# ADVANCES IN GENETICS

## VOLUME III

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# Observations on the Cytology of Bacteria

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## I. INTRODUCTION

The structure of bacteria is a subject which has stimulated the investigations of many workers since the earliest days of bacteriology; none of them, however, has provided a completely satisfactory explanation. The very long history of this question has been described in several reviews (among them, Guilliermond, 1907; Delaporte, 1939b and 1940, which contains references, numbering about 440, to almost every paper on bacterial cytology up to 1938; Knaysi, 1938, 1949; Lewis, 1941). I will mention here only that the very first papers written on the subject —between 1886 and 1890—gave the following diverse descriptions of bacterial structure: (*a*) bacterial cells do not have a nucleus; (*b*) the cell contains a small granule, which is the nucleus; (*c*) the entire cell is a

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nucleus; (d) the greater part of the cell is a nucleus, and is surrounded by a thin layer of cytoplasm; (e) the nucleus is composed of a great number of small chromatic granules scattered inside the cell. Many of these opinions were still being supported quite recently.

A recent study by C. Robinow has attracted much attention. During the war Robinow (1942, 1944, 1945) used on several species of bacteria a technique described by Piekarski in 1937; namely, fixation of the cell by osmium tetroxide vapor, hydrolysis in a normal solution of hydrochloric acid at 60°C. for 7 to 10 minutes (the same as for the Feulgen technique), and staining with Giemsa solution. This technique, although not as specific as Feulgen reaction, is rapid, and results in remarkably clear and sharp figures, but the images of the nuclear substance are consistently larger than with other staining procedures, and in particular much larger than those given by the Feulgen reaction.

Robinow (1945) diagramed the changes in the nuclear structure of *Escherichia coli* occurring during the first hour after transfer from an 18-hour agar-slant culture onto a fresh agar plate. He has also described the early hours of growth after transfer of cells from old cultures of *E. coli* to a fresh medium, during which he observed 2, 4, or 8 chromatinic bodies having the shape of transverse rods or horseshoes, which divide lengthwise in a plane more or less parallel with the short axis of the bacterium.

## II. Useful Methods of Studying Bacterial Cytology

The bacterial nucleus is not easy to identify, since it does not have the characteristic shape and appearance that we know in the cell nuclei of all other organisms. It is therefore desirable to examine first the various elements inside the cell that we can easily find and identify. The bacterial cell is not composed merely of a nucleus and some cytoplasm inside a membrane. There are in addition other elements, which are easily identified when stained, especially with basic stains. For example, some cytologists have described a "nucleus," which in actuality was simply a metachromatic granule; they had not made a critical study of what they had stained. Very few cytologists have made studies of these other structures in the bacterial cell.

### 1. *Living, Unstained Bacteria; Observations of Lipids*

Microscopic examination of a culture of living bacteria, between slide and cover glass in a drop of either plain water or broth, shows, in certain species, one or several spherical, somewhat refractive globules inside the cells. These globules are stainable by the specific stains for lipids: Sudan III, Sudan black, Sudan red, and nascent indophenol blue

(Nadi reagent). In a great many bacterial species, these lipids are formed, for the most part, by polymerization of $\beta$-hydroxybutyric acid (Lemoigne *et al.*, 1944). In sulfur bacteria (Beggiatoa) the lipids seem to be phospholipids (Delaporte, 1939b, p. 766). In some species we can also see refractive ovoid or spherical bodies which are spores. Round, extremely refractive globules of sulfur are visible in sulfur bacteria.

## 2. *Vital Staining; Metachromatic Granules and Vacuoles*

Living bacterial cells may also be observed in a very dilute solution of a nontoxic vital stain. There are, unfortunately, very few such vital stains for bacteria; almost all stains kill the cell at the time they enter it. We can use neutral red, which is nontoxic and stains the vacuoles only when the cell is living; at the moment of death the vacuoles lose their color and the whole protoplasm—cytoplasm and nucleus—becomes colored. Brilliant cresyl blue and methylene blue likewise stain vacuoles in living cells; they are slightly toxic, and they can be reduced to the leuco-base by living cells. They can also stain the living nucleus and the cytoplasm slightly. Because bacterial cells are so small, it is advisable, in order to understand the action of a stain, to study it first on cells whose structure is well known.

