecting 31,725 Wild D. melanogaster *from Gelendzhik, Russia; Populations of 1933, 1934 and 1935.* (From Dubinin and collaborators.)

	Samples of Gelendzhik *D. melanogaster* populations					
	Year 1933	Sample 10,000	Year 1934	Sample 14,765	Year 1935	Sample 6,960
Variant	Total	Per Cent	Total	Per Cent	Total	Per Cent
1. Trident	2,096	20.96	1,096	6.95	1,001	14.3
2. Extra brist.	252	2.52	94	.64	15	.21
3. Small brist.	102	1.02	13	.09	..	..
4. Semi-small brist.	101	1.01	33	.22	4	.06
5. Brist. comb	19	.19	..	..	..	..
6. Wavy brist.	55	.55	..	..	..	..
7. Reduced bris.	11	.11	..	..	..	..
8. Small eye	37	.37	..	..	5	.07
9. Rough eye	41	.41	1	.01	..	..
10. Dark eye	16	.16	..	..	5	.07
11. Mottle eye	5	.05	1	.01	..	..
12. Sepia eye	2	.02	..	..	..	..
13. Garnet eye	1	.01	..	..	..	..
14. Dark body	3	.03	7	.05	1	.01
15. Yellow body	1	.01	..	..	..	..
16. Dachs legs	1	.01	..	..	..	..
17. Extra vein	5	.05	10	.07	..	..
18. Analis inc.	16	.16	..	..	..	..
19. X-veinless	2	.02	..	..	2	.02
20. Upturn wing	14	.14	12	.08	..	..
21. Diverg. wing	..	..	1	.01	..	..
22. Extra-x-vein	..	..	..	..	1	.01
23. Extra-analis	..	..	8	.05	..	..
24. Extra-media	..	..	..	..	1	.01
25. Light eye	..	..	1	.01	..	..
26. Notch wing	..	..	2	.01	..	..
27. Truncate	..	..	1	.01	..	..
28. Comma	..	..	1	.01	..	..
29. Tumor	..	..	5	.04	..	..
Total changes (trident exc.)	684	6.84	190	2.07	34	.49

of homozygotes appearing in the population was equal to the number expected from the percentage of heterozygosis of the population for the gene in question. These studies indicated that a considerable number of flies in the Russian populations were morphological variants, that many of these changes were due to mutation and that populations differed somewhat from year to year and from place to place in the proportions of these mutant types.

TABLE IV

Results of Genetic Tests of Variants Found in the Gelendzhik and Other 1933 Populations. Phenocopies, Non-Inherited Variants, Tested through 2 Generations. (From Dubinin and collaborators.)

Variant	Total Tests	Phenocopies	Mode of Inheritance
Trident	Not tested		
Extra brist.	Not given	..	Semi-dominant
Small brist.	43	43	
Semi-small brist.	63	49	17 Dominant
Brist. comb	9	..	9 Dominant
Wavy brist.	19	19	.....
Reduced brist.	16	14	2 Recessive
Small eye	29	29	.....
Rough eye	30	25	5 Recessive
Dark eye	14	12	2 Recessive
Mottle eye	9	2?	3 Recessive; 4 dom.
Dark body	9	4	5 Semi-dominant
Plexus-II	12	..	12 Recessive
Analis inc.	14	..	14 Recessive
Upturn wing	7	7	.....
Diverg. wing	11	2	9 Recessive
Extra-analis	2	..	2 Recessive
Yellow body	1	1	.....
Garnet eye	1	..	1 Recessive

Note. — In F_1 under laboratory conditions, phenocopies were rare.

12. *A Study of Russian Populations of* D. melanogaster *by Method 9*

Dubinin and collaborators (22; 23) also carried out a very extensive analysis of samples from populations in 10 localities in the Caucasus and one in central Russia for mutants present in heterozygous form. They used a stock for testing which carried Curly and cross-over suppressors in one second chromosome and Lobe in the other; Dichaete-Stubble in one third chromosome and cross-over suppressors in the other. A mating system similar to the one described in method 9 was used, whereby all lethals in the second chromosomes tested were found and all visibles in the second and third chromosomes, except those carried in the lethal-bearing second chromosomes, could be found. In a total of about 4000 second chromosomes tested they found that over 10% of them carried lethals. The number of visibles recovered was much higher than that of lethals. However, two of these visibles, "extra bristles" and "comma" mark on the thorax, formed the bulk of all visibles recovered. While it is true that these two visibles were absent from one or two collections, it seems to the reviewer that

they come under the designation of species-specific or at least geographic-specific variants and that the data gain in value for comparisons between populations when such variants, forming in population after population the bulk of the mutants recovered, are omitted from the tables or treated separately. When this is done the ratio of lethals to visibles falls pretty well in line with that of other studies.

A special study of the Gelendzhik area was made in 1933, 1934 and 1935. 7.98% of 877 second chromosomes carried lethals in 1933; 12.86% of 616 second chromosomes in 1934; 8.78% of 797 chromosomes in 1935. No sex-linked lethals or visibles were found among a total of almost 4740 sex-chromosomes tested. This seems much more remarkable than the finding of several sex-linked mutants in the material analyzed by the Timofeeffs. An examination of the data (Dubinin and collaborators 23) shows that lethals and visibles were looked for by counting F_1 offspring from each female tested. There were 83,384 offspring from 2370 females or an average of 35.2 flies per culture. With the variation which must have occurred in numbers from culture to culture it seems likely that many of the test cultures contained too few flies to recognize a lethal by counts of males and females even if it were present. Nor does the small experiment involving an exact test of 576 X-chromosomes reported as confirming their findings settle the point. Their failure to find sex-linked lethals in this very large experiment has often been quoted as evidence for an extremely low incidence of this type of mutant in natural populations. Sex-linked lethals certainly have a lower concentration than autosomal lethals in wild populations but other investigators have repeatedly found them and there is no reason to believe that they are not continually occurring, although subject to rigid selection under most environmental conditions.

Cross-tests of 51 of the 70 lethals found in the Gelendzhik population in 1933 showed that 22 different lethals were represented once, 5 twice, 1 four times, and 3 five times. Similar tests of the 78 lethals found in 1934 gave 45 lethals represented once, 5 twice, 3 three times, and 1 nine times. Of the 33 separate lethals of 1933 and the 55 lethals of 1934, 9 were identical in the two lots. In all, only 34 different visibles were found in over 4300 autosomes tested, but some of these were recovered many times. Aside from "extra bristles" and "comma," only 2, "divergent" and "crossveinless," were recovered all three years.

The data reported indicate that the Gelendzhik population was a continuous one, but the high concentration of a few mutants, both visibles and lethals, shows that the population underwent sharp reduction at one or more seasons of the year, followed by expansion. The other Russian populations sampled seemed to be of much the same breeding structure, although, as might be expected, each population appeared to have its own

peculiar constellation of mutant genes with a few widely spread mutants common to all. This extensive study of both visibles and lethals carried on over a period of years has added much to our knowledge of the natural variability of this species.

13. *A Large Scale Study of American Populations of* D. melanogaster

Ives (29) has reported the results of an analysis of several American populations of *D. melanogaster* carried out on a scale comparable to that of the Russian experiments. His work included a study of lethal mutation rate in several stocks derived from the wild populations.

He confined his investigations to mutants present in the second chromosome, both visibles and lethals. Two collections came from a farming district in South Amherst, Massachusetts, one made in October, 1938, and the other in September, 1941. Two collections were made from a fruit orchard in Winter Park, Florida, one in April, 1940, and the other in March, 1942. One collection was made in a fruit warehouse in Massillon, Ohio, in September, 1941, one near a restaurant kitchen in Gallup, New Mexico, in September, 1941, and one from a refuse dump near Belfast, Maine, in September, 1938. The Florida collections were made in the spring and all the others in the fall. Ives used a breeding technique similar to that outlined in Fig. 4. He classified as lethals all chromosomes which failed to appear as homozygotes among 200 test flies from two generations, as semi-lethals those which gave between 0 and 17% homozygotes, and as deleterious mutations those which gave between 17 and 30% homozygotes where 33% of the flies were expected to be of this class if no viability-lowering mutant were present. He found that most of the semi-lethal mutants and very few of the deleterious class were marked by visible effects. Very few visibles that were not at the same time deleterious or semi-lethal were found. This is in line with the known lowered viability of most visible mutant types.

An extremely high incidence of lethals and semi-lethals grouped together was found in all populations, ranging from a low of 34.2% in the Maine stock to a high of 67% in one of the Florida collections. The data on lethals and semi-lethals found are given in Table V. Of the 1202 chromosomes tested 663, or 55.2%, showed lethal or semi-lethal effects. As there are two second chromosomes in each fly, these data indicate that, on the average, there were more than one second chromosome lethal or semi-lethal per fly in the samples of the populations studied. Assuming an equal number of such mutations in the third chromosomes, an average of more than two autosomal lethals and semi-lethals per fly was present.

To analyze further the probable structure of some of the populations studied, Ives made a large scale test of the identity of many of the pure

TABLE V

Results of Lethal Tests on 1202 Second Chromosomes of D. melanogaster *from 6 Wild Populations from the United States and from the Maine 42 Stock after 3½ Years of Laboratory Culture.* (From Ives.)

Collection	Chromosomes Tested	Number of Lethals	Per Cent Lethals
Massachusetts 38	151	68	45.0
Massachusetts 41	108	64	59.3
Florida 40	227	152	67.0
Florida 42	110	68	61.8
Ohio	177	88	49.7
New Mexico	203	126	62.1
Maine 38	115	59	51.3
Maine 42	111	38	34.2
Total	1202	663	55.2

lethals present in the samples from these populations. These data are shown in Table VI. He first made a study of allelism in lethals arising spontaneously in the second chromosomes of two laboratory stocks. As seen in the table, in one sample of 75 newly-arisen lethals in Lab stock 2, 7 cases of two lethals arising at one locus, lethal alleles, were found. In the Massachusetts 38 and 2 Florida stocks he concludes that the rare cases of recurrent lethals were due to independent mutation rather than to common origin and inbreeding in the populations. He considers the one

TABLE VI

Frequency of Different Lethal Genes Determined by Cross-Tests within a Sample of Lethals from Each Population and within Two Samples of New Lethals Arising in the Laboratory. Not quite all the cross-tests were made for the Lab 2 and the Florida 40 samples. (From Ives.)

Origin	*le* Chrom's	*le* Genes	Frequency of Appearance							
			1	2	3	4	5	6	7	13
Lab 1	27	27	27	0	0	0	0	0	0	
Lab 2	(75)	68	61	7	0	0	0	0	0	
Massachusetts 38	49	46	43	2	1	0	0	0	0	
Florida 40	(50)	46	42	4	0	0	0	0	0	
Florida 42	47	41	35	6	0	0	0	0	0	
Ohio	48	44	42	1	0	1	0	0	0	
New Mexico	48	19	7	3	4	3	0	1	1	
Maine 38	41	10	2	1	1	1	1	3	1	
Maine 42	34	17	13	1	0	1	1	0	0	1

case in the Ohio collection in which a lethal was recovered 4 times as probably due to inbreeding in part of the fruit warehouse population. A test of lethals present in the New Mexico sample and in stocks which had been established from the Maine 38 collection at the time of the collection and 3.5 years later, showed many lethals represented three or more times, and evidently due to inbreeding in the stocks and not to recurrent mutation. This type of analysis requires a tremendous amount of work in cross-testing lethals as most of these are indistinguishable from one another phenotypically. It might be mentioned here that the number of cross-tests involved, $\frac{n^2-n}{2}$, where n is the number of lethals being tested for allelism, runs into the thousands where adequate samples of lethals from a population are involved. To test 100 lethals 4950 cross-tests are necessary. Ives actually made 10,125 cross-tests of lethals in these studies.

On the basis of the large number of lethals present and the non-identity of these lethals, Ives has concluded that the American populations of *D. melanogaster* in the north as well as in Florida are continuously large. Certainly the structure of the American populations seems to differ radically from that of the Russian populations studied by Dubinin and collaborators. It may be noted, however, that lethal cross-tests have been made on only three of the northern collections, that from Belfast, Maine, which did show indications of inbreeding; that from the fruit warehouse in Massillon, Ohio, where some degree of inbreeding was indicated, and the collection known as Massachusetts 38. To quote Ives on the nature of this collection: "The first collection, Mass. 38, consisted of about 400 flies, mostly old in appearance, which were caught early in October of 1938 in a half-pint milk bottle culture of fly food during a two-day exposure period on the porch of a farmhouse harboring several bushels of decaying fruit. The fruit had not been decaying long enough to culture a generation of flies. These must have flown in from neighboring orchards which, through the late summer months, swarmed with literally hundreds of thousands of flies, but which at this time were nearly barren of both fruit and flies because of fruit harvesting and the first severe frosts of fall." During September and October in good fruit years in the northern United States *D. melanogaster* populations expand to enormous numbers and may approach a panmictic condition with subpopulations overlapping. The way in which this Massachusetts collection was made from migrants from surrounding orchards rather than flies taken on the breeding grounds would favor heterogeneity. We suspect that the analysis of a spring collection made at one point where the flies were breeding would show a somewhat different picture. It is certainly true that *D. melanogaster* undergoes enormous changes in the size of the populations throughout

the year, but Ives' very extensive study seems to have established that the over-wintering populations of this species in the northern United States are much larger than was formerly supposed and that the species may over-winter outdoors. There is, however, an earlier upsurge in population size in our rapidly breeding true native species, such as those belonging to the *affinis* group, than in *D. melanogaster*, indicating an over-wintering survival of the former in greater numbers than of the latter.

In September, 1940, the author (Spencer, unpublished) collected and inbred 102 *D. melanogaster* from 6 grocery stores in Wooster, Ohio. He recovered 20 different visibles from the 408 long autosomes tested and, on the basis of numbers of F_2 cultures reared, an average of 4 visibles went undetected. In contrast, Dubinin and co-workers found only 34 different visibles in over 10 times as many autosomes analyzed from Gelendzhik populations over a three-year period. Very little recurrence of identical mutants was found in the Wooster material. It seems likely that there was a real difference in the Wooster population structure and that of Gelendzhik, a difference of the same sort as that demonstrated by Ives in other American populations of *D. melanogaster*.

14. *Mutation Rate and Selection Studies in* D. melanogaster *Populations*

R. L. Berg has been interested in the possible role of mutations found in natural populations of *D. melanogaster* in the evolution of the population and perhaps eventually of the species. She tested (Berg 1) 94 lethals and semi-lethals extracted from wild populations in the Nikita Botanical Gardens, Crimea, Delizhan, Armenia and Kashira, near Moscow, and found that six of these were more viable in heterozygous condition than a vigorous standard stock known to be free of lethals. She argues from these and other data that mutants which may be deleterious in homozygous form may have above normal vigor as heterozygotes, particularly under certain environmental conditions.

Berg (2) studied a wild population of *D. melanogaster* from two canneries in Kashira. Among 1282 wild males taken, 7 or 0.55%, carried visible sex-linked recessive mutations. Laboratory bred females from the Kashira population showed a high sex-linked visible mutation rate. Among 5349 males from these females 15 sex-linked visibles were found, including 12 mutations at the yellow locus. Apparently this population differed from all those populations studied by Dubinin and resembled more closely the population studied by the Timofeeffs in respect to sex-linked mutants. Possibly mutator genes, like those demonstrated by Demerec (9), Neel (37) and Mampell (31), account for such differences in general or locus-specific mutation frequency.

Berg (4) has carried out an excellent study indicating a change in the

TABLE VII

Variation in the Per Cent of Aberrant Forms in the Phenotype of a Population along with its Numerical Growth (Uman Distillery), and in the Phenotype along with its Numerical Reduction (Nikitsky Gardens Distillery). (From Berg.)