Cells of yeast (for example, Saccharomyces) may be used for this purpose. When they are placed in a dilute solution of neutral red no staining is observed at first, but the large central vacuole (*v*) and many granules in the cytoplasm can be distinguished. After a short time, inside the unstained vacuolar sap, we see one or a few globules (*m*) of different sizes, which are stained red (Fig. 1a) and show Brownian move-
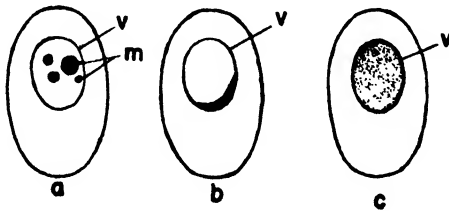


FIG. 1

ment. Apparently the dye has passed through the cytoplasm without staining it. After a few minutes these red globules migrate to the border of the vacuole and spread into the shape of a crescent (Fig. 1b). Later the stain diffuses completely through the vacuole, so that it is stained uniformly red (Fig. 1c); sometimes the whole vacuole stains red in this way without the previous staining of granules.

These red granules of yeast cells are metachromatic granules, formed by precipitation of the metachromatin that was in solution in

the vacuolar sap. (The name "metachromatin" is derived from the red color it gives with certain blue stains. Sometimes this substance is called "volutin," but the name metachromatin has priority.) It is very easy to confuse a metachromatic granule with a nuclear body, because their staining affinities are nearly the same; there is, however, a specific reaction for the identification of metachromatic granules developed by Meyer (1904). If, after staining with methylene blue, the cell is treated with a 1% solution of sulfuric acid, all the stain is removed except from the metachromatic granules, which change from red to black.

Except for unusually large cells (*Achromatium oxaliferum*), the bacterial cell has no large vacuole containing metachromatic granules, as in yeasts, but it has within the cytoplasm strongly staining metachromatic granules. These correspond to very small vacuoles, containing a high concentration of metachromatin. Another reason for considering the metachromatic granules as vacuoles is that they are stained black by silver impregnation with the Da Fano technique (Delaporte, 1939b). Metachromatin is a reserve substance composed—at least mainly—of ribonucleic acid (Van Herwerden, 1917; Delaporte, 1939a), probably in the form of ribonucleoprotein.

### 3. *Glycogen*

A third reserve substance of many species of bacteria is glycogen which is easy to recognize because of the mahogany color it takes with iodine. It is identified by its behavior when the material is warmed slightly. The glycogen always appears to be in a diffuse state inside the cytoplasm of the bacteria in which it is found; the quantity may vary greatly among cells. Some species of anaerobic bacteria (Clostridium) contain another type of carbohydrate (granulose) which is stained red-purple with iodine and occurs in the form of small granules.

It is necessary to consider these non-nuclear elements within the bacterial cell in order to understand and explain the structure and shape of the nucleus, because their presence can alter its shape. Some of these bodies have a surface tension greater than others. For example, we never see globules of lipid or sulfur that are deformed by nearby elements; they are always spherical since their surface tension is very high.

### 4. *Vital, or Sublethal, Staining; Observation of Nuclear Substance*

If we observe living bacteria (for example, *Bacillus cereus*, 16-hour culture; *B. enterothrix*; *B. camptospora*) in a drop of a dilute solution of a vital stain (methylene blue or brilliant cresyl blue) over a period

long enough for the stain to enter the cell without killing it, we can often observe the presence of a reddish thread inside the light-blue cytoplasm. This thread is situated in the long axis of the cell (Figs. 30, 33); it is often a little wavy and sometimes slightly granular. It is the nuclear element of the cell.