Population	Time of Collection	Size of Collection	Per Cent of Aberrants
Uman Distillery	July 22–29, 1937	3,793 flies	1.48 ± 0.20
Expanding Population	July 31–Aug. 25, 1937–Optim. Env.	6,787 flies	4.97 ± 0.26
Nikitsky Gard. Dist.	Oct. 21–Nov. 9, 1937–Optim. Env.	4,771 flies	8.70 ± 0.41
Contracting Population	July26–Aug.2,1938	554 flies	2.53 ± 0.67

stringency of natural selection on dominant mutations in a population during different periods of the seasonal population cycle. Her data on two populations studied are shown in Table VII. It is clear that the proportionate number of visible dominants was greater in the population at the time when food and other environmental conditions were at an optimum, when competition and natural selection were at the lowest ebb. This worker has clearly realized and amply demonstrated the interplay between the genetic variability present in populations and the seasonal ecological factors to which those populations are subjected.

Berg (3) has studied mutation rates in *D. melanogaster* populations of various breeding structures and comes to the conclusion that the mutation rate is higher in populations broken up into micro-populations and subject to inter-group competition than in more isolated populations. Taken at face value her data seem convincing enough. Yet one must carefully sift fact from theory and remember that, while the conclusions are based on large scale studies in regard to the number of flies examined, the actual number of populations studied is very small. Until her data receive corroboration from other and independent studies it would seem wise to suspend judgment on the validity of the conclusions drawn.

15. *Polygenes in Populations of* D. melanogaster

On the basis of selection experiments on the number of bristles on the fourth and fifth abdominal sternites of *D. melanogaster* Mather (32; 33; 34) has emphasized the difference between major mutations in oligogenes, producing so-called qualitative effects, and minor mutations in polygenes, acting on so-called quantitative characters. The discussion of these poly-

genes is in line with what has long been known concerning the subjects of multiple factors and systems of linked genes dealt with in breeding for quantitative characters in such plants as wheat and cotton. It seems to the reviewer that there is little need for the introduction of new terms at this point; that many, if not most, so-called oligogenes will react as polygenes and often in more than one polygenic system (see Dobzhansky and Holz 14). A series of cases could be demonstrated in many populations ranging from monogenic phenotypic effects through digenic and trigenic to polygenic effects. The reason digenic and trigenic characters are relatively unknown is because of their difficulty of analysis and not because they are absent. Many monogenic factors, for instance, the lethals found by Ives, Dubinin and others, have marked negative selective value in certain population patterns. On the other hand the balanced polygenic combinations discussed by Mather could have positive selective value, particularly if they were held together by close linkage or by inversions. Mather's work serves to demonstrate constellations of genetic changes present in wild populations and not demonstrated by the cruder methods of population analysis by which genetic variants with larger phenotypic effects are brought to light. In view of the rarity of major inversions in wild *D. melanogaster* autosomes it is not clear how polygenic systems covering the length of one of the long autosomes could hold together long enough to serve as potent factors in natural selection, unless the sudden origin of such a system by crossing-over resulted in the initiation of an isolating mechanism. In the populations studied by Ives it is difficult to see how the balance between homozygous and heterozygous polygene combinations could possibly be considered without at the same time taking into account the lethal, semi-lethal and deleterious oligogenes with which the populations were saturated.

16. *Conclusions from the* D. melanogaster *Studies*

The several investigations of *D. melanogaster* populations in Russia, Germany, England and the United States indicate that they all contain a large amount of hidden genetic variability; that the individual populations differ, however, in their mutant gene contents, and that there are real differences in population structure under different geographical and environmental conditions. The author feels that the apparent contradictions in the findings of different investigators regarding proportions of dominants and recessives and of total numbers of mutants reflect (a) the results of different methods of sampling; (b) sampling at different seasonal points in the population cycle; (c) real differences in population structure under different environmental conditions. The extremely short life cycle of this fly and its close association with the complex activities of man are

both likely to lead to variety in population size and breeding structure over short time periods.

In concluding this section on mutants in *D. melanogaster* populations it should be pointed out, as Dobzhansky (10) has long since done, that all of these studies have been carried out on the species outside of its native range. Actually *D. melanogaster* is an introduced and semi-domestic form in all of the geographical areas where the studies reported have been made. We turn now to a large scale study of a species in its native habitat.

17. Drosophila pseudoobscura *Populations*

The studies on the genetic variability of *Drosophila pseudoobscura* populations have been so voluminous and comprehensive in scope, so logically planned in temporal sequence and so thoroughly and painstakingly made that it will be impossible in this brief review to report them adequately.

Dobzhansky and Epling (13) have presented the facts concerning the taxonomy, geographic distribution and ecology of this species. *D. pseudoobscura* is native to the western United States, ranges southward into Mexico and Central America, northward into British Columbia and eastward to central Texas, Nebraska and western South Dakota. Over much of the semi-arid West, populations are found concentrated in wooded mountain ranges and sparse or absent in the intervening desert regions. Even more attention has been paid to the presence and distribution in wild populations of chromosomal changes in the form of inversions and combinations of inversions, particularly in the third chromosome (Dobzhansky and Sturtevant 18; Dobzhansky 12), than to the presence and distribution of gene mutations. Obviously investigations of these two types of mutants have often been carried on simultaneously. We shall confine our review to the work on point mutations.

Preliminary studies on the species had shown that lethals were present in some of the autosomes of wild strains when Sturtevant (47) undertook to determine the lethal frequency in the second and third chromosomes. A breeding method similar to that employed by Dubinin and Ives for *D. melanogaster* was used. For the third chromosome lethals a single male to be tested is crossed to a female homozygous for the third chromosome recessive, orange eyes. An F_1 male is then mated to an orange-Scute-purple female from stock. The non-orange flies from this cross carry the third chromosome to be tested and an orange-Scute-purple chromosome. When two such flies are paired all of their offspring are orange-Scute-purple and wild-type. If a lethal is present in the chromosome being tested the wild-type does not appear except for a few cross-overs. Crossing-over is largely eliminated in most tests because of the numerous inversions in the wild

third chromosomes of this species, generally, though not always, resulting in a different gene sequence in the wild chromosome from that in the tester chromosome. Where cross-overs do occur, lethals can still be detected as Scute lies near the center of the chromosome and the non-Scute class will be much lower than expected when a lethal-bearing chromosome is tested. The addition of another dominant marker, Blade, to the tester chromosome has added to the efficiency of the method in later studies on lethals. The second chromosome tester used was less efficient due to the absence of inversions.

Sturtevant found a total of 7 lethals in 37 second chromosomes tested and 43 lethals in 216 third chromosomes tested. The chromosomes came from old laboratory wild stocks, the Rocky Mountains, the Mexican Plateau and southern California. He thus found that about 20% of the wild autosomes carried lethals and the incidence was about the same for the chromosomes from different sources.

Dobzhansky and Queal (16) tested 849 wild third chromosomes, coming from ten different forested mountain ranges in the Death Valley region of California, and found that 127 of them, or 15%, carried recessive lethals or semi-lethals. They also found that an additional 39% of the wild chromosomes contained factors which reduced the viability below normal when homozygous. Over 4% of the chromosomes not carrying pure lethals and, therefore, subject to test carried visible mutants. If the other long autosomes, the second and fourth chromosomes, contained an equal number of visibles this would mean that, on the average, one fly in four from these populations carried a visible autosomal recessive. This is the proportion of autosomal visibles which the author has found to be present in the average population of several other species. The populations all showed concealed genetic variability of about the same magnitude.

On testing 38 wild chromosomes from Guatemala and 82 from Mexico, Dobzhansky (11) found that about 30% of them carried lethals or semi-lethals. He concluded that the higher incidence than in the Death Valley populations was due to the greater effective breeding size of the Mexican and Central American populations, resulting in a slower rate of lethal elimination.

At this stage in the investigation Dobzhansky teamed up with Dr. Sewall Wright, whose classic studies on the statistics of evolution in Mendelian populations (Wright 54; 55; 56; 57) formed a theoretical background for the remainder of the work. Dobzhansky and Wright (19) reported extensive tests for allelism of lethals found in the 10 desert mountain ranges of the Death Valley region. Seven hundred seventy-two cross-tests of 123 lethals from these regions, in which each cross-test was between two lethals from the same locality (intra-locality tests) revealed

an average of 3.11±0.42% allelism for all the localities tested. The 105 different lethals found by these tests were then tested for allelism with all the lethals coming from other mountain ranges and the inter-locality tests revealed an average of only 0.407±.061% allelism. Furthermore, the results of the tests showed that "the lethals found repeatedly within a population are not significantly commoner in the species at large than are the apparently rare lethals while, *vice versa*, the lethals recovered from samples from two or three localities show no tendency to accumulate in any particular sample." These observations showed that the accumulation of lethals in a population is largely based on the breeding structure of the population rather than on differential mutation rates.

Extensive mutation rate studies, in which 40 mutations were found in 13,472 chromosomes of 18 Death Valley lines and 25 mutations were found in 7699 chromosomes of 11 Mexican and Guatemalan lines, indicated that there was no marked difference in the frequency of origin of lethal mutations in the two regions. The conclusion that population structure and not mutation rate accounted for the difference in accumulated lethals in the two regions was thus confirmed. Using the data on mutation rate, accumulation of lethals in the populations, estimated number of mutating loci in the third chromosome, postulated migration into the population, inbreeding and selection against heterozygotes, Wright concluded that the effective breeding size of the populations in the Death Valley areas from which the samples were taken was less than 2500.

Wright, Dobzhansky and Hovanitz (58) reported 179 lethals from 1292 wild third chromosomes from nine collecting stations in three widely separated localities on Mount San Jacinto in Southern California. There was no significant difference in lethal frequency among the localities, stations or collections made at different seasons of the year. Thousands of inter-cross tests for allelism showed that it was lowest where the two lethals came from different localities, higher where they came from the same locality, and highest where they came from the same station. The effective size of the population for the largest locality where collecting was done, an area of about two square miles, was estimated at about 20,000 or 30,000 individuals.

A study of genetic variability in the second and fourth chromosomes of wild flies collected on Mount San Jacinto showed an even higher percentage of lethals and semi-lethals than in the third chromosomes (Dobzhansky, Holz and Spassky 15). Most of the remaining chromosomes tested carried factors which, in homozygous form, lowered the vitality or the rate of development, or showed visible characters. From the figures for all the long autosomes it was possible to calculate that less than 3% of

the flies in the population studied carried no factors which, in homozygous form, would be markedly deleterious.

An extensive experiment by Dobzhansky and Spassky (17) was designed to test the viability of 26 second and 22 fourth chromosomes in homozygous condition under a variety of environmental conditions, including temperature changes and different population densities, and in the presence of systems of genetic modifiers. Great differences in viability under different conditions, both genetic and environmental, were demonstrated. For instance, one chromosome when homozygous was lethal at 25.5° C., semi-lethal at 21° C., and nearly normal at 16.5° C. From these data it may be concluded that the population unit of *D. pseudoobscura* carries a reserve of genetic variability sufficiently diverse and extensive to meet the exigencies of seasonal changes in the environment to which it is subjected.

Experiments on the release and subsequent trapping of marked and mutant flies (Dobzhansky and Wright 20) have added information on the rate of migration and the population density of flies on Mount San Jacinto. These studies, in conjunction with the work on lethal alleles in the populations, have made possible for the first time an extensive application of Wright's theories on the distribution of genes in animal populations breeding under natural conditions. We can only mention that comprehensive studies on the geographical distribution of inversions in the third chromosome and on morphologically different Y-chromosomes have been carried out parallel to the work reported here. This monumental work on the mutations in wild populations of *D. pseudoobscura* will long stand as an important landmark on the road to a clearer understanding of the dynamics of evolution.

18. *Populations of Species Closely Related to* D. pseudoobscura

Small samples of a population of *D. subobscura* from the Biological Field Station at Slough, England, were collected in the summers of 1933 and 1934 and analyzed for visibles by the pair-mating inbreeding method (Gordon 27). Gordon found 24 visibles carried by 29 impregnated females. On the basis of the number of F_2 cultures examined the sample of 58 flies examined carried about one visible mutant per pair of flies. One pair carried 3 mutants and seven pairs carried 2 mutants each. Three mutants were found twice in the 1934 collection, indicating a population with small effective breeding size.

Gordon, Spurway and Street (28) analyzed three southeast England populations for visibles by inbreeding. From 47 impregnated females from Slough they extracted 19 autosomal recessives and one sex-linked recessive. From 42 New Forest females they recovered 16 autosomal recessives and

from 55 Studland females they secured 20 autosomal recessives. About 20% of the flies analyzed carried recessive visibles or a little less than one recessive visible per four flies. Certain minor venation changes, not included in the above summary, were found many times in all three samples. These showed weak dominance in many cases and diverse phenotypic effects in the presence of genetic modifiers and may be considered species-specific characters. Mutants at one eye color locus, poppy, were found in all three populations and twice in one of them. In general, each population appeared to carry its own group of mutant genes but all were alike in the amount of heterozygosis present.

Sturtevant (48) has published genetic data on *D. affinis* and states that "wild females have been brought in, and F_2 generations reared from each one separately. In this way a number of mutant types have been isolated." These strains came from many widely separated regions of the eastern United States. Of the 31 autosomals reported 11 have been recovered from F_2 cultures and most of the rest from wild stocks soon after their collection. While Sturtevant has made no quantitative determination of the percentage of heterozygosis for visibles in the populations his work has demonstrated that wild flies of this species frequently carry visibles in heterozygous condition. In fact, Sturtevant was one of the first students of Drosophila to recognize that wild strains carried visibles in heterozygous form and has bred out mutants from many species by the F_2 mass culture inbreeding method.

In 1922 Sturtevant found a strain of *D. affinis* in which certain males gave almost exclusively female offspring (Morgan, Sturtevant and Bridges 35). Gershenson (25) described a strain of the European species, *D. obscura*, which exhibited the same behavior. Males carrying the mutant X-chromosome produced few sons, regardless of the female to which they were mated. Females, either heterozygous or homozygous for this X produced a normal complement of sons and daughters when mated to normal males. This "sex ratio" factor has been found by Sturtevant and Dobzhansky (49) in wild strains of *D. pseudoobscura*, *D. persimilis*, *D. affinis*, *D. athabasca* and *D. azteca*. They showed that the factor was associated with an inversion in the X of *pseudoobscura*, *persimilis* and *affinis*. In *D. pseudoobscura* the "sex ratio" factor showed a high concentration in populations from the southern area of distribution. Dobzhansky (12) has found that the "sex ratio" X of *D. pseudoobscura* carries three inversions in the right limb and that one strain with the two sub-basal inversions but without the terminal inversion did not carry "sex ratio." In *D. azteca* he has also found three inversions in the "sex ratio" bearing X. How such a factor can attain a frequency in the neighborhood of 20% in some populations is difficult to understand and no explanation has been found for the north-south

gradient of distribution. However, the widespread occurrence of the factor not only in *pseudoobscura* populations but in related species indicates an ancient origin in the evolution of the group.

VII. Populations of the Subgenus Drosophila

19. *Populations of* D. repleta

Sturtevant (46) found that about 17% of the wild specimens of *D. repleta* taken in New York City carried a sex-linked factor, light, which reduced the size of the dark spots around the bristles on the thorax. Heterozygous females showed an intermediate phenotype so that the mutant may be described as an incomplete recessive. As the character is expressed in heterozygotes there is evidently little selection against it in populations. Sturtevant (Morgan, Bridges and Sturtevant 35) reported that about 15% of the specimens taken in New York City in 1923 and 1924 were light. C. V. Beers found the same mutant in Los Angeles and the author has found it in populations in Wooster, Ohio, and in Pittsburgh. It is evidently a species-specific character, although absent from some populations. Wharton (53) in a study of sexual isolation in 7 stocks from widely separated areas of Texas and stocks from Connecticut, Guatemala and Ankara, Turkey, did not report the light character. The author has found it only once in extensive collections of the closely related species, *D. hydei*. Wharton, however, did find genetic heterogeneity for sexually isolating genes widely spread in the species, with no correlation between the point of origin of the geographical strains and the degree of isolation between two strains. No attempt has been made to study the general genetic variability of populations of this species.

20. *Populations of* D. immigrans

Dr. A. H. Sturtevant first called the author's attention to the fact that wild strains of *D. immigrans* often carried a net-like venation mutant and supplied a stock of an extreme "net" mutant which had been extracted by inbreeding from a female collected near Azusa, southern California. The author (Spencer 42) made a comparative study of genetic variability in five populations of the species by the crude technique of rearing F_2 mass cultures from pairs of flies from samples of these populations. From a population from Azusa, California, breeding on a citrus dump, 16 distinct visibles were recovered from 60 wild flies tested. In contrast, only 4 visibles were recovered from 156 flies from Woods Hole, Massachusetts. The greater genetic variability of the Azusa population was interpreted as due to the larger effective breeding size. From what is known of the ecology of this fly it seems certain that its populations in the northern part of the

United States undergo severe winter reduction and pass through a sharp bottle-neck each year. The data secured from populations from Camp Rincon in the San Gabriel Mountains, Gatlinburg, Tennessee, and Wooster, Ohio, were consistent with this hypothesis. In all, 362 flies were tested and 32 mutants recovered, an average of about one mutant per 11.3 flies. The number of mutants recovered would probably have been much larger if F_1 pair-matings had been made up. Net-veins was found in 4 of the 5 populations and may well have been present in the other population from which only two mutants were recovered from the small sample of flies tested. It seems that "net" is a species-specific mutant widely spread through populations of this species.

In some respects the most interesting wild population analyzed by the author was one of this species in New Wilmington, western Pennsylvania. While trapping for *D. robusta* in a small woodland plot in this village in early September, 1944, a large sample of *D. immigrans* was taken from three traps a few yards apart. The species was very abundant in tomato patches in the village and the collection may be assumed to be a sample from the surrounding area rather than a population breeding at the point where the collection was made. The results of inbreeding 55 pairs of these flies by the F_1 pair-mating method are shown in Table VIII. This table is also presented to illustrate the statistical analysis of variability in populations by the F_1 pair-mating method. The vertical columns represent the 1st, 2d, 3d, *etc.*, pair-matings of the F_1 of each original mating. It will be noted from the total mutants recovered in each vertical column that, within the limits of experimental error, about the same conclusions can be derived concerning the variability of the population from each vertical column.

The method furnishes a check on the subjective error introduced by the tendency of the investigator to overlook mutants. If this were large the totals in the columns 4, 5 and 6 would be greater than for the early columns as, obviously once a mutant is found in an early column, it will not be overlooked in a later one, whereas if mutants are overlooked in early columns they may still appear in the later ones. The writer has repeatedly used this check and concludes that with the numbers of mutant flies appearing in the F_2 cultures there is little chance of overlooking them in any one culture. He believes that this method furnishes a valid check on the subjective factor. This is not to say that some mutants will not be missed; that will depend upon the magnification used and the time spent in examining cultures; but as any method yet devised reveals only a certain class of mutants the subjective error for that class need not be large and is itself subject to this objective test.

On the basis of the first 5 columns 76% of the mutants should have

TABLE VIII

Analysis of 55 P_1 Pairs of D. immigrans *from New Wilmington, Pa., by 7 F_1 Pair-Matings Each.* Mutants appearing in F_2 cultures shown. Only numbered P_1 pairs from which mutants were recovered tabulated.

P_1	F_1-1	F_1-2	F_1-3	F_1-4	F_1-5	F_1-6	F_1-7
2	..	..	..	Brick	..	..	..
4	..	..	cveinless	..	..	Brick	..
5	Minute	..	Stubble	..	Minute	..	..
6	..	..	..	Stubble	..	..	Stubble
9	..	Brick	..	..	..	..	..
15	..	Dubonnet	..	..	Stubble	..	..
17	..	..	Brick	..	Brick	..	..
18	..	..	Stubble	Stubble	..	..	Stubble
19	Brick	Brick	..	..	..	..	..
21	..	..	..	..	..	Stubble	..
22	..	Stubble	Brick	Stubble	..	..	..
24	..	..	..	Stubble	Stubble	..	..
(26)	Stubble	..	Stubble	(Stubble)	Stubble	..	..
28	..	..	..	..	Sepia-spineless	..	..
29	..	Brick	..	..	..	..	..
30	Stubble	Stubble	..	..	..	..	..
32	Broken	Stubble	Broken Stubble	Broken	..	Broken	Purple net-short
33	..	..	..	..	Stubble	Stubble	..
34	..	..	Tiny	..	..	Tiny	Tiny
35	..	..	..	..	..	..	Stubble
36	..	Dubonnet	..	..	..	Dubonnet	..
38	..	..	..	..	Brick	..	..
40	Stubble	..	..	..	Stubble	..	Stubble
42	Double	..	..	Double	..	Double	..
43	..	..	Stubble	..	Stubble	..	Stubble
46	2-Bristle	2-Bristle stubble short-5	..	Stubble	2-Bristle short-5	Short-5	Short-5
47	..	Stubble	..	..	..	Stubble	..
48	..	..	Small	..	..	..	
51	Stubble	..	..	Purplish-thin-sing	..	Stubble	Stubble
52	..	Stubble	Stubble	..	..	..	..
Total	9	13	12	10	12	10	9

Note. Both parents of P_1 26 were heterozygous for stubble. Both parents of F_1 26–4 were homozygous stubble.

been recovered from the sample (See Table I). There were 36 mutants recovered, which would indicate that there were about 47 present in the sample. Had 2 more pair-matings, or 7 in all, been reared, then from Table I it will be seen that 87% of the mutants present should have been found. In other words, 2 more pair-matings should have added 11% of the total mutants present, or 5 more mutants. Actually 4 of these appear for the first time in columns 6 and 7 and we can assume that the 7 not recovered at all were present in the sample and would have turned up if an indefinitely large number of F_2 cultures had been examined. It will be noted that relatively more information concerning the population could have been gained for the work expended by making 3 F_1 pair-matings rather than 7. Had the extra work been put into analyzing a sample twice the size of the original one by 3 F_1 pair-matings for each pair of flies more knowledge would have been gained concerning the population. However, this would not have given as complete information on the maximum numbers of mutants carried by an individual pair of flies.

Another point of interest is the validity of the data from the sample in regard to the incidence of individual genetic factors in the population. The factor, stubble bristles, not a species-specific character, was recovered 17 times in the first 5 columns. It would then be present in about 22 of the flies analyzed or in approximately 20% of the flies in the sample. This gives "stubble" a gene frequency of 10% in the population, a higher frequency than any non-species-specific visible or lethal found in any Drosophila population yet analyzed. About the same conclusions as to the incidence of "stubble" would have been drawn whether 3, 5 or 7 F_1 pair-matings had been reared. However, inspection of the table will show that the figure for the incidence of "stubble" would only have been half as great had the first 23 pairs of flies been analyzed than had the last 22 pairs been studied. Thus, even for this gene with a high frequency, our sample is perhaps still too small. Obviously most of the samples in the experiments reported in this review have been too small to give an adequate picture of the variability at a single locus. Unless much larger samples are analyzed the proportions of individual genes in the populations studied are not established. One gains an impression from some of the work published that invalid conclusions have at times been drawn from inadequate sampling. It will be noted that another gene, brick eyes, was recovered 8 times and dubonnet eyes was found twice. Taken in conjunction with the small number of different visibles recovered, the data on "stubble," "brick" and "dubonnet" indicate a population with a small effective breeding size, probably due to sharp winter bottle-necks. Adequate allelic tests on "stubble" and "brick" showed that two loci and not a series of mimics were involved.

It might be pointed out that, had 36 lethals rather than 36 visibles been extracted from the population, a total of 630 cross-tests for allelism would have been necessary before gaining comparable information concerning the genetic structure of the population. Tests by rearing 2 F_1 mass cultures were run concurrently with the above experiment and less than one-fourth as many mutants found, indicating clearly the superiority of the pair-mating method in recovering mutants. Finally, it may be noted that the presence of lethals in the population was observed through high and low counts of "stubble" in some of the segregating cultures. Where the chromosome carrying "stubble" also carried a lethal the "stubble" count was low; where the chromosome opposite "stubble" carried a lethal the count was high, provided both parent flies of a culture carried the lethal-bearing chromosome.

21. *Populations of* D. robusta *and* D. funebris

The author (Spencer, unpublished) has recovered 89 visibles from 632 wild *D. robusta*, the samples having come from 10 wild populations from widely separated points in Ohio. In many cases 1, 2 or 3 F_1 pair-matings were made up. Analysis of the data indicates that in this species in this part of its breeding range an average of one fly in five carries an autosomal visible. None of the populations showed a high incidence of individual mutant genes, which is consistent with the facts that the species is very abundant in all woodland areas in the state, is truly native and spring collections show large over-wintering populations in contrast to *D. immigrans*, *hydei*, and other introduced forms.

Timofeeff-Ressovsky (52) has reported on geographical temperature races in *D. funebris*. This experiment does not deal with the discovery of individual mutations in the populations studied, but rather demonstrates that races of the same species may develop genetic complexes which result in physiological characters adapting the organisms to some particular environmental factor. The strains came from the British Isles, northern Europe, the territories surrounding the Mediterranean and several widely separated points in Russia. In each culture bottle he placed an equal number of eggs of the strain being tested and of *D. melanogaster* so that the larvae would be competing with those of the latter species. Such cultures of each strain were reared at 15° C., 22° C. and 29° C. The ratio of emerging *funebris* and *melanogaster* gave an index of viability at each temperature for each strain of *funebris*. It was found in general that northern strains had a higher viability index than their southern relatives at low temperatures, while the reverse was true when the strains were reared at high temperature. The experiment was not designed to demon-

strate the effect of individual genes and it seems likely that polygenic combinations were responsible for the differences.

22. *Populations of* D. hydei

The author (Spencer 40) reported a population of *D. hydei* from Wooster, Ohio, in which, among 1843 males collected, 120, or 6.5%, were vermilion eyed. Representative tests showed the character to be sex-linked vermilion. Among 1898 females, 5 were vermilion where 8 would have been expected from the proportion of vermilion-bearing X-chromosomes carried by the males. Populations of this species distant from one another as much as two miles were found to contain vermilion in proportions approaching that reported. The gene persisted in appreciable numbers in Wooster *hydei* populations for at least six years. The gene may have entered the semi-wild population in the vicinity of Wooster through laboratory escapes but, in any case, became widely disseminated and well-established in local populations. That this is not a species-specific factor comparable to light in *D. repleta* is attested by the fact that it has been found only once outside this area in collections of many thousands of flies made by the author in widely separated geographical areas.

In a series of reports (Spencer 41, 43, 44, 45) the author has presented data on mutants present in three populations of *D. hydei*, one inhabiting a citrus dump near Azusa, southern California, one near Gatlinburg, Tennessee, and one in the environs of Wooster, Ohio. Inbreeding by the F_1 pair-mating method was used to discover recessives present in wild flies. From tests of about 1200 wild flies from the 1937, 1938 and 1939 Azusa populations and the 1937 and 1938 Wooster populations, more than 180 autosomal recessives were recovered. Table IX gives the results of mating 111 wild males from the Wooster 1938 population each to a stock female containing markers in each of the long autosomes, chromosomes II, III, IV and V and rearing 7 F_1 pair-matings from each. Male 111 when collected showed a dominant wing-vein character which turned out to be in chromosome VI, the dot, and is probably homologous to cubitus interruptus in *D. melanogaster* and to Gap in the VI of *D. virilis*. This same chromosome, as will be seen from the table, carried a recessive, grooveless, which is shown by the mating system to lie in chromosome VI, as it did not show linkage with any of the markers. Gray body, which had turned up repeatedly in analysis of the 1937 Wooster population, was recovered 5 times in this 1938 sample and, of course, showed up in the F_1 flies since gray was used in the marker stock. Javelin, another of the marker genes, was recovered once in the F_1.

All *hydei* populations studied by the author have contained a complex series of alleles at the bobbed locus in the X-chromosome. This is a species-

TABLE IX

Analysis of 111 Males from Wooster, Ohio, 1938 Population of D. hydei *by Mating Each Male to a Female with Markers in Each Long Autosome;* II-scarlet; III-gray; IV-pearly; V-javelin. 7 F_1 pair-matings made from each. F_1 or F_2 cultures in which mutants appeared are marked —. Linkage groups marked —. Only males from which mutants were secured are tabulated.

Male	Mutant	F_1	F_2-1	F_2-2	F_2-3	F_2-4	F_2-5	F_2-6	F_2-7	Linkage Group				
										II	III	IV	V	VI
9	Abnormal				—			—			—			
11	Gray	—									—			
11	Squat				—	—					—			
18	Rough				—	—						—		
21	Taxi		—					—		—				
23	Gray	—									—			
23	Tiny					—				—				
27	Rose		—	—	—	—	—		—				—	
28	Gray	—									—			
44	Nicked		—	—			—		—	—				
49	Nicked		—			—				—				
54	Tiny				—					—				
56	Gray	—									—			
56	Orange-like				—						—			
64	Rose		—	—	—	—			—				—	
71	Facet			—	—		—					—		
97	Facet				—							—		
100	Javelin	—											—	
101	Gray	—									—			
111	Grooveless								—					—
Total	20									5	8	3	3	1

specific variant. Wild populations also contain iso-alleles at this locus which have no phenotypic effect in homozygous form but which have been demonstrated by the use of strong bobbed testers. The Azusa population gave a higher number of mutants at different loci, the Wooster population a higher concentration of mutants at a few loci. The distribution of bobbed alleles in the two populations ran parallel with this finding on autosomal recessives. It was thus established that the Azusa population approached more closely a panmictic structure with a larger effective breeding size. The Wooster population passed through a sharp winter bottle-neck. From considerable experience the author finds that, on the average, about one wild *hydei* in four carries a recessive autosomal visible in heterozygous form. It should be mentioned that, except for the work on *D. robusta* and possibly *D. funebris*, all of the studies on naturally occurring mutants in

the subgenus Drosophila reported here have been done on populations of species which have been introduced. The populations are in one sense semi-domestic as they are breeding in and around towns or in orchards or other food stores supplied by the activities of man.

VIII. Discussion and Summary

It has not seemed advisable to include in this report a summary of the interesting and significant work on genetic factors demonstrated in the speciation studies of J. T. Patterson and collaborators, Th. Dobzhansky, A. H. Sturtevant and others. The reader is referred to the publications of Dr. Sewall Wright, several of which are listed in the bibliography, for a discussion of the statistics of population structure and evolution at the Mendelian level.

From the work reviewed here it becomes clear that the populations of all species of Drosophila investigated carry a rich store of genetic variability which furnishes adequate material not only for long-time evolutionary changes but also for rapid adjustments of populations to the environmental extremes to which they are subjected during the seasonal cycles through which they pass.

However, just as the individuals within populations can be shown to differ from one another by genetic factors, so each population has impressed upon it a peculiar genetic complex owing to its breeding structure and the nature of its constituent parts. The mutants carried by wild populations include those which are species-specific and extremely common; others which are rare and sporadic in distribution; many so extreme in phenotypic effect as to be lethal; and many others so slight as only to act or at least to be demonstrable in the presence of certain environmental and/or genetic complexes.

Certainly much progress has been made in the field of Drosophila population genetics since those days not so long ago when many sceptics considered the "bottle" mutations of the geneticist as interesting phenomena but with little or no bearing on what was occurring to species in the woods, the fields and the mountains. Yet the studies reviewed here should indicate that the work is in its incipient stage. Laboratory genetics, ecology, field studies and the application of statistical methods must all be brought to bear upon the unsolved problems. It is to be hoped that some young geneticists may turn to the field of Drosophila population genetics where "the harvest indeed is rich but the laborers are few." With the incomparable cytological tool of salivary chromosomes, the many cases of incipient speciation mechanisms which have been demonstrated, the opportunity to combine field work with a background of genetic knowledge not even approached in any other animal organism, here, if

anywhere, a study of the dynamics of evolution may be put on a sound experimental basis.

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Types of Polyploids: Their Classification and Significance

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CONTENTS

I. INTRODUCTION

Although polyploidy has long been recognized as a very important factor in plant evolution, many differences of opinion have existed and still exist concerning the nature of its role, and the relative importance of different types of polyploidy. The development of the use of colchicine to produce polyploids freely has not only made possible a thorough reëxamination of these points of view using experimentally controlled material but, in addition, has made such a reëxamination of great practical as well as theoretical importance. There is no doubt that the more we know of how polyploidy has operated in the past to produce new species and races of plants, the better we shall be able to use experimental polyploidy as a means of improving our useful cultivated plants. The whole subject of polyploidy is much too large to be discussed in any one article, so that this contribution will be confined to those aspects in which the relation between natural and artificial polyploidy is of major theoretical and practical importance.