*Explanation of the Expression "Nuclear Element."* By "nuclear element" I mean a cellular component which has many of the properties of a nucleus and which contains substances having the staining characteristics seen in every nucleus. The nuclear element of the bacterial cell, as understood here, has the following characteristics, which are also the characteristics of the true nuclei of the higher forms. It never appears *de novo*, but always is formed by the division of a preexisting similar element; it is found in every cell and spore; it divides when the cell divides; and it is deeply chromatic. It is stained by the Feulgen reaction, and with ferric and other hematoxylins, Giemsa, etc. The positive reaction with the Feulgen technique indicates that the nuclear element contains desoxyribonucleic acid, which is the nucleic acid characteristic of the nucleus of higher forms. Nothing is known, however, about structures in the nuclear element having hereditary functions. We do not know whether the nuclear element is composed of a single or of several nuclear units and consequently whether it is a simple or a multiple nucleus—in other words, whether it is one nucleus or a group of several nuclei. Therefore, it is advisable not to use the word *nucleus*, which designates something more precise than our knowledge justifies, but provisionally to use the more general expression "nuclear element." The extremely varied shapes it assumes, and the fact that no consistent structure is seen, render it difficult to identify this nuclear element of the bacterial cell with the true normal nucleus of higher forms.

At the time that the nuclear element can be observed with vital staining, the cell has already begun to deteriorate; it seldom recovers, and generally dies after a short while. At the moment of death there is a rapid and pronounced change; the cytoplasm congeals and stains dark blue, and the axial thread (in rod cells)—or central body (in cocci)—disappears from view, although it can sometimes be seen with the help of a green filter.

### 5. *Staining of Nuclear Substance after Fixation*

The most common method of studying the nucleus in bacteria is to fix the cells with one fixative, stain, and then make observations only with regard to the nucleus, entirely neglecting the other bacterial structures.

**6.** *The Comparative Method of Scrutinizing the Cytology of Bacteria*

A better method is to try a number of different combinations of fixatives and nuclear stains; to observe on the same culture not only nuclear structures but also metachromatic corpuscles, lipids, glycogen, etc.; and to consider all observations before coming to a conclusion. A more detailed study of observations based on such procedures can be found in earlier papers (Delaporte, 1934, 1935, 1936a, 1936b, 1939b, 1940).

### III. Cytological Observations

#### 1. *Cytology of Round Bacteria*

When these different techniques of studying and staining are applied to round-shaped bacteria (Micrococcus, Streptococcus, Sarcina, etc.) they suggest that all such cells have quite comparable structure: namely, that they may contain one or several metachromatic granules (Fig. 2, *m*), and one or several globules of lipid (Fig. 3, *l*); and that they have one nuclear body, in the shape of a large globule, which occupies the whole center of the cell (Fig. 4, *n*), or else is situated somewhat eccentrically (Fig. 4, cell at lower center with lipid globule, *l*, in the middle). In a very young culture—one with actively multiplying cells—there are a great many cells in which this nuclear element is dividing, by lengthening and constricting in the middle (Fig. 4, right); and often two nuclear bodies are visible in one cell, because the transverse membrane has not yet formed (Fig. 4, cells at lower right and left). In some species the nuclear element may have divided twice before transverse membranes are visible, so that three or four nuclear elements are seen in one cell (Fig. 5). In older cultures, however, one almost never observes division figures, and consistently finds only one nuclear element in each cell (Fig. 6, *Leuconostoc mesenteroides;* Fig. 7, Sarcina—two cells are dividing).

#### 2. *Cytology of Ovoid Bacteria*

The large cells of ovoid shape (*Azotobacter agile, Achromatium oxaliferum, Chromatium weissei*) have their whole central region filled with globules. In *A. agile* the most numerous of these are fat globules (Fig. 9, *l*), some are metachromatic granules (Fig. 8, *m*), and the center of the cell contains much glycogen (Fig. 10, *g*). Stainings to demonstrate the nuclear substance in these cells show several small granules, more or less at the periphery of the cell (Fig. 11, *n*), making these cells very different in structure from coccus forms. The Feulgen reaction shows a few positive granules and an apparently diffuse positive reaction, chiefly in the peripheral part of the cell (Fig. 12). However, after a few trans-