II. BASIS FOR THE CLASSIFICATION OF POLYPLOID TYPES

In most literature on cytogenetics, polyploids are divided into two types, autopolyploids (recently contracted to autoploids by Clausen, Keck and Hiesey, 1945), and allopolyploids or amphidiploids (amphiploids in Clausen, Keck and Hiesey, 1945). These two categories have been variously defined and redefined, but no system yet devised has proved satisfactory. There are three main reasons for this. First, the two categories usually recognized are not sharply distinct from each other, no matter on

what basis the distinction between them is drawn. The usual criterion is whether the component genomes are similar or different from each other, and the common symbols used are AAAA for auto- and AABB for allopolyploids, the letters referring to a set of chromosomes or genomes, containing the basic haploid number for the group. Obviously, however, all degrees and types of differences can exist between two genomes, so that the decision as to whether a polyploid is auto- or allopolyploid must depend on the amount and type of differences that are considered significant in separating the two categories. Differences in both genic content and structural arrangement of the chromosomes are so widespread both within and between species, that the component genomes of a polyploid are not certain to be identical with each other in either of these respects unless the polyploid originated by somatic doubling from a homozygous diploid.

The second difficulty arises from the fact that genic and chromosomal differentiation are independent of each other, so that two genomes widely different in genic content may be very similar in structural arrangement, and *vice versa*. A polyploid may, therefore, contain, when first formed, two chromosomal sets that are highly dissimilar in genic content but are, nevertheless, structurally identical and form a fertile hybrid on the diploid level. Such a polyploid would be termed an autopolyploid under any definition of the term, but it could differ morphologically from any stabilized diploid form, and could produce many new, and still more different, types of segregation in its progeny. Furthermore, even when genic content is disregarded and similarities or differences between genomes are judged solely on the basis of chromosome structure or segmental arrangement, reliable estimates of the amount of difference between two genomes are hard to obtain. The type of structural changes best known, and those which are easiest to detect because of meiotic abnormalities in structural hybrids involving them, are interchanges and inversions of relatively large segments. There is, however, a good deal of evidence now available that a large proportion, if not a majority of the structural differences between genomes, involve segments so small that they do not give the typical meiotic configurations such as multivalents and bridge-fragments in structural hybrids. The writer has pointed out elsewhere (1945) that many of the numerous examples now known of diploid hybrids that are partly or wholly sterile in spite of nearly regular meiosis are best explained on the basis of this phenomenon of cryptic structural hybridity. Furthermore, this condition is the most common one in diploid hybrids between closely related species. It follows, therefore, that tetraploids derived from such hybrids will possess four genomes that have the majority of their chromosomal segments in common and are capable of pairing more or less com-

pletely with each other. The type of polyploid which has the strong genomic differentiation usually associated with allopolyploidy or amphidiploidy is in most cases derived from a hybrid between species belonging to different sections, subgenera or genera.

The third difficulty encountered in classifying polyploid types involves those polyploids higher than tetraploids, many of which are of very complex origin. A hexaploid, for instance, may contain only two different chromosome sets, one of them duplicated, or three distinct sets (AABBCC), while an octoploid may contain one, two, three or four different sets, variously differentiated from each other. At these higher levels, therefore, one and the same plant may possess autopolyploid characteristics, due to duplication of chromosome sets, in addition to the characteristics of allopolyploidy.

The obvious conclusion from these facts is that the classification of a plant simply as an auto- or an allopolyploid, based on such criteria as the resemblance in external morphology to certain diploids, the gross morphology of the chromosomes or the behavior of the chromosomes at meiosis, does not provide any basis for conclusions about the cytogenetic behavior or the phylogenetic origin of the species concerned and, therefore, is of little practical value.

Amplified classifications of polyploid types have been made by Simonet (1935), Lilienfeld (1936), Fagerlind (1937, 1941), and Clausen, Keck and Hiesey (1945). The first three are based primarily on the nature of chromosome pairing in the polyploid or its diploid ancestor, and the amount of differentiation between its component chromosomal sets, while the last is based on biosystematic principles. These involve principally the development of barriers to gene interchange between the component genomes of a polyploid or, in other words, whether the polyploid is derived from a fertile species or a more or less sterile diploid hybrid. This criterion was also used by Lilienfeld (1936) and the writer (1940), but its implications were not fully understood at that time. Since barriers to gene interchange often fail to affect visibly chromosome pairing at meiosis (Stebbins 1945), it is obvious that classifications based primarily on chromosome behavior will give very different results when applied to a particular group from those based on biosystematic principles which emphasize the origin of the polyploid.

The nearest approach to a complete classification of these various polyploid types is presented by Clausen, Keck and Hiesey (1945, Table 12). This, however, includes only the less complex types and tells little about their cytogenetic behavior. The present discussion, therefore, although based largely on their classification, aims to supplement and broaden it and, in doing so, to review some of the more important recent literature on the cytogenetics of polyploids.

III. Autopolyploidy

The number of autopolyploids artificially produced is now very large, so that their morphological and cytogenetic characteristics are well understood. Morphologically, the effects of chromosome doubling vary greatly with the nature of the original material (Kostoff 1939a, Barthelmess 1941, Randolph 1941, Pirschle 1942). The characteristics most consistently present are thicker leaves, larger flowers, and larger fruits. The plant as a whole, on the other hand, is as often smaller and less vigorous as it is larger and more vigorous than its diploid progenitor. It generally, but not always, flowers and fruits later than the diploid. Because of their lateness and partial sterility, artificial autopolyploids of crop plants are as a rule distinctly undesirable. Autotetraploids of garden flowers are likely to have desirable qualities of greater size, sturdiness, durability and lateness (Emsweller and Ruttle 1941), while valuable qualities associated with alterations in their chemical composition have been found in the autotetraploids of tomatoes, maize, sugar beets, and other crop plants (Randolph 1941).

Cytological studies of several artificially produced autopolyploids have changed the opinions once held on the cause of their sterility. Darlington (1937) considered that the sterility of autopolyploids is due to the formation of multivalents, which divide irregularly at meiotic anaphase and so produce gametes with abnormal chromosome combinations. Kostoff (1939b), following this opinion, postulated that, in plants with small chromosomes and low chiasma frequency, polyploids would be less sterile than in species with large ones, and later (1940) cited a few examples which seemed to bear out this opinion. That this is not always true, however, is evident from the sterility of some autopolyploids with small chromosomes, such as those of *Antirrhinum majus* (Emsweller and Ruttle 1941), *Gossypium herbaceum* and *G. hirsutum* (Beasley 1940), *G. arboreum* (Stephens 1942), and *Stipa lepida* (Stebbins 1941 and unpubl.). Myers (1943), and Myers and Hill (1942) concluded that the reduced fertility in the tetraploid *Dactylis glomerata*, which behaves cytologically as an autopolyploid, is due in large part to meiotic irregularities but that these irregularities do not depend on irregular segregation of multivalents. Variance between clones in frequency of univalents at first metaphase was positively correlated with that in frequency of tetrads with micronuclei but not with frequency of quadrivalents. Similar results were obtained by Sparrow, Ruttle and Nebel (1942) in *Antirrhinum majus*, and by Myers (1945) in autotetraploid *Lolium perenne*. Randolph (1941) concluded that the sterility in autotetraploid maize is, to a large extent, genically controlled and is largely physiological in nature. Nevertheless, irregular chromosomal distribution does occur in this tetraploid, giving rise to unbalanced, partly sterile, aneuploid

types. There are apparently at least three different causes of sterility in autotetraploids: (1) irregular chromosomal distribution caused by unequal separation of multivalents; (2) irregular distribution caused by meiotic abnormalities of a physiological nature, presumably controlled genetically; and (3) genetic-physiological sterility of an unexplained nature, but not associated with meiotic irregularity. The relative importance of these three causes varies with the tetraploid in question, but in most examples the first is less important than the last two.

None of the artificial tetraploids has been carried through enough generations so that its future evolution can be predicted, except for the tetraploid *Lycopersicum esculentum* (Lindstrom 1941). This has not given rise to anything new, and no fully fertile lines have been selected. On theoretical grounds the evolutionary future of an autopolyploid from an essentially homozygous diploid is likely to be limited (Stebbins 1940, Huskins 1941). On the other hand, if an autotetraploid should arise by somatic doubling from a diploid intervarietal hybrid exhibiting hybrid vigor, the tetraploid would retain and might augment this vigor. Furthermore, as was pointed out by Randolph (1941), the smaller amount of segregation in intervarietal autotetraploids should lead to a longer persistence of hybrid vigor in their progeny as compared with the offspring of a diploid intervarietal hybrid, according to any of the current hypotheses concerning the nature of hybrid vigor. We should expect, therefore, that the most successful autopolyploids, both for the plant breeder and in natural evolution, would be derived from hybrids between varieties or subspecies. This seems to be the case in *Antirrhinum majus* (Sparrow, Ruttle and Nebel 1942), in *Fragaria bracteata* × *F. vesca* var. *rosea* (*cf.* review in Clausen, Keck and Hiesey 1945), and in *Allium paniculatum-oleraceum* (Levan 1937).

This frequent occurrence of intervarietal autotetraploids adds to the difficulties in the way of interpreting the evolutionary significance of autopolyploidy *per se* on the basis of naturally occurring polyploids. Before the differences in external morphology and geographic distribution between a natural autopolyploid and its nearest known diploid relative can be ascribed to the polyploid condition, one must be certain that no diploid variety or subspecies exists which possesses the morphological and ecological characteristics in question. And in many discussions of the evolutionary significance of autopolyploidy, this point has not been considered. Clausen, Keck and Hiesey (1945) have already indicated (p. 130) that most of the supposed autopolyploids listed by Müntzing (1936) are actually allopolyploids, and state that "relatively few natural polyploids reported in the literature can be regarded as clear examples of autoploidy." They cite ten examples of possible or probable natural autopolyploids, but of one of these, *Cuthbertia graminea* (*cf.* Giles 1942), they say that

(p. 144) "this is a case in which the earliest known facts pointed to autoploidy, but where an assembling of additional data . . . is likely to revise the first impression." Of the remaining nine, of which the morphological differences and ecological preferences of the tetraploid are listed in Table 14 (p. 151), eight are, in the opinion of the writer, either doubtfully autopolyploid or else represent intervarietal autopolyploids which have acquired their supposedly divergent characteristics as a result of hybridization with a diploid variety or subspecies different from that to which their origin has commonly been ascribed. These eight cases are as follows:

1. *Zea perennis* (Hitchcock) Reeves et Mangelsd. has been found only once in nature, and its supposed diploid parent, *Z. mexicana* (Schrad.) Reeves et Mangelsd., is itself believed to be of hybrid origin (Mangelsdorf and Reeves 1939). There can be no certainty, therefore, that the presence of rhizomes in *Z. perennis* is due to the polyploidy, and not to admixture with a rhizomatous species of Tripsacum, or from some other source.

2. The strictly autopolyploid origin of *Dactylis glomerata* L. is doubted by Clausen, Keck and Hiesey. The writer has seen herbarium specimens, identified as *D. hispanica* Roth. or *D. juncinella* Bory, from Spain, Morocco, Sardinia and Asia Minor, which have to an extreme degree the various morphological characteristics by which, according to Müntzing (1937), tetraploid *D. glomerata* is distinguished from diploid *D. Aschersoniana*. These specimens from the Mediterranean region, moreover, have pollen grains as small as those of *D. Aschersoniana*, and may therefore be diploids. If so, nearly all of the morphological and ecological divergence of *D. glomerata* from *D. Aschersoniana* could be explained on the basis of hybridization between *D. Aschersoniana* and these Mediterranean forms.

3. The example of Eragrostis, first given by Hagerup (1932) and widely cited in general references as an example of the effects of autopolyploidy, is particularly doubtful. Eragrostis is a very large and complex genus, in which good taxonomic characters for separating species are particularly hard to find. Furthermore, it is very poorly known cytologically. Hence, until more is known about the cytology of other African desert species of this genus, the status of the three cited by Hagerup must be considered ambiguous.

4. *Biscutella laevigata* L. The fine analysis by Clausen, Keck and Hiesey of Manton's classic work on this example overlooked one point, namely, that two of the western European diploid "species" of the group, *B. arvernensis* and *B. Lamottii*, share with the tetraploid the ability to produce stolons, or "root buds" (Manton 1937, p. 449). It seems likely, therefore, that the stoloniferous character of the tetraploid was acquired through crossing with one of these forms, so that *B. laevigata* must be considered an intersubspecific autotetraploid, and its differences from the

diploids in both external morphology and distribution are due to gene recombination as well as to doubling of the chromosome number.

5. *Tradescantia canaliculata, T. occidentalis, T. humilis, etc.* The genus Tradescantia has served as a classic example of one in which autotetraploids exist side by side with diploids of the same species, and have enabled the species to spread into new territory (Anderson and Sax 1936). This situation must, however, be reëxamined, particularly in the light of the experiments of Skirm (1942) with doubling artificially the chromosome number of the natural hybrid ("Oakhill") between *T. canaliculata* and *T. humilis*. This hybrid possesses cytological abnormalities usually associated with hybridization (Anderson and Sax 1936), and that are probably caused by structural hybridity for small chromosomal segments. When doubled somatically it produces a tetraploid with very few multivalents, resembling cytologically an allotetraploid. This is due, as Skirm pointed out, to differential affinity, and preferential pairing of exactly similar chromosomes (see discussion below). On the other hand, if doubling is accomplished through the medium of gametes with the unreduced chromosome number, which were produced after meiosis and crossing over had taken place, the resulting tetraploid behaves cytologically like an autotetraploid. Such a tetraploid, although allotetraploid in that it was produced from an undoubted interspecific hybrid, could nevertheless segregate in the direction of one or other of its parents, so that some of its descendants could come to appear both morphologically and cytologically like autopolyploids. The probability of such an event is increased by the evidence of Giles (1941), who found that the triploid hybrid between tetraploid *T. canaliculata* and diploid *T. paludosa* was morphologically indistinguishable from *T. canaliculata* and had as many trivalents as an autotriploid. It showed more evidence of structural hybridity than its parents, but this is also greater in tetraploid *T. canaliculata* than in any diploid. There is no doubt that autotetraploids occur in Tradescantia. But diploid interspecific hybrids also occur, which could give rise to allotetraploids, and hybridization between tetraploids, followed by backcrossing, has been described by Anderson and Hubricht (1938) as introgressive hybridization. These introgressive types are, as Clausen, Keck and Hiesey pointed out, partial allopolyploids. Since the evidence of Skirm and Giles has shown that neither morphological nor cytological evidence is reliable in this genus for distinguishing between partial allo- and true autopolyploids, it is not certain whether the widely distributed "weedy" tetraploids are strict autotetraploids, or whether they are partial allopolyploids, the new characteristics of which are due to the presence of genes from other species as well as to the doubling of the chromosome number.

6. *Galium mollugo, G. verum, et aff.* Fagerlind (1937, pp. 342–345) recognizes that in this genus certain known allopolyploids, such as *G. lucidum-mollugo,* are nearly indistinguishable from one of their diploid ancestors and, furthermore, that many of the "intraspecific" tetraploids, particularly those of *G. mollugo* and *G. verum*, show definite signs of admixture with genes from another species. Furthermore, quadrivalent frequency is low in all of the tetraploids, both putative auto- and known allotetraploids. Nevertheless, because of the fact that the diploid species are unable to hybridize with each other, Fagerlind correctly concludes that true intraspecific polyploidy, *i.e.*, autopolyploidy, must be present. The important question from the evolutionary point of view is, however, what proportion of the tetraploids are true autopolyploids, and what proportion of them, particularly those which diverge from the diploids in their ecological requirements and geographical distributions, owe their new characteristics wholly or in part to an admixture of genes from another species. Since the diploid species, and consequently the newly arisen tetraploids, often occur together, and since the latter intercross freely, there is plenty of chance for such admixture on the tetraploid level. Furthermore, the intermediate allopolyploid between *G. mollugo* and *G. verum* is less vigorous than the apparent autopolyploids and would, in nature, give way to backcross types, which produce segregates indistinguishable from the supposed autopolyploids. Hence, although in Galium, as in Tradescantia, true autopolyploids certainly exist, the supposed autopolyploid types which have greatly extended the range of the species may actually be partial allopolyploids which owe their new characteristics in large part to the presence of combinations of genes derived from different ancestral diploid species.

7. *Vaccinium uliginosum* L. The subgenus to which this species belongs has its center of variability in western North America, where large-leaved forms similar to the European tetraploid also occur. Hagerup's (1933) evidence must therefore be considered definitely incomplete, and the status of the European tetraploid is doubtful until its American relatives have been studied.

8. *Empetrum nigrum* L. and *E. hermaphroditum* (Lange) Hagerup. This famous case, which has been widely cited in discussions of the relationship between polyploidy and sex, must also be considered doubtful. The genus Empetrum is predominantly a North American one, and the forms on this continent are mostly bisexual and unknown cytologically. Until a good series of chromosome counts has determined whether these North American forms are diploid, tetraploid, or both, the origin of *E. hermaphroditum* must be considered doubtful. Significantly, Blackburn

(1938) has found diploid hermaphroditic plants of *E. nigrum* to occur occasionally in Great Britain.

The removal of these eight from Clausen's list of undoubted natural autopolyploids would leave only *Galax aphylla*, representing a monotypic genus (Baldwin 1941). In this genus, the morphological differences between the diploids and autotetraploids are about like those seen in artificial autotetraploids, and the tetraploid race differs only slightly from the diploid in geographic distribution. Beside those cited by Clausen *et al.*, still others originally interpreted as autopolyploids have turned out to be entirely or partly allopolyploid when more fully investigated. Some of the most notable of these are *Nasturtium officinale* (Manton 1935, Howard and Manton 1940), *Lilium tigrinum* (Stewart and Bamford 1943), *Rubus caesius* and its relatives (Thomas 1940a, Gustafsson 1943), *Oxycoccus quadripetalus* (Hagerup 1940, Camp 1944), and *Solanum tuberosum* (Lamm 1945). *Allium oleraceum* and *A. carinatum* remain as true autopolyploids which have acquired a geographic distribution distinct from that of their diploid ancestors (Levan 1937), but these are probably derived from intervarietal hybrids, and they maintain their heterozygosity and hybrid vigor by means of asexual reproduction, which is unknown in the diploids of this group. The evolutionary future of such asexually reproducing autopolyploids is of course decidedly limited. The evidence is mounting, therefore, that autopolyploidy by itself rarely produces morphologically distinct species. Furthermore, the divergence of an autopolyploid from its diploid ancestor by means of mutation and other genetic changes without hybridization has taken place seldom if at all. The role of autopolyploidy in evolution has been primarily as a means of preserving vigorous intraspecific hybrid combinations and, secondarily, as a means of enabling species to hybridize which are incompatible on the diploid level.

IV. Typical Allopolyploids

This term will be used here in the same sense as the term amphiploid of Clausen, Keck and Hiesey (1945), though not as a synonym of amphidiploid in the sense of Navashin (1927), as will be explained further below. The difficulty of using the widely accepted criterion of structural similarity or dissimilarity in the chromosomes as the primary distinction between auto- *vs.* allopolyploidy does not lie only in the fact that the categories based upon this criterion cannot be used for evolutionary studies. In addition, there is no way of measuring quantitatively the amount of structural difference between two chromosome sets, so that the only possible dividing line between the two categories on this basis would have to be, as Müntzing (1936) has suggested, whether or not the component genomes are structurally identical. Since, however, structural differences, both

inversions and translocations, are commonly found in wild plants of pure species (*cf.* Dobzhansky 1941, p. 126), and can in most cases be detected only if they are so large or numerous that they affect chiasma formation and metaphase pairing, this structural identity could never be determined with certainty. Fagerlind's criterion (1941a), namely, whether the diploid ancestor of the polyploid has or has not perfect pairing, presumably at metaphase, is even more unreliable. A single large translocation or inversion will undoubtedly affect metaphase pairing more than several small ones, so that many polyploids derived from interracial hybrids showing quadrivalents or bridge-fragment configurations would, on the basis of Fagerlind's criteria, be considered more nearly allopolyploid than those derived from interspecific hybrids having cryptic structural hybridity (Stebbins 1945). For instance, Bergner (1944) found, as expected, many different types of configurations in meiosis of an artificial tetraploid produced from a hybrid between prime types 1 and 2 of *Datura stramonium*, but the designation of such a polyploid as an allopolyploid or even part allopolyploid would be very misleading.

Among allopolyploids at the tetraploid level two general types may be recognized, although these are, of course, connected by a whole series of intermediates. The best known type is defined on the chart of Clausen, Keck and Hiesey (1945, p. 72) as inter-cenospecific with no intergenomal pairing and the identity of the parental genomes preserved. To this type belong the classic examples Triticale, Raphanobrassica, *Gossypium hirsutum, barbadense et aff.*, and *Nicotiana Tabacum*. These are the only type which Fagerlind (1941a) recognizes as allopolyploid, and are the typical allopolyploids or amphidiploids of textbook accounts. An extensive review of the literature on these types is that of Goodspeed and Bradley (1942). They emphasize the fact that most representatives of this type are highly constant because chromosome pairing is between similar chromosomes derived from the same species. This type of pairing is termed allosyndesis by Darlington (1937, p. 199), following the original definition of Ljungdahl (1924). He refers to the fact that when it occurs in an established allopolyploid the chromosomes which pair have been derived from different parental gametes. Sharp (1943) uses for it the similar term allosynapsis. On the other hand, Lawrence (1930), Sansome and Philp (1932, p. 178), Dobzhansky (1941, p. 232) and Goodspeed and Bradley (1942, p. 287), call this type of pairing autosyndesis because it is pairing between chromosomes derived from the same species. Waddington (1939, p. 73) has suggested that Ljungdahl's definitions be followed very strictly, and that the terms auto- and allosyndesis be used only for pairing between chromosomes derived from the same or different immediate parents of the plant involved, whether it be an autopolyploid, a newly formed allopolyploid or

an allopolyploid species of long standing. Used in this sense, these terms have no reference to either the structural similarity or the phylogenetic relationship of the chromosomes concerned. In an allopolyploid newly formed from a diploid hybrid by somatic doubling, pairing between chromosomes derived from the same parental gamete, or autosyndesis, is the pairing of similar chromosomes, while in the later progeny of this allopolyploid autosyndesis in this strict sense is the pairing of dissimilar chromosomes. Waddington has introduced the terms homogenetic and heterogenetic association to replace auto- and allosyndesis as used in the phylogenetic sense. The writer believes that the use of these terms will eliminate the confusion that has centered around the use of the older ones.

If we follow the system of Waddington, therefore, we have two different series of terms with different uses. Autosyndesis and allosyndesis are purely genetical terms without phylogenetic connotations. In a diploid species, pairing is always allosyndesis and free genetic segregation is, therefore, possible. In an established allopolyploid autosyndesis is predominant and segregation is restricted. In an autopolyploid auto- and allosyndesis occur with equal frequency, while in intermediate polyploid types and in hybrids between polyploids these two types of pairing occur with various relative frequencies. Homogenetic and heterogenetic association are terms with definite phylogenetic connotations and cannot be used unless something is known, or can be inferred, about the origin of the polyploid in question. In diploid and autopolyploid species only homogenetic association can occur. In F_1 hybrids between distinct diploid or allopolyploid species only heterogenetic association can occur, unless the parental species have identical chromosome arrangements and are separated from each other by isolation barriers other than chromosomal sterility. A hybrid between two distinct partial allopolyploids can have two different types of heterogenetic association, namely, allosyndesis, or pairing between chromosomes derived from different parents, or autosyndesis between the different genomes derived from the same parental gamete. Within an established partial allopolyploid species, on the other hand, heterogenetic association will be mainly allosyndesis and homogenetic association will be the commonly occurring autosyndesis.

Heterogenetic association has long been known to occur as an occasional anomaly in otherwise true breeding allopolyploids (Darlington 1937, p. 200) and to be responsible for genotypic aberrations in these species. In new allopolyploids, even between widely different parents, a small percentage of heterogenetic association may occur regularly, as in Howard's (1938) strain of Raphanobrassica and in *Gossypium Thurberi — arboreum* (Beasley 1942). Since even a small amount of this type of pairing usually leads to some sterility as well as to inconstancy, its absence or

rarity in old, established allopolyploids is probably due to selection in the past of mutations and other genetic changes in this direction. From the cytogenetic point of view, therefore, the raw allopolyploid becomes progressively "diploidized" until its behavior resembles that of a diploid species. The nature of this diploidization has been accurately determined by R. E. Clausen (1941) for certain chromosomes of *Nicotiana Tabacum*. This species was derived from a hybrid between the diploids *N. sylvestris* and *N. tomentosiformis* or a close relative, as has been demonstrated by Greenleaf (1941), through comparison of the experimentally produced allopolyploid between these two species with *N. Tabacum*. Nevertheless, *N. sylvestris—tomentosiformis* has in duplicate certain factors, such as MM (dominant allele for mammoth growth), the normal allele to an asynaptic factor, and another to a recessive white-seedling character, which are all present only singly in *N. Tabacum*. Since the F_1 between the raw amphidiploid and *N. Tabacum* has perfectly normal meiosis, the elimination of these duplicate alleles appears to have been either through mutation or the loss of very small chromosomal segments. In self-pollinated plants, this diploidization apparently proceeds differently in different inbred lines, as evidenced by the fact that hybrids between different pure lines of such polyploids as hexaploid wheat and oats often have multivalents and other cytological irregularities (Huskins 1941).

Although the presence of heterogenetic pairing in allopolyploids usually causes some sterility, its absence by no means assures fertility. Sterility due to the interaction of genic factors in upsetting one of the developmental processes necessary for seed production, or genic sterility (*cf.* Dobzhansky 1941, p. 293) may be superimposed on chromosomal sterility in hybrids between distantly related species, and only the latter is removed by doubling the chromosome number. This condition was first noted by Greenleaf (1941, 1942) in allopolyploids between *N. sylvestris* and various members of the *N. tomentosa* complex. In these, the sterility involves the abortion of the female gametophyte, or embryo sac, at the 2 to 4 celled stage. In *Aegilops umbellulata — Haynaldia villosa* (Sears 1941) there is genic sterility affecting meiosis and producing partial asynapsis, in spite of the presence of an exact homologue for each chromosome.

All of the allopolyploids which show genic sterility have been produced from a diploid hybrid by doubling in the somatic tissue. On the other hand, a fertile allopolyploid of *Nicotiana sylvestris* × *tomentosiformis* was produced by Kostoff through gametic doubling, using as an intermediate stage a triploid derived from backcrossing the F_1 diploid to *N. sylvestris*, and crossing this triploid to *N. tomentosiformis*. Greenleaf (1942), based on his analysis of an F_1 hybrid between this allopolyploid and the one which he produced by somatic doubling, concluded that during the process of

gametic doubling in the Kostoff allopolyploid one of the two complementary factors for genic sterility was transferred by heterogenetic association and crossing over onto the chromosome that carried the other factor and, consequently, the homologue of this chromosome was neutral and viable in the female gametophyte. It seems likely, therefore, that if heterogenetic association occurs to any degree, this can act as a sieve to eliminate genic sterility from allopolyploids produced by gametic doubling. On the other hand, the presence in a diploid hybrid of several different bivalents formed by heterogenetic association between chromosomes that have only certain segments in common will produce unreduced as well as reduced gametes that differ from each other widely in the arrangement of chromosomal segments. The union of two such unreduced gametes, therefore, will produce an allopolyploid with considerable structural hybridity and consequent chromosomal sterility, as has been found in Triticum-Agropyron (Love and Suneson 1945). It can be said, therefore, that, if any pairing at all occurs in the diploid hybrid, the allopolyploid produced from it by somatic doubling may have genic sterility but not chromosomal sterility, while that resulting from gametic doubling will rarely if ever have genic but is very likely to have chromosomal sterility. The sterility reported by Clausen, Keck and Hiesey (1945) in *Layia pentaglossa*, which resulted from gametic doubling, is undoubtedly chromosomal. Allopolyploids produced by somatic doubling may have chromosomal sterility in later generations, but by means of differential affinity and preferential pairing (Darlington 1937, p. 185) this may be reduced enough to permit the survival of the line until the diploidization process has eliminated heterogenetic association.

Heterogenetic association in the ancestral diploid hybrids may be responsible for part of the peculiar phenomena found by Müntzing (1939) in hybrids between different strains of *Triticum aestivum — Secale cereale* (Triticale). This allopolyploid has been produced several times by different workers, and each of the initial allopolyploids has given rise after several generations of selfing to a distinct strain of Triticale. All of these strains have a somewhat irregular meiosis and are partially sterile as to both pollen and seeds. Interstrain hybrids, moreover, are harder to obtain than cross pollinations within a strain. The resulting F_1 plants are less fertile than their parents, indicating that these different strains of Triticale have developed new barriers of partial isolation. Müntzing explained these results as due to physiological sterility and incompatibility resulting from inbreeding of the rye set of chromosomes. Rye is a self-incompatible, normally cross-pollinated species, in which inbreeding is known to produce partial sterility and a reduction in chromosome pairing. Wheat, on the other hand, is normally self-fertilized, and carries this characteristic into

the Triticale allopolyploids, thus enforcing inbreeding of the rye as well as the wheat genome. There is no doubt that some of the sterility, which is apparently physiological in nature, is due to this cause, but the high number of univalents (up to 18 in some strains and interstrain hybrids) must be due in part to reduced homology between some of the chromosomes, which must also account for some of the haplontic sterility.

This evidence shows that many typical allopolyploids have a very different cytogenetic behavior from that of diploid species. Further evidence of this fact is the ability of all which have been so tested to tolerate much larger chromosomal deficiencies than can diploid species. Clausen (1941; Clausen and Cameron 1944) has been able to obtain monosomic plants deficient for one of each of the 24 different chromosomes found in the haploid set of *Nicotiana Tabacum*. Furthermore, although nullisomic (23-paired) plants are never viable in Nicotiana, in the case of one chromosome (the F) the vital portion is only a small region near the centromere. In *Triticum aestivum*, on the other hand, plants nullisomic for any one of the 21 pairs are viable (Sears 1944), and in the case of some chromosomes these nullisomics are fairly fertile. Sears has shown that the use of nullisomics provides a new rapid method for analysis of the gene content of individual chromosomes of this species. He also was able to show with striking clarity the presence of duplications in chromosomes belonging to different genomes. Plants which were tetra-II, nulli-XX were nearly normal and highly fertile, indicating that these chromosomes, one homologous to a chromosome of *T. durum*, and the other a chromosome of the Aegilops (*C*) genome in *T. aestivum*, have many genetic factors in common, in spite of the fact that they do not pair, even when both are monosomic. Even allopolyploids which behave cytologically as diploids under normal conditions are therefore actually quite different from them and can be expected to show more complex genetic ratios as well as reacting less strongly to cytogenetic disturbances of various sorts.

For allopolyploids of this usual type the term amphidiploid is often used. This term, however, is not synonymous with allopolyploid. It was first used by Navashin (1927) for the hypothetical doubled hybrid of *Crepis capillaris* ($n=3$)$\times$*C. setosa* ($n=4$). Since the haploid number ($n=7$) of such a doubled hybrid is not a multiple of any basic number, it could not be called a polyploid in the strictest sense of the word. On the other hand, the type of allopolyploid to be discussed below, which undergoes segregation because of regular heterogenetic association, could not be termed an amphidiploid, since it does not behave like a diploid in any respect. Therefore, according to the original definitions and connotations of the two terms they are overlapping but not synonymous in meaning. The introduction by Clausen, Keck and Hiesey (1945) of the new, abbre-

viated term amphiploid, which they have defined exactly according to modern biosystematic concepts, which covers the meaning of both of the old terms, and has no connotations, is a simplification of terminology which has much in its favor.