fers on very poor medium (agar with tap water, and possibly a few hours thereafter on very dilute nutritive medium), the cells show only one mass of nuclear substance (or two in dividing cells), located in the center of the cell (Fig. 13, *n*) and scalloped by fat globules (Fig. 13, *l*) which appear as empty spaces in such preparations.  This mass divides by lengthening and constricting in the middle (Fig. 13, the cell at the top), as in cocci; it gives a positive Feulgen reaction.  This leads one to suppose that the nuclear substance, which in this species theoretically consists of a single mass, is habitually deformed, displaced, and even fragmented into several parts, which are located wherever there is room for them between the fatty globules and other globules.  The first diffuse Feulgen reaction is easily explained in this case: the nuclear substance, being displaced by globules, surrounds them in a thin layer, with tiny masses between the globules—thus giving the impression of a partially diffuse substance with Feulgen, and of granules with hematoxylin stain.  This does not mean that there are several nuclei in the cell, but only that the nuclear substance does not have a definite shape and may be deformed and fragmented by other elements of the bacterial cell. Exactly the same thing is observed in cells of *Chromatium weissei* and *Achromatium oxaliferum*, but, in addition, these have sulfur globules, and the latter has concretions of calcium oxalate (or, according to certain authors, calcium carbonate).  Figs. 14-17 show cells of *A. oxaliferum* enlarged 900 times.  The central part of these cells contains large concretions of calcium oxalate (*o*), and around these are very refractive sulfur globules (*S*).  Fig. 14 shows the vitally stained large vacuoles (*v*) found in this species.  Fig. 15 shows stained lipid globules (*l*).  In Fig. 16 the cell is fixed with Lenhossek's fluid, and the nuclear substance (*n*), situated between and around the calcium oxalate masses, is stained black with ferric hematoxylin; the cytoplasm (*c*), at the periphery of the cell, stains pink with erythrosin.  Fig. 17, in comparison, shows a Feulgen positive reaction (*n*) only at the center, because this is a young cell from a very rapidly growing culture.  In such a culture the nuclear substance is in one mass, centrally located.  It divides by stretching and partition in the middle, as in cocci and Azobacter.  (For other figures see Delaporte, 1939b, Pl. IV, Figs. 1-14, 29-36; Pl. VIII, Figs. 27-42; and Delaporte, 1940, p. 41.)

### 3. *Cytology of Rod-Shaped Bacteria*

Distribution of the nuclear substance in the form of several granules is usually also observed in rod-shaped cells when they contain a large amount of reserve material.  The cytoplasm includes fat globules (*l*), sometimes very many (Fig. 23) or very large ones (Fig. 19), as well

as metachromatic granules (Figs. 18, 22, 26, *m*), and sometimes glycogen (Fig. 28). In these cells the nuclear substance is generally divided into several granules, located in the interstices between the globules (Figs. 24, 46); and the Feulgen reaction often seems to be diffuse, although occasionally the masses of nuclear substance are evident (Fig. 25). In a few cells of these cultures, however, particularly those that have a very small number of globules and a dense cytoplasm (often these are also smaller in diameter), the nuclear substance is in one single mass, and appears as a more-or-less granular thread extended in the center of the cell, its length proportional to the length of the cell (Figs. 24, 49, 74, 76—middle cell, 78—right).

In a rod-shaped species of bacteria (Figs. 18-21) that has a few small metachromatic granules (Fig. 18, *m*), a few large lipid globules (Fig. 19, *l*), and no glycogen, the nuclear element (24-hour culture) has the shape of an axial thread (Figs. 20 and 21). In Fig. 20, the nuclear element (*n*) is stained black with ferric hematoxylin. In Fig. 21, the nuclear element is stained purple by the Feulgen reaction; the lower cell of the two-cell rod is dividing. The axial thread is deformed by lipid globules; the unstained areas (*l*) show the location of lipid globules, which have been dissolved during the successive treatments. In another rod species (Figs. 22-25), which has a few small metachromatic granules (Fig. 22, *m*), many lipid globules (Fig. 23, *l*), and no glycogen, the nuclear element (Fig. 24, ferric hematoxylin; Fig. 25, Feulgen) appears most often as several granules scattered throughout the cell or between the lipid globules; exceptionally it appears as a thread. In a third species (Figs. 26-29), which has very few metachromatic granules (Fig. 26, *m*) and lipid globules (Fig. 27, *l*), but much glycogen (Fig. 28, *g*), the nuclear element is nearly always displaced (Fig. 29, 1 to 3-day cultures, stained with ferric hematoxylin).