V. Segmental Allopolyploids

The second type of allopolyploid or amphiploid is that defined in the chart of Clausen, Keck and Hiesey as inter-ecospecific or inter-cenospecific with intergenomal pairing partial or complete, and the identity of the parental genomes lost in recombination. The best known example of this type is *Primula kewensis*, and Clausen, Keck and Hiesey list *Aquilegia Janczewskii*, *Crepis rubra — foetida*, *Crepis capillaris — tectorum* and *Layia pentaglossa* as additional artificially produced examples. To this list may be added *Nicotiana glauca — Langsdorffii* (Kostoff 1938), several combinations in Aegilops and Aegilotriticum (Sears 1941), *Allium cepa — fistulosum* (Jones and Clarke 1942), *Tradescantia canaliculata — humilis* (Skirm 1942), *Solanum Douglasii — nodiflorum* (Paddock 1942), *Lycopersicum esculentum — peruvianum* (Lesley and Lesley 1943), *Nicotiana paniculata — solanifolia* (Bradley and Goodspeed 1943), *Melica imperfecta — Torreyana* and *M. californica — imperfecta* (Joranson 1944), *Triticum durum — Timopheevi* and *T. vulgare — Timopheevi* (Zhebrak 1944 a,b), *Elymus glaucus — Sitanion jubatum* (Stebbins, unpubl.), and several combinations in Bromus, sect. Ceratochloa (Stebbins, unpubl.). Natural tetraploids of this type are *Zauschneria californica* (Clausen, Keck and Hiesey 1940), *Galium mollugo — verum* (= *G. ochroleucum*, Fagerlind 1937), *Aesculus carnea* (Upcott 1936) and *Lilium tigrinum* (Stewart and Bamford 1943). In external morphology, these allopolyploids usually differ from typical ones in resembling more closely one or both parents. This is both because their parents are more closely related and therefore differ less from each other in appearance, and because segregation of interspecific differences occurs, so that an initial intermediate allopolyploid of this type may in later generations produce segregates resembling more or less closely one or the other of its original parents. Cytologically, they are characterized by the presence of multivalents in varying numbers, so that in meiosis they often resemble autopolyploids more than true allopolyploids.

Clausen, Keck and Hiesey (1945, pp. 68–73) have advanced two reasons why these allopolyploids between closely related species might be expected to be unsuccessful and therefore infrequent in nature. In the first place, segregation of interspecific differences, particularly the incompatibility and sterility barriers which existed between the parental diploids, would result in the appearance of many weak or sterile types in their progeny. Secondly, this segregation would prevent the polyploid from

breeding true and, therefore, of maintaining the proper physiological balance with its environment. They recognize, however, that such types might be successful if they became stabilized in later generations through the elimination of weak, sterile and unfit combinations. As a test of this hypothesis, the cytogenetic behavior of the above mentioned artificially produced examples of this type will be summarized. Of the 14 different examples or groups of examples, 5 — those in Crepis, Layia, Allium, Lycopersicum and Solanum — were either themselves weak and completely sterile or produced offspring entirely of this type, so that they fulfilled in every respect the prediction of Clausen, Keck and Hiesey. Six — those in Aquilegia, Nicotiana, Aegilops, Melica, Elymus-Sitanion and Bromus — segregated or varied in respect to both morphological characteristics and fertility, while the remaining 3 — *Primula kewensis*, *Tradescantia canaliculata — humilis* and *Triticum durum — Timopheevi* — are highly fertile, but later generations, when they have been produced, show considerable segregation for morphological characteristics. Only two of these segregating types — *Nicotiana glauca — Langsdorffii* and *Triticum durum — Timopheevi* — have been carried on for a sufficient number of generations so that their ultimate fate can be ascertained. From both of these, relatively constant, highly fertile types have been secured after four to six generations of inbreeding and selection. Furthermore, the great amount of segregation for morphological characteristics in the early generations permitted the production of a whole series of different lines, the number of which is limited only by the number of plants which the breeder is able to grow. There is good reason to believe, therefore, that these segregating allopolyploids are a valuable source of new variants for the plant breeder and well worth his attention, although the production of useful types from them obviously requires considerable time. Another valuable feature of these allopolyploids is that, in contrast to non-segregating allopolyploids derived from distantly related parents, they can be crossed to autopolyploids derived from their parental species and the resulting hybrids will in many cases be vigorous and reasonably fertile (Zhebrak 1946). This makes it possible to transfer genes or groups of genes from one species to another on the tetraploid level when this transfer is impossible on the diploid level because of the sterility of the F_1 hybrid. These same qualities give the segregating allopolyploids certain unique evolutionary possibilities. In the first place, the variability of these allopolyploids in early generations would give them an opportunity of exploring and occupying "adaptive peaks" in the sense of Wright (*cf.* Dobzhansky 1941) that might lie between or apart from those occupied by the parents (*cf.* Müntzing 1932). Secondly, if autotetraploids of the parent species should exist in nature, these could, by hybridization with the segregating allopolyploid, increase their vari-

ability and adaptability to potential new habitats and, in addition, lose some of the well-known drawbacks of most new autopolyploids, such as slow growth, irregular meiosis and consequent sterility.

For these reasons, allopolyploids of this type are sufficiently important so that they should be designated by a distinctive name. The name applied by Fagerlind (1937, 1941) interspecific autopolyploid, is inappropriate, as has been indicated above. A more satisfactory term is segmental allopolyploids. A segmental allopolyploid may, therefore, be defined as an allopolyploid of which the component genomes bear the majority of their chromosomal segments in common, so that the diploid hybrid from which it is derived has good pairing at meiosis, but in which these genomes differ from each other by a large enough number of chromosomal segments or gene combinations so that free interchange between them is barred by partial or complete sterility on the diploid level. The examples of segmental alloploids, both artificial and natural, have been cited above.

From the standpoint of both evolution and plant breeding, it is important to know what factors contribute to the success of segmental allopolyploids and which ones are responsible for their failure. Our knowledge of these factors is as yet very imperfect, but certain considerations are undoubtedly of paramount importance. These are, first, the nature of pairing, as determined by the amount of differential affinity between the chromosomes as well as their size and genically determined factors of chiasma frequency and distribution; second, the amount and nature of the sterility in the diploid hybrid from which the polyploid arose; and third, certain physio-ecological features of the plant group concerned which determine its ability to pass through the "bottleneck" of partial sterility which must intervene between the formation of the "raw" allopolyploid and the stabilization of constant, fertile lines from it.

The phenomenon of differential affinity (Darlington 1937, pp. 198–200) is the most characteristic feature of segmental allopolyploids and the degree to which it is developed is one of the most important factors determining their success or failure. As Darlington has pointed out, it is caused by the fact that chromosomes pair segment by segment, so that those which are completely homologous have a greater affinity for each other than those which differ in respect to large or small non-homologous segments. In diploid hybrids having nearly complete pairing and regular distribution of the chromosomes at meiosis, as in *Primula verticillata—floribunda*, and various hybrids in Galeopsis (Müntzing 1938), the sterility seems to be produced chiefly by the random segregation of small non-homologous segments, so that the gametes come to possess non-viable duplications or deficiencies. This is the chromosomal sterility of Dobzhansky (1941, p. 293), and it is partly eliminated in polyploids from such hybrids by means of

homogenetic pairing, which results from differential affinity. It does not follow from this, however, that the fertility of segmental allopolyploids is directly correlated with the lack of heterogenetic association. Sears (1941) has clearly shown in a series of 21 allotetraploids of the Triticinae that this is not the case. Although in this group there is a general correlation between the amount of heterogenetic association, as measured by the frequency of multivalents and the degree of pollen and seed sterility, there are striking exceptions. The example of *Aegilops umbellulata — Haynaldia villosa*, a true allopolyploid in which genic sterility is found, has been mentioned above. Three other allopolyploids obtained by Sears which have a high number of univalents in spite of perfect homology of the chromosomes are *Ae. speltoides ligustica* II—*Ae. uniaristata*, *Ae. caudata-Ae. speltoides lig.* II, and *Ae. speltoides lig.* II—*Ae. umbellulata*. The first two of these had much lower seed fertility than any of the other allopolyploids except for the Aegilops—Haynaldia example mentioned above and, therefore, genic sterility connected with partial asynapsis or desynapsis may exist in them also. A more significant exception is the difference between two different allopolyploids involving the same two species, *Ae. caudata* and *Ae. umbellulata*. One of these (produced in 1938), obtained from a diploid hybrid characterized by a relatively low amount of pairing, had the high average of 5.81 chromosomes per cell in multivalents, while the other (produced in 1939), of which the diploid hybrid had closer pairing, had only 3.86 chromosomes per cell in multivalents. Nevertheless, the pollen and seed fertility in the two allopolyploids was nearly identical. A similar example is the pair of allopolyploids involving two different strains of *Ae. speltoides ligustica* (I and II) and *Ae. umbellulata*. Finally there is the example of *Ae. comosa—uniaristata*, which had the relatively high seed fertility of 78% in spite of the fact that the number of chromosomes in multivalents, 6.64%, was the next to the highest recorded. It is perhaps significant that *Ae. comosa* and *Ae. uniaristata* are placed by all monographers in the same taxonomic section (*cf.* Kihara 1940), while all but one of the other allopolyploids are intersectional. This evidence from Aegilops shows that, even within groups that are relatively homogeneous as to chromosome size and chiasma frequency and distribution in the diploid species, one cannot predict accurately the chromosome behavior or the fertility of an allopolyploid on the basis of the pairing in the diploid hybrid from which it is to be derived.

The factors of chromosome size and chiasma distribution, as they affect multivalent formation and fertility in polyploids, have already been discussed above in connection with autopolyploidy. These same factors obviously hold, with similar qualifications, for segmental allopolyploids. For instance, the low frequency of multivalents in *Primula kewensis*

(Upcott 1939) and *Galeopsis tetrahit* (Müntzing 1932), in spite of the high degree of pairing in their ancestral diploid hybrids, is probably due to the small size of the chromosomes and the low chiasma frequency in these genera. Nevertheless *Lycopersicum esculentum—peruvianum* var. *dentatum* has as high a multivalent frequency as autotetraploid *L. esculentum*, in spite of the fact that chromosome size and chiasma distribution are approximately the same in Lycopersicum as in Primula. Undoubtedly, therefore, unknown factors, in addition to the recognized ones, affect the frequency of multivalent formation and heterogenetic association in segmental allopolyploids.

The final factor determining the success of all new polyploids, and particularly segmental allopolyploids, is the character of the plants themselves. The writer (1938) pointed out that the chance of chromosome doubling in a sterile hybrid is much greater if the plant is a long lived perennial than if it is an annual. This chance would be increased still more if the hybrid had an efficient means of vegetative propagation, such as rhizomes, tubers or bulbs. Furthermore, the partly sterile descendants of an unstabilized autopolyploid or segmental allopolyploid would also have a much greater chance of survival if they were perennials with vegetative means of reproduction. Botanists are well aware that many wild perennial species which produce rhizomes, tubers or bulbs, such as those of Acorus, Agropyron, Elymus, Ammophila, Fritillaria and Tulipa, often set little or no seed and propagate themselves in the main vegetatively. In such species the partial sterility which accompanies the segmental allopolyploid condition is only a slight, or even a negligible, selective disadvantage which could easily be counterbalanced by the vigor and evolutionary possibilities of polyploids of this type. We should, therefore, not expect either autopolyploidy or segmental allopolyploidy to occur commonly in annual species, because of their difficulty in passing through the bottleneck of partial sterility which always accompanies these conditions in their initial stages. In perennials, on the other hand, these conditions should be more common, and perhaps as frequent as true allopolyploidy. This agrees with the evidence previously obtained by the writer (1938) that perennial groups have a higher percentage of polyploid types than annual ones, and that wherever they have been sufficiently investigated, these polyploid perennials can be seen to be descended from perennial diploid ancestors.

At levels of polyploidy higher than tetraploidy, types can occur which combine completely the characteristics of auto- and allopolyploidy. If, for example, an autotetraploid is crossed with a different diploid species, and the resulting triploid hybrid is doubled, a hexaploid will be produced which will be autopolyploid with respect to one genome, but allopolyploid

in that it contains a different genome. This type has been called by Kostoff (1939c) an autoallopolyploid, and the example given by him is *Helianthus tuberosus*, which has a diploid chromosome number of 102. When it is crossed with *H. annuus* ($2n=34$), the resulting tetraploid hybrid has usually 34 bivalents. Kostoff interprets this result as due to the fact that the haploid complement of *H. tuberosus* has one genome, *Bt*, homologous with that of *H. annuus* (*Ba*), and two, *AtAt*, that are entirely different. *Phleum pratense* apparently has a similar constitution. Clausen, Keck and Hiesey (1945), after a review of most of the literature on this much disputed case, agree with the original opinion of Gregor and Sansome that it is an allopolyploid, and consider that the parental forms belong to different cenospecies. Nordenskiold (1941), however, concludes that *P. pratense* is an autopolyploid of *P. nodosum*, while Myers (1944), after finding in *P. pratense* both multivalents and tetrasomic genetic ratios considers it to be at least partly an autopolyploid. Critical evidence, in the writer's opinion, is provided by two different haploids of this species described by Nordenskiöld (1941) and Levan (1941). Both have typically fourteen bivalents and seven univalents, indicating that their genomic formula is *AAB*, and that normal *P. pratense* is *AAAABB*. This interpretation would reconcile the apparently conflicting evidence which has suggested on the one hand an autopolyploid and on the other an allopolyploid origin for *P. pratense*.

Another type of autoallopolyploid is the autopolyploid produced by somatic doubling from the allopolyploid species *Nicotiana tabacum* (Clausen 1941) and the similar one produced from *Gossypium hirsutum* (Beasley 1940). A natural octoploid of this type is *Rubus ursinus* (="*R. vitifolius*", Thomas 1940a, b). There are, moreover, many examples of high polyploid species which have some autopolyploid characteristics, such as the presence of multivalents and a close morphological resemblance to certain diploid species, but which are known more or less definitely to contain genomes derived from more than one species. Typical of these are *Iris versicolor* (Anderson 1936), *Agropyron elongatum* (Wakar 1935), *Pentstemon nectericus* (Clausen 1933), *Rubus lemurum* (Brown 1943) and *Bromus arizonicus* (Stebbins, Tobgy and Harlan 1944). These do not fit the definitions of either auto- or allopolyploids, and had best be designated either as partial allopolyploids, or undefined secondary polyploids. The observations of Love and Suneson (1945) on hybrids between *Triticum* and *Agropyron* have shown that interspecific hybrids involving these higher polyploids may produce fertile derivatives with euploid numbers between the undoubled and the doubled one. Thus the 41-chromosome F_1 *T. macha*×*A. trichophorum* gave rise to a fertile plant with 70 chromosomes, presumably through the functioning of a partially reduced gamete with 28 chromo-

somes. It is possible, therefore, that some of these higher polyploid F_1 hybrids may give rise in later generations to several distinct species all descended from the same hybrid combination. The limitless possibilities and complexities of such a situation can only be imagined. In many genera containing these high polyploids we must, therefore, be content with assuming that most of these species contain various combinations of autopolyploidy, segmental allopolyploidy and true allopolyploidy, and that their phylogenetic relationships will be difficult or impossible to unravel.

VI. Summary and Conclusion

The various polyploid types may now be summarized as follows. On the tetraploid level there are autopolyploids, segmental allopolyploids and true allopolyploids. Autopolyploids usually are characterized by the presence of multivalents at meiosis, of tetrasomic ratios and, in the examples artificially produced, of slower development and reduced fertility. They may be descended from relatively homozygous diploids, or from hybrids between varieties or subspecies of a diploid species. The latter are more likely to be successful in nature, so that differences between wild autopolyploids and their nearest diploid relatives may be genetic in nature as well as the result of chromosome doubling *per se*.