When bacterial cells have little or no accumulation of reserve material, the nuclear substance is in the form of a single mass, rod-like or thread-like in long rod cells and more or less round in short rod cells (*Bacillus enterothrix*: Fig. 30, vital staining; Fig. 31, ferric hematoxylin; Fig. 32, Delafield hematoxylin; Fig. 36, Feulgen reaction; 900 X. *B. camptospora*: Fig. 33, vital staining, 900 X. *Bacterium* sp.: Fig. 38, Feulgen reaction, 1800 X. *Escherichia coli*: Fig. 34, 2700 X; and Figs. 82, 82A, and 82B.)

In *E. coli* we find cells of quite different lengths, depending on the strain and the culture conditions. Sometimes they are very short and almost spherical (Figs. 34, 39—bottom, 82—photograph, 82A top left—explanatory drawing), sometimes longer (Figs. 39-45). The nuclear element appears to divide by stretching. When the cell is short, the globular

nuclear element enlarges (Fig. 82—right and Fig. 82B), takes the shape of a dumbbell, and breaks in the middle (Figs. 35, 40, 44, 45). When the nuclear element is shaped like a thread (Fig. 39—top; Figs. 83 and 83A), only a break in the middle is visible (Fig. 40—top). Often a second nuclear division is accomplished before the formation of the transverse membrane that divides the rod after the first nuclear division (Figs. 44 and 45; 82 and 82B, which show beginning of division). Under different conditions (of strain, age, and culture medium), when very active multiplication occurs, the observed events are different. The nuclear substance takes the shape of transverse rods, as seen in Fig. 41-43 (strain B/r, 4¾ hours in nutrient broth without aeration), and in Figs. 79-81 (photographs) and Figs. 80A and 81A (drawings). Two or three nuclear divisions occur in one cell before the cell divides, and then four, six, or eight nuclear transverse rods are found in one bacterial cell. When these nuclear rods divide they divide lengthwise, forming V shapes or U shapes (Figs. 42 and 43).

a. *Formation of Spore in Bacillus.* A more complex cycle of development is seen in species that form spores. If we observe stained cells from a 16-hour culture of *Bacillus cereus,* for example, we see many cells in which several granules of nuclear substance are located between the globules; that is, the nuclear substance is fragmented between the globules in two to six (occasionally more) main centers. Each of these nuclear granules of a cell seems to be completely separate from the others, but perhaps they are linked by nonapparent ties. A few cells have only one nuclear element, in the form of an axial thread (Fig. 49); and many cells are just beginning to form their spores (Figs. 46-48). The successive stages of this process can be observed during the next few hours. At first, all the nuclear granules inside the cell seem alike, but at the very beginning of spore formation, one granule, situated nearest an end of the cell, appears a little larger, for example, the one at the extreme right in Fig. 46. Then the cytoplasm around this granule becomes more homogenous and denser, without globules, and stains pink with Giemsa (Fig. 47, two prespores, *p,* in the two-cell rod at right); this region, which is to develop into the spore, grows larger and becomes ovoid in shape (Figs. 46, 47, 48, and 51, showing twelve stages of spore formation; photographs in Figs. 84 and 85, and explanatory drawings in Figs. 84A and 85C). The nuclear element of the future spore stretches into a short rod (Fig. 51), which migrates to the periphery of the spore (Figs. 50, 52) and takes the shape of an oval ring with one side slightly larger than the other (Fig. 52). At this time the staining properties of the cytoplasm of the interior of the spore change; it stains sky blue with Giemsa, and the spore becomes refractive. Most often this oval

ring is seen in side view, appearing as a kind of curved rod with slightly
thickened extremities. In Fig. 85, lower right corner, there are two free
spores with dark peripheral nuclear element and ovoid grey cytoplasm;
the spore coat is not stained. Fig. 53 shows spores after liberation; the
spore coat is not stained and not visible.

At the beginning of spore formation, only a small portion of all the
nuclear substance in the cell constitutes the nucleus of the future spore.
In a cell that has a nuclear thread, only a small part of this thread—
approximately a third in *B. cereus* (Fig. 47) or *B. macerans* (Fig. 54),
and much less in species having longer cells (Figs. 30, 33, 36, 37)—be-
comes the prespore nucleus. As the prespore enlarges and occupies a
larger part of the cell, it possibly assimilates by a chemical process more
of the nuclear substance of the rest of the cell. Almost always, however,
some remainder of nuclear substance is found outside the spore (Figs.
54, 55; *B. macerans*), sometimes the largest part of the axial thread
(Figs. 33, 36, 37; bacteria from intestine of tadpoles). The spore is lib-
erated after disintegration of the cytoplasm, the remainder of the nuclear
substance, (Figs. 37, 52), and the cell membrane. (See also *Spirillum
praeclarum*: (Figs. 98-101; and Delaporte, 1939b, Pl. VI, Figs. 1-17.)

b. *Germination of Spore*. In subcultures from an old culture, one
made up mostly of spores, the spores swell, and during this process
nuclear changes may be observed inside them. In *Bacillus mycoides*, for
example, the nucleus, which was at the periphery (Figs. 56, Giemsa; 57,
Feulgen; 65, vital staining), migrates to the center of the spore and en-
larges (Figs. 58, Feulgen; 59, Giemsa; 66, vital staining; 60, Giemsa,
one hour later). Then the new cell comes out like a bud, grows bigger,
and emerges from the envelope of the spore, while the nucleus undergoes
a first and a second division (Fig. 60, 3-hour culture, Giemsa; Fig. 61,
4-hour culture, Giemsa; Fig. 62, 4-hour culture, Feulgen). The first
transverse membrane is sometimes formed as soon as the first nuclear
division is completed, but more often after the second or the third
division. The nuclear element is larger at this time than it was inside
the resting spore.

In the interior of many spores, however, before the new cell emerges,
the nuclear element is in the form of a mass of three or four granules
(Fig. 59, bottom row, *B. mycoides*); and later, in the first cells that
emerge, the nuclear element is granular (Figs. 60-62). In many other
spores the nuclear element has the shape of a thick thread, often greatly
distorted, or of a mass that seems to be pulled in many directions (Fig.
59, several spores); such nuclear elements may divide before the cell
emerges (Fig. 59, right). In some spores, for example, we see two short,
bent threads, transversely and symmetrically situated (Figs. 58, 59);

the rods emerging from these spores have two nuclear elements, each consisting of a twisted, angular thread (U-shaped, square, Z-shaped, or V-shaped), with granules located especially at the angles (Fig. 60). When the nuclear element does not divide before germination, there is only one nuclear axial thread (Fig. 61, right). Rods that are a little older and longer have four similar nuclear elements (Figs. 61, 62). Although these rods have no visible transverse membrane, they must be considered as virtually two or four cells, because they are *dividing* and not *resting* cells, and cell division has not been completed at this time. Consequently, each actual cell is very short, and is square or slightly rectangular in shape. In such cultures there are a few rods that consist of only one cell, and in these the nuclear element is a wavy thread situated in the long axis of the cell (Fig. 61, lower right).

It can nearly always be shown that scallops in the outline of the nuclear element are made, or appear as if made, by globules of unknown nature (Fig. 63, 5-hour culture, Giemsa). What are these globules in the very young rods? Probably they are not yet true reserve substances, as are the lipid globules in older cells; possibly they are centers of more rapid metabolism, which enlarge locally and thereby displace the nuclear substance and other nearby substances.