Segmental allopolyploids in which, by definition, all of the component genomes have a majority of chromosomal segments in common, will resemble autopolyploids to a greater or lesser degree in possessing multivalents and tetrasomic ratios, but these will be less common. They may also come to resemble morphologically one or other of their diploid ancestral species, as a result of heterogenetic association and the consequent segregation for interspecific differences, as well as of the proven adaptive value of the gene combination possessed by these ancestral diploids. There is, therefore, no certain way of distinguishing between autopolyploids and segmental allopolyploids except by finding out through systematic studies and experimental verification the actual origin of the polyploid in question.

True allopolyploids may rarely have multivalent associations and tetrasomic ratios, but they usually do not, and they, therefore, resemble diploids to a large extent in their cytogenetic behavior. All of them, however, differ from diploids in that they can tolerate much more easily deficiencies of chromosomal material, in particular monosomic and nullisomic types. This doubtless is an important cause of the fact that fertility is usually higher in interspecific hybrids of polyploid species than it is between diploid species of about the same degree of relationship.

On levels of polyploidy higher than tetraploidy, complete autopolyploidy may exist, but since, on these higher levels, experimental

autopolyploids are nearly always weak and aberrant, the success of such types in nature is highly problematical and no unquestionable examples are known to the writer. True allopolyploids, which contain three or more strongly differentiated genomes, derived from as many sharply distinct species, definitely exist in nature (*e.g.*, *Madia citrigracilis*, Clausen, Keck and Hiesey 1945), and have been synthesized artificially (*e.g.*, *Nicotiana digluta*, Triticale). It is likely, however, that a large proportion, if not a majority, of hexaploids, octoploids and higher polyploids represent some variant of the autoallopolyploid condition, in other words, that they have resulted from autopolyploids, segmental allopolyploids and true allopolyploids combined in different ways. The complete cytogenetic and phylogenetic analysis of such higher polyploids will probably be made in only a few clear and important examples.

These considerations lead to a revaluation of the importance of polyploidy in evolution and plant breeding. From the scientific point of view, it is important to know to what extent evolution in polyploid groups has been affected by chromosome doubling *per se*, with its attendant morphological and physiological alterations of the genotype and its creation of an isolation barrier which would permit divergent evolution, and to what extent divergence of polyploids from their diploid ancestors has been due to hybridization, with polyploidy acting as a stabilizer of hybrid combinations and as a means of obtaining fertility. The practical breeder needs to know how much he can expect from autopolyploidy and subsequent selection within a single variety as compared with the use of polyploidy as a tool in hybridization, either for transferring gene complexes from one species to another (Clausen 1941), or as a means of either fixing or rendering fertile hybrid combinations.

The best answer that can at present be given to the evolutionary question is that earlier estimates of the importance of autopolyploidy must definitely be revised. When we recognize the fact, first that true allopolyploids may often resemble diploid relatives so closely that taxonomists place the two in the same species (Clausen, Keck and Hiesey 1945, p. 130), and second, that segmental allopolyploids may resemble autopolyploids in cytogenetic as well as morphological characteristics, we must be more critical of most previously assumed examples of autopolyploidy. Much more work will have to be done before the extent of this necessary revision will be clear. Two facts, however, supply indirect evidence unfavorable to the assumption that polyploidy *per se* has played a large part in the differentiation of plant species. In the first place, the only known natural sexually reproducing polyploid which does not belong to a group of more or less closely related subspecies or species, including various diploids or putative diploids from which it could be descended, is the autotetraploid

Galax aphylla (Baldwin 1941). This strain resembles closely the diploid of the species in morphological and physiological characteristics as well as in distribution and, from the evolutionary point of view, is of distinctly minor importance. Secondly, all polyploid complexes fully analyzed follow the pattern suggested by the writer (1940, 1942), namely, that the morphological, physiological and ecological characteristics of their polyploid members are entirely, or almost entirely, recombinations of characteristics present in the ancestral diploids, with little or no evidence of divergence along a new evolutionary path (*cf.* Gustafsson 1943). The importance of polyploidy in fixing and spreading hybrid combinations is undoubted, and this is very likely its major role in evolution.

This conclusion leads directly to a suggestion to plant breeders that they will find much more profit in the use of polyploidy as a tool, combined in various ways with hybridization and selection, than in the creation of polyploids which might be expected to have immediate value. Viewed in this light, the importance of polyploidy in both plant evolution and plant breeding is by no means diminished, but it must be considered as one factor which is integrated with many others in producing various types of change.

The conclusion that polyploidy in nature is nearly always associated with hybridization, either intervarietal or interspecific, is of far-reaching evolutionary significance. About half of the species of Angiosperms have chromosome numbers that clearly indicate polyploid origin, and in some families, like the Gramineae, three-fourths of the species are polyploids. These polyploids are distributed through about two-thirds of the genera of Angiosperms. Hence in this proportion of genera, species formation has been associated in part with hybridization and the phylogenetic "tree" of the genus is partly reticulate in nature. And in genera like Rumex, Dianthus, Thalictrum, Mentha and Salix, in which the majority of the species are polyploids, the interrelationships of the species and the phylogenetic pattern of their evolution must be largely reticulate.

Of even broader significance is the possibility, which has arisen from recently counted chromosome numbers, that a large proportion of genera and even higher groups of Angiosperms may be of polyploid and therefore principally of hybrid origin. The writer pointed out (1938) that the basic chromosome number of woody genera is on the average higher than that of herbaceous ones, and that, of the nearly 150 genera that were well enough known to be listed, 35 had basic numbers of $x=16$ or higher, numbers which strongly suggest a polyploid origin. Furthermore, 78 more of these woody genera had basic numbers of $x=10$ to $x=15$, which is higher than the modal numbers found in herbaceous genera. The prevailing evidence was and is against the hypothesis that these woody genera origi-

nated through polyploidy from herbaceous types. However, there is some reason for revising the estimate made by the writer of the relative probability of the two remaining hypotheses, namely, first, that the woody genera with basic numbers of $x=11$, 12, 13 and 14 were derived from more ancient woody types with numbers of $x=5$, 6 and 7, and second, that the numbers 12, 13 and 14 are themselves primitive. In 1938, the writer favored the latter hypothesis. The following facts, however, now favor the former. In the family Anonaceae, which is entirely woody, predominantly tropical and phylogenetically primitive, basic numbers of $x=7$, 8 and 9 have been found (Bowden 1945, Asana and Adatia 1945). In the primitive, tropical subfamily Caesalpinoideae of the large and relatively primitive family Leguminosae one ancient, woody genus, Cercis, has the basic numbers $x=7$ and 6, while the related, also ancient genus Bauhinia has $x=14$ (Senn 1938, Pantulu 1942). Finally the opinion of Avdulov (1931), that the original basic number of the Gramineae is $x=12$, is now questionable. This opinion was based on the fact that this number is prevalent in the tribe Bambuseae and the series Phragmitiformes, in which the numbers 7 and 6 were unknown. Recently, however, these latter numbers have been determined as basic for Danthonia (Calder 1937, Stebbins and Love 1941), one of the clearly primitive and ancient genera of the Phragmitiformes and one which connects this series with the Festuciformes, containing the familiar northern genera with $x=7$. It seems likely, therefore, that the number 12 in the Phragmitiformes is of very ancient polyploid origin. The well-established phylogenetic sequence by which Avdulov derived the series Sacchariferae from the Phragmitiformes through a gradual reduction in the basic number can still be accepted, so that the important tribes Oryzeae, Eragrosteae, Chloridae, Paniceae, Andropogoneae and Maydeae may well be derived secondarily from ancient polyploids. It therefore seems likely that reticulation is characteristic not only of the phylogenetic pattern within genera of this important family, but also of the pattern of relationships between genera and tribes.

If future evidence continues to favor the hypothesis that basic numbers of 10 and higher in the woody Angiosperms are originally or secondarily of polyploid derivation, we must conclude that within this group the pattern of relationships between genera of a family, and even between the families themselves, is to a large extent reticulate. If this is true, an explanation is at hand for the fact that plant systematists have never been able to construct a satisfactory system of relationships for the flowering plants. Although the genera and families of insects, vertebrates and other groups of animals have been arranged according to orders and classes that have become relatively stabilized and have met with general approval, the arrangement of the Angiosperms into groups higher than families and even

the grouping of genera into families has been interpreted in widely different ways by such competent authorities as Bentham, Engler, Wettstein, Bessey and Hutchinson, and none of these systems has been considered satisfactory by more than a small fraction of modern systematists. The cytogeneticist will never be able to resolve this confusion, but he may be able to point to its cause. This is that any genus or family of flowering plants may share some genes with two, three or more other genera or families which otherwise have little in common. Hence the system will depend on the characters emphasized by its maker and, from the cytogenetic and phylogenetic point of view, any one of several systems is as nearly correct as another.

These speculations do not, however, alter the concept expressed by the writer (1940) and affirmed by a number of other cytogeneticists, that polyploidy is a conservative rather than a progressive force. Even if reticulation through allopolyploidy has played a major role in the origin of genera and families, this role has probably involved chiefly the production of new combinations of characters, rather than the origin of the characters themselves. Such fundamental changes as from polypetaly to sympetaly, from hypogyny to epigyny, and from actinomorphy to zygomorphy have probably been produced by successive genetic changes on the diploid level or in secondarily diploidized polyploids, while hybridization and polyploidy have acted mainly to put together the various resulting conditions in innumerably different ways, and the favorable combinations have been preserved and stabilized by selection. We can, therefore, conclude that, in the flowering plants as in other organisms, mutation, recombination, selection and isolation have been the chief agents of evolution, but that the prevalence of polyploidy has given recombination a more predominant and basic role than it plays in most other organisms.

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Cytogenetics of Gossypium and the Problem of the Origin of New World Cottons

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CONTENTS

I. GENERAL CYTOLOGICAL CLASSIFICATION OF THE GENUS

The genus Gossypium includes all the species to which the cultivated cottons of commerce belong and taxonomically allied species which can be hybridized with them. All the species under cultivation bear true lint (partially thickened, convoluted hairs) on their seeds at maturity; the allied species, with the exception of a single wild species endemic in Hawaii, have seeds bearing fully thickened, unconvoluted hairs which are incapable of being spun (36, 38). The native Hawaiian species (*G. tomentosum* Nutt.), though uncultivated, bears true lint.

Comprehensive classifications of the genus, based on geographical distribution and taxonomic, cytological and genetic studies, have been made by Harland (23) and Hutchinson (33). From the cytogenetic point of view it is clear that the genus is monophyletic, as nearly all the possible combinations of interspecific crosses can be performed artificially (3, 4, 19, 55, 56, 63, 68) — some very readily, others with great difficulty, sometimes involving *in vitro* culture of the immature hybrid seed (4, 55). From these crosses hybrids are obtained which exhibit a great range of fertility according to the parental species employed. Some combinations give very vigorous, fully fertile hybrids which, however, always yield very inferior "unbalanced" progenies on selfing (21, 24, 35, 52, 53); others give completely sterile hybrids. Cytological studies (1, 7, 14, 54, 57, 67, 68) have shown that even in the completely sterile hybrids there is a fair amount of pairing between chromosomes at meiosis.

By 1937, thanks chiefly to the work of Skovsted (54, 57) and Webber (67, 68), the general cytological relationships within the genus were fairly well understood. It had been found that all the species could be classified

TABLE I

Cytological classification of Gossypium adapted from Skovsted (57) and Beasley (7)

Primary Group	Location	Genome Class	Chromosome Size	Gametic Chromosome Number	Species
Old World	Asia	A_1	large	13	*G. herbaceum* L. (Cultivated)
	Asia	A_2	large	13	*G. arboreum* L. (Cultivated)
	Africa	B_1	medium	13	*G. anomalum* Wawr. and Peyr.
	Australia	C_1	very large	13	*G. sturtii* F. Muell.
	Indo-Arabia	E_1	large	13	*G. stocksii* Mast.
American	N. America	D_1	small	13	*G. thurberi* Tod.
	N. America	D_2	small	13	*G. armourianum* Kearney
	N America	D_3	small	13	*G. harknessii* Brandg.
	Galapagos and N America	D_3	small	13	*G. klotzschianum* Anderss.
	N. America	D_4	small	13	*G aridum* (R & S) Skovsted
	S. America	D_5	small	13	*G. raimondii* Ulb.
New World and Polynesian	N. America	$(AD)_1$	13 large:13 small	26	*G. hirsutum* L. (Cultivated)
	S. America	$(AD)_2$	13 large:13 small	26	*G. barbadense* L. (Cultivated)
	Hawaii	$(AD)_3$	13 large:13 small	26	*G. tomentosum* Nutt.

The primary grouping and the classification according to chromosome size is due to Skovsted; the subdivision of the primary Old World Group into four distinct genome classes, *A*, *B*, *C* and *E*, follows Beasley. Following Hutchinson (33) *G. klotzschianum* includes var. *davidsonii* (formerly *G. davidsonii* Kell.) from which it shows no cytological and little genetic difference. *G. raimondii* was not included in Skovsted's classification and *G. tomentosum* was omitted by Beasley.

in three major cytological groups corresponding to their geographic distribution (see Table I). On the basis of meiotic pairing in interspecific hybrids and the comparative sizes of the chromosomes in the different genoms, Skovsted (57) suggested that the New World ($n=26$) cottons had arisen by amphidiploidy from a hybrid or hybrids of Asiatic ($n=13$) and American ($n=13$) parentage. This theory, though attractive, was not at first universally accepted, since it was open to criticism on two grounds. First, degree of meiotic pairing might be genotypically controlled (11) and hence not an adequate measure of general homology. Second, distinguishing chromosomes that are rather small and not readily fixed in a satisfactory condition is a highly subjective process and particularly so when allosyndetic and autosyndetic bivalents are classified on this basis (15, 64). Nevertheless, Skovsted's findings were consistent and agreed very well with the results of taxonomic and cross-compatibility studies. Then, too, they had independent genetic evidence to support them. Harland (20) by the successive backcrossing of an Asiatic to a New World species was able to transfer a gene for anthocyanin pigmentation (petal spot) from the former species and show that it was an allele of one of the two anthocyanin series (R_1) in the New World group. Later, by similar methods (22), he

showed that the American diploid species carried anthocyanin alleles that were homologous with the other anthocyanin series (R_1) in New World cottons.

Such was the situation prior to the discovery of the colchicine technique in 1937 (8). A ready method of synthesizing polyploids had an obvious application to the testing of Skovsted's theory. It was clear that a direct proof of the theory awaited the successful synthesis of Asiatic×American amphidiploids which should be to some extent interfertile with naturally occurring New World cottons. This crucial evidence was obtained independently in 1940 by Harland (25) and Beasley (6), who successfully synthesized an ($n=26$) amphidiploid by colchicine treatment of the sterile hybrid *G. arboreum* (Asiatic)×*G. thurberi* (American). The amphidiploid, which was partly male sterile but female fertile, crossed readily with New World cottons, giving hybrids that were highly, though not completely, fertile and in whose pollen mother cells the majority of the chromosomes were paired as bivalents (7). Subsequent genetic evidence by Harland and Atteck (26), who showed that three American diploid species carried "Normal" alleles of the New World "Crinkled" mutant, and by Silow (53a), who discovered duplication of a linkage group in New World cottons (the duplicated group involved the R_1 and R_2 loci and thus added considerably to the value of Harland's earlier evidence), only served to confirm the validity of Skovsted's original hypothesis.

Beasley (7) extended Skovsted's cytological analysis of the genus to include various colchicine-synthesized polyploids, in particular hexaploids involving the genomes of New World and various diploid species. He concluded that diploid species could be divided into five clear-cut genome classes (A to E in Table I). Within each class practically no cytological differentiation could be detected, but all hybrids between members of different classes showed evidence of gross structural differences at meiosis. Four of his classes are subdivisions of Skovsted's Old World group (see Table I), while his D class is equivalent to Skovsted's American diploid group. Beasley's refinement of Skovsted's classification is important in connection with the question of the amphidiploid origin of New World cottons; his results not only show that the *A* and *D* genomes are together very similar to the New World *AD* genome (in support of Skovsted's hypothesis) but also rule out the possibility that any other combination of genomes (*AB*, *AC*, *etc.*) may be as closely related. For instance, the fact that his hexaploids, New World×*G. sturtii*, 2[(*AD*) *C*], and New World×*G. anomalum*, 2[(*AD*) *B*], tend to give 39 bivalents at meiosis with little allosyndetic pairing shows clearly that the *B* and *C* genomes are not closely related to either the *A* or the *D* subgenome of *AD*. This conclusion is supported by the results of other workers (40, 61, 63).