*Remnants of sporangium*: In certain old cultures having living resting spores (particularly in *B. mycoides*, either unstained, or, better, with vital staining), it is possible to observe that the spore remains, perhaps permanently, inside the remnant of the sporangium membrane (*spg*), which can be seen, like two short fingers, protruding beyond the poles of the spore (Figs. 64, 65). Occasionally, a slightly refractive granule (*a*) is seen inside the sporangium membrane, most frequently located at a pole on the surface of the spore; very rarely, there are two such granules. This granule is vitally stained purple with cresyl blue. No change occurs in it during germination, and it is discarded on the surface of the spore coat. It stains red-purple with Giemsa after hydrolysis, and shows a positive Feulgen reaction. It is probably a remnant of nuclear substance of the sporangium. It is seen (*a*) on resting spores in Fig. 56 (Giemsa after hydrolysis), Fig. 64 (beginning of a vital staining), and Fig. 65 (vital staining of spores); and during germination in Fig. 66 (1-hour culture, vital staining), Fig. 59 (2-hour culture, Giemsa after hydrolysis), and Fig. 61 (4-hour culture, Giemsa after hydrolysis).

Vital, or sublethal, staining of the nuclear element (*n*, Fig. 65) in resting spores may be obtained only in certain nonrefractive spores, such as may be found, for example, in cultures of *B. mycoides*. It is never possible to stain the nuclear element, without previous hydrolysis, in refractive spores.

c. *Shape of Nuclear Element during the First Divisions in a New Bacillus Culture.* In a very young culture made from actively multiplying cells, the globules of reserve materials are not readily visible. There may be only a few lipid globules, occupying the most central part of the cell (black globules in Figs. 68-71, stained with Sudan black). In such cases the nuclear substance (n) surrounds these globules in the shape of cups, horseshoes, V's, or rings (Figs. 72, 73, 77, 78, and their explanatory drawings 72A, 73A, 77A, 77B, 78A; Figs. 72, 73, 77 are from the same culture as Figs. 68-70, stained for lipid globules). In the rare cells that have extremely few lipid globules, they apparently do not modify the shape of the nuclear element, which then is seen as a round or more-or-less ovoid body (Fig. 67); the nuclear element divides as a dumbbell (Figs. 75, 76, 78, 75A, 76A, 78A, 78B), or through apparent fission by lipid globules (Figs. 72, 75, 77, 78, 72A, 75A, 77A, 77B, and 78A). When the culture becomes a little older, the nuclear divisions are less frequent and the cells have time to lengthen without so many of these occurring, then we find in many cells, if the center of the cell is not occupied by reserve substances (lipids, metachromatic granules, glycogen), a nuclear axial thread (Figs. 74 and 74A).

## IV. DISCUSSION AND EXPLANATION OF OBSERVATIONS

What is the explanation of the transverse rods that divide longitudinally and are now believed by many cytologists to be chromosomes and to constitute the nucleus of bacteria? We have seen that the nuclear substance of bacteria is more "fluid" than the elements that surround it—that it is very easily deformed by nearby elements and displaced or penetrated by globules. No exterior membrane has ever been observed. Furthermore, whenever the edge of a nuclear mass presents a concave surface, it can usually be seen that a granule of lipid or some other substance is present, for example, in the horseshoe structure. It seems likely, therefore, that these lengthened shapes, more or less perpendicular to the long axis of the cell, are due to the presence of other adjoining elements, that they are not determinate structures but altered, passive ones. Many observers think that the lengthwise division of these rods is an argument in favor of their being chromosomes. However, this may be regarded as a normal consequence of cellular division, when lengthening of the long axis of the cell must result in lengthwise division of an element that is perpendicular to it. At the time of division of this nuclear element it is often easy to see one or two globules being formed and insinuating themselves between the two future halves.

Many cytologists have shown that the element that is strongly chromatic with Giemsa after hydrolysis is the same one that gives a

positive Feulgen nuclear reaction. This Feulgen-positive element, made of desoxyribonucleic acid, the nucleic acid of animal and plant nuclei, has already been demonstrated (Delaporte, 1939b) to be the same one that is strongly chromatic and in particular stains with ferric and other hematoxylins. Stainability with ferric hematoxylin has always been considered one of the characteristics of nuclear chromatin. The constant presence of this element, its division (at the time of cell division) always from a preexistent element, and its characteristic stainings and chemical composition, considered together, strongly suggest that it is the true nucleus of the bacterial cell.