II. The Problem of the Origin of Amphidiploid Cottons

The cytological evidence considered so far settles only part of the problem of the origin of New World cottons. Their amphidiploid origin is known, but how it took place is still open to question. The American diploid group carrying the *D* genome (see Table I) consists of six isolated species confined to the Pacific slopes of North and South America and neighboring islands scattered along the Pacific Coast. As far as in known, each species shows remarkably little variability and has an extremely limited geographical range. In only one case do the respective territories overlap, and even here an ecological separation is probable (33). The *A* genome carried by *G. arboreum* and *G. herbaceum*, which include the cultivated Asiatic cottons, is widespread throughout subtropical Africa, Asia and India, extending as far east as the Philippines and Japan, but is absent from the Western Hemisphere. It is clear, then, that the groups which, according to the cytological evidence, must have provided the parents of the amphidiploid New World cottons are now separated by the width of the Atlantic Ocean plus the main body of the American continent on the one hand, and by the total expanse of the Pacific Ocean on the other. Furthermore, being subtropical species, they are confined to that belt of the world's surface in which the separation of New World and Old World land masses is at a maximum. How and where did they come in contact in order to give rise to the New World cottons?

III. Harland's Theory of Ancient Origin

The New World cottons consist of three species, according to Hutchinson's recent classification (33) — *G. barbadense* and *G. hirsutum*, which have centers of variability in Peru-Bolivia and Mexico-Guatemala respectively, and *G. tomentosum*, which is endemic in Hawaii. This taxonomic classification is in full agreement with the genetic evidence available (33, 35). Since the first two of these species include the most important cottons of commerce, they are now very widespread throughout subtropical regions. They are found not only in the Western Hemisphere, but also in various islands of the Pacific and also in Asia, Africa, India and Australia. There seems little doubt that their presence in the Old World as a whole is due to introduction by man in post-Columbian times (35). It is known, for instance, that the Portuguese introduced many crop plants into their Old World colonies from Brazil, and many introductions of a more recent date are known (2, 29, 65, 66). Of the cottons found in the Pacific Islands, some are known to be introductions (Sea Island cotton in Fiji and Upland cottons in Hawaii) but the origin of others is unknown. With the evidence at the time available, Harland put forward what seemed to be the only plausible explanation of the origin of New World

cottons; namely, that they originated in Polynesia before the establishment of the Pacific Ocean in its present form:

"The question arises: if the modern Central American amphidiploids contain an Asiatic genome, how did they get it, since it is now confined to Asia. The answer is provided by the present distribution of the amphidiploids. In addition to the three mainland species, there are endemic species in Polynesia, in the Galapagos Islands, in Hawaii, and in the Marquesas Islands. These three Polynesian endemics share the Asiatic sub-genome with the mainland species. Consequently it is highly probable that the original union of the ancestral genomes took place somewhere in Polynesia in late Cretaceous or early Tertiary times, and that migration subsequently took place into the American continent along a land bridge which according to Schuchert extended at this period from Australia — New Zealand through Samoa and the Paumotas to South America. In any case, the origin of the amphidiploid must have taken place prior to the time when these islands first became isolated, *i.e.* several million years ago." (From "Abstracts of Papers by Sydney Cross Harland, 1915–1941," Institute of Cotton Genetics, Lima, Peru, Pub. No. 1, July, 1942.)

The facts (*a*) that American diploid cottons are confined to the Pacific seaboard and hinterlands and (*b*) that one of the Asiatic species, *G. arboreum*, extends into the Philippines and Japan, suggest that migration occurred over a Pacific route. But the evidence for a Polynesian origin of amphidiploidy, based on the presence of ancient endemic species there, is now very much weakened. Careful taxonomic and genetic studies convinced Hutchinson and Silow (35) that "*G. darwinii* Watt," the supposed endemic species of the Galapagos Islands, is no more than a subspecies of *G. barbadense*, and the discovery in Puerto Rico of a type morphologically indistinguishable from "*G. taitense* Parl.," formerly supposed to be endemic in Fiji and the Marquesas, suggests that it is only a form of *G. hirsutum* var. *punctatum* Hutchinson, to which it is genetically and morphologically allied (33). This leaves *G. tomentosum* in Hawaii as the sole endemic species in the Pacific and, apart from this fact, all the remaining evidence points to South America as the most likely center of origin of the amphidiploids (35).

Whether a South American or a Polynesian center of origin is postulated, the difficulty of accounting for a Pacific crossing still remains. The existence of a land bridge of such magnitude is purely speculative and runs counter to current information (46, 48, 69). Furthermore, as Hutchinson and Stephens have recently pointed out (36), if such a land bridge did exist, presumably one or both of the diploid parents must have colonized it successfully and, if so, it is perhaps surprising that they have now completely disappeared from the Pacific Islands while the amphidiploids

are well established. Again, the land bridge requires an extremely ancient origin of New World cottons (in the Tertiary Period). Yet the high interfertility of colchicine-synthesized amphidiploids with present-day New World cottons and the complete interfertility among species of the latter, shows conclusively that cytological differentiation of the genomes involved has been slight during the ensuing period. It is difficult to reconcile such an extremely slow rate of evolution in natural and widely different environments with the enormous genetic variability found by breeders in cultivated New World cottons prior to their being subjected to intensive selection (27, 30, 34, 37).

An important point which the theory overlooks is worth consideration. The production of an amphidiploid is a "double-event" and not a "single-event" process. If one accepts Mayr's views (47) on the mechanism of speciation and applies them to the present problem, one must visualize the following sequence:

(*a*) Geographical isolation of the American and Asiatic groups followed by a (presumably long) period during which the respective genomes became cytogenetically differentiated (allopatric speciation).

(*b*) Geographical reunion, by which the two groups became once more sympatric, followed by interspecific hybridization and polyploidy. To these should be added, in the case of Gossypium, a third period during which the New World species became genetically differentiated and the parental diploid species became geographically isolated from each other a second time.

If (*b*) is placed in the Tertiary, an earlier, distinct mechanism must have accounted for (*a*); and since in the intervening period the major cytological differentiation of the genus occurred, this earlier mechanism must have been operative in the remote past. Alternatively, if (*a*) occurred in the Tertiary, (*b*) can only be accounted for by some method of trans-oceanic transport in geologically recent times.

IV. The Theory of Recent Origin

A consideration of the above alternatives has lately led Hutchinson and Stephens (36) to suggest the possibility of a much more recent origin of New World cottons. Since the genus as a whole is confined to sub-tropical regions, any crossing more recent than the establishment of the Pacific Ocean in its present form would necessitate transport over thousands of miles of water. A chance crossing by air or ocean currents is possible (18, 46, 48, 69) but seems unlikely over such immense distances when one bears in mind that the seeds of Gossypium are heavy and rapidly lose germinability on exposure to moist air. This latter factor should be stressed, as it is important enough to constitute a major difficulty in

carrying out experimental work with cotton. Furthermore, superimposed on the small likelihood of this initial event is the fact that establishment over a wide area (or, alternatively, fortuitous establishment in the neighborhood of the other parental species) would be a necessary preliminary to interspecific hybridization and polyploidy. Natural outcrossing, even between nearly related species, does not amount to more than 20% (2, 41); there is a gap of several hours between the initiation of pollen shedding in Asiatic and American diploids (59) and there is some evidence that certation occurs to the disadvantage of foreign pollen in mixed pollinations (42, 43, 63). Any such combination of rare events would surely require that the parental species should be in contact either in large numbers or over a long period of time. The absence of Asiatic cottons from the New World and the extremely limited ranges of present-day American diploid species negate the possibility that an isolated chance colonization provided means of parental contact in geologically recent times.

The only remaining possibility in accordance with a recent origin would appear to be that proposed by Hutchinson and Stephens (36). They suggest that cultivated Asiatic cottons were carried by an early civilization across the Southern Pacific to the New World in prehistoric times, and that natural hybridization of cultivated crop with a neighboring wild American species gave rise there to the first amphidiploid. Such an explanation would remove three major difficulties encountered by Harland's theory. Firstly, the fertility found in hybrids between synthetic polyploids and naturally occurring New World species, and the absence of major cytological differentiation among the latter, are in accordance with a recent origin. Secondly, it offers a plausible interpretation of the absence of Asiatic cottons from the Pacific Islands and American continents. A wild species must, in the face of natural competition, take part in the gradual process of becoming part of a climax vegetation if it is to establish itself permanently in a given area, but no such time limitation is imposed on a species in cultivation. Consequently, a crop can spread rapidly under culture without undergoing competition with the native climax vegetation. In fertile areas, cleared and cultivated land reverts to scrub or secondary forest when cultivation is abandoned. There is abundant evidence that no species of Gossypium can, in the wild, survive competition and shading — they form part of a climax vegetation only in semidesert areas as members of sparse, xerophytic associations (31, 32, 33). Although cultivated strains of Asiatic cottons were introduced into the southern United States and British Guiana during the 17th-19th centuries, they have now disappeared without trace (17, 65, 66). There is no reason to suppose they would stand any better chance of survival at the sites of more ancient cultivations. Thirdly, the

chances of natural hybridization and amphidiploidy, though low, would be most favorable where one of the parental species was concentrated in large numbers, *i.e.*, under cultivation, and in the neighborhood of a native wild species. All New World species have spinnable lint (36), their centers of variability coincide with the primary centers of American cultures (35), there is no sharp distinction between "wild" and cultivated forms and in many cases the former appear to be recent escapes from abandoned cultivations (31, 32, 35, 36); these facts all suggest that the New World cottons may have been used by man at the outset and not independently developed from ancestral wild species.

On the negative side, it must be admitted that there is no direct proof and no unequivocal circumstantial evidence that any early civilization crossed the Pacific (12). Polynesian races reached the Marquesas and Hawaii but there is no evidence of their having reached the South American mainland. In any case, Polynesians are users of bark cloth and there is no evidence of their ever having been acquainted with spinning (9, 13). Although a minority of authorities regard cultural parallels between Old World and American civilizations as evidence of common origin (10, 16, 50, 58), the majority regard these as independent developments and see no need for postulating any other population of the Western Hemisphere than by way of the Behring Straits (28, 44, 45). To the layman in anthropological matters, it would appear that the balance of the evidence at present available is against a Pacific crossing and, although there is no disproof of it, the possibility must be considered quite speculative at the present time.

V. Cytogenetic Studies as a Method of Attack on the Problem

Probably the most fruitful and least speculative line of future attack on the problem will be afforded by cytogenetic studies. In broad terms such studies should be capable of deciding whether or not a recent origin of New World cottons is more probable than an ancient one. If it could be shown that an amphidiploid, cytogenetically no more differentiated from New World cottons than the latter are differentiated from one another, could be synthesized from present-day Asiatic and American diploid species, then a theory of recent origin would have strong support. It would indicate that amphidiploidy occurred *after* speciation of the American diploids, which themselves appear to be greatly differentiated genetically but show little evidence of gross structural differentiation (7). Alternatively, if such evidence should not be forthcoming, the problem would remain an open one. Synthesis of all the possible Asiatic/American amphidiploid combinations is clearly required, followed by cytological and genetic analysis of their hybrids with New World cottons. At present

direct evidence is lacking but Hutchinson and Stephens (36) have drawn attention to the indirect evidence available in colchicine-synthesized hexaploids. This evidence suggests that an amphidiploid synthesized from the Asiatic species, *G. arboreum*, and the South American diploid species, *G. raimondii*, would show a close cytological homology with New World cottons. Briefly, the argument developed is as follows:

Using Beasley's (7) nomenclature, the New World genome is AD, and the various American diploid genomes are D_1, D_2, *etc.* The hexaploids derived by colchicine treatment will therefore have the constitution $2[(AD)D_1]$, $2[(AD)D_2]$, *etc.* If the D, D_1, *etc.*, genomes are differentiated from each other cytologically, such hexaploids may be expected to be highly fertile giving an approximation to 39 bivalents at meiosis. On the other hand if the D, D_1, *etc.*, genomes are alike they should give associations characteristic of autopolyploids (7, 39, 49, 59), and prove less fertile. A similar argument applies to hexaploids $2[A_1(AD)]$, *etc.*, involving an Asiatic species. From an examination of their own and Beasley's data (5, 7), Hutchinson and Stephens find that of the hexaploids which have been synthesized, those having the least regular bivalent associations and the lowest fertility appear to be those involving New World and *G. raimondii* and New World and Asiatic genomes.

More recently Iyengar (40) has carried out a detailed analysis of meiosis in hexaploids of the constitution $2[(AD)D_1]$ (New World×*G. thurberi*); $2[(AD)D_2]$ (New World×*G. armourianum*); and $2[A_2(AD)]$ (New World×*G. arboreum*). Pooling data from the first two, he concludes that chromosome conjugation (as measured by mean number of associations per pollen mother cell) does not differ significantly from that in $2[A_2(AD)]$. It is evident, however, that his pooling of data is unjustified, since, while 20% of his $2[(AD)D_1]$ pollen mother cells had 39 bivalents, no

TABLE II

Chromosome conjugation in the pollen mother cells of two hexaploids, New World×G. thurberi *and New World*×G. armourianum *(Data from Iyengar (40))*

	Frequency of Associations						
	I	II	III	IV	V and VI	Total	PMCs examined
N.W.×*thurberi*	16	815	10	48	1	890	24
N.W.×*armourainum*	65	917	24	49	3	1058	28
Total	81	1732	34	97	4	1948	52

Contingency $\chi^2_{(5)} = 29.19$, $P < .001$

such "all bivalent" conjugations were found in the other two hexaploids. Furthermore, a contingency test carried out on his data shows clearly (Table II) that his 2[$(AD)D_1$] and 2[$(AD)D_2$] hexaploids are quite different in type of chromosome conjugation, the difference being as expected if the D subgenome of New World cottons is less differentiated from *armourianum* (D_2) than it is from *thurberi* (D_1). In this case the issue can scarcely be complicated by cross-homologies between A and D_2 sets, since there is good evidence (59) that triploids of the constitution $A_2A_2D_2$ show no allosyndetic pairing. It seems in fact reasonably certain that the genomes of existing diploid species differ appreciably in their cytological affinities with the New World genome, and detailed comparative studies of the type carried out by Iyengar should repay future study. Although the evidence at present available suggests that the *raimondii* genome is most nearly akin to the New World D subgenome, strictly comparable data have not yet been obtained, it being well known that chromosome conjugation in similar material may vary under different experimental conditions (7, 39).

Evidence from other sources appears to favour *G. raimondii* as a probable parent of New World cottons. Its sterile hybrids with Asiatic cottons show a close morphological resemblance to perennial New World cottons, particularly with regard to leaf shape, general habit and bracteole characters (60). Studies of leaf-shape development (60, 62) suggest that the D subgenome in New World cottons carries the gene, or genes, for an entire (undivided) leaf, and *G. raimondii* is one of the three American species that share this character. Finally, studies of the development of lint (36, 38) suggest that of all the possible Asiatic×American amphidiploids that can be synthesized from existing species, only Asiatic×*G. raimondii* would be expected to bear true lint, capable of being spun. Since all New World cottons bear true lint, this evidence has considerable weight.

Whatever bearing synthesized amphidiploids may have in the future on the origin of New World cottons, it is evident that they will be interfertile enough with the latter to permit detailed genetic studies of the type already carried out by Harland (21, 24) and Silow (51, 52, 53). The former has shown how speciation in the genus is brought about by the selection of distinct modifier (polygenic) complexes, which result in the production of inferior segregates when fertile interspecific hybrids are inbred. The latter has demonstrated that this differentiation is a continuous process, which may be considered on a quantitative basis; *i.e.*, the amount of "breakdown" resulting from interspecific hybridization and inbreeding is a measure of the divergence of the specific genotypes. It should be possible, therefore, to study quantitatively on a form of evolutionary scale the differentiation of the New World subgenomes from their nearest prototypes.

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