What is the *usual* shape of this nucleus, which has no membrane and no constant shape? This depends on the shape of the cell and on the age and conditions of the culture. In round resting cells, it is a round body, generally centrally located. In ovoid resting cells, it is a central, more-or-less ovoid mass. In very short resting rods, it is an ovoid body or a very short rod. In long resting rods, it is an axial thread. These shapes are the theoretical ones, however, because in a cell containing abundant reserve substances the nuclear element is displaced, deformed, constricted, or (apparently) fragmented; this is the normal condition for many species. Lipid globules, metachromatic corpuscles, and glycogen, if they occupy the center of a cell, prevent the nucleus from being there.

In new cultures, when the cells are multiplying very rapidly, the shape of the nuclear material is generally different from that in old cells, and may vary a great deal; no constant or determined structure is observed. After a few divisions, the nuclear substance of a rod appears as two, four, or eight distinct masses—either roundish, or in short rods perpendicular to the long axis of the cell, or U- or V-shaped. The cells in which these last forms are found are often about to divide in the middle to form two rods. At this time, when the bacterial cell contains two, four, or eight nuclear elements, it seems convenient to consider it, not as a normal cell, but as a cell undergoing the process of division. Many examples are known of plant cells in which the nucleus divides into two or four, sometimes eight, nuclei before the separating membranes of each new cell are formed. Each of these bacterial "cells" contains two, four, or eight potential cells, which are consequently very short, their length often less than their width. In such short cells it is normal that the nuclear substance should be disposed as we see it here.

Later, when the frequency of the divisions has slowed down, the nuclear element lengthens little by little in the direction of the long axis of the bacterial cell. In a majority of species, the nuclear element

is then in the form of a more-or-less thick thread, whose length is proportional to the length of the cell.

In spores of some species of Bacillus (for example, *B. mycoides, B. cereus, B. megatherium, B. macerans*), the shape of the nuclear element seems to be more constant; it is not altered by reserve substances and, in resting spores, appears most often as a curved rod with slightly thickened extremities (side view) or an oval ring (front view), but sometimes as two or three closely related granules. In large Bacillus species (*B. enterothrix, B. collini, etc.*), it seems always to be a wavy axial thread.

Can the observation that only a fragment of the nuclear substance of a bacterial cell becomes the nucleus of the future spore help to clarify the question of whether the nuclear element in the bacterial cell is haploid, diploid, or polyploid? I actually do not believe so. Even though it is possible to interpret the formation of a number of nuclear fragments (such as is often observed in cells having several lipid globules) as indicating that a reduction division precedes sporulation, still it is difficult to believe that this happens in most of these bacteria, because the number of nuclear fragments in a cell is not constant. Another possible explanation is the following: With the stains used, it is not possible to observe a difference between the several nuclear fragments present in such a cell; but, inasmuch as only the granule near a pole of the cell will become the nuclear element of the future spore, we may suppose that only this granule contains the complete hereditary stock of characters. That is to say, inability to discern a morphological heterogeneity does not necessarily imply a similarity of function of all nuclear granules.

Several observations may be cited in support of this latter possibility (heterogeneity in the intimate structure of the nuclear element), such as the constant position of spores in *B. enterothrix, B. camptospora*, etc., and, in *Spirillum praeclarum*, the accumulation and condensation of nuclear substance in the region of the cell where the spore will be formed. In this last organism, the nuclear substance and cytoplasm, which until then are equally distributed throughout the cell (Fig. 98), accumulate in a definite region just at the beginning of sporulation (Figs. 99 and 100). Fig. 101 shows a cell during spore formation. From such observations it is obvious that all the hereditary characters exist in a smaller volume than the total volume of the nuclear substance in a Bacillus cell.

It is also possible, however, that in a cell having a polyploid nuclear element only one (or two) of these nuclear units would be able to develop into a spore.

The cytological observations do not permit us to judge whether the bacterial nuclear element is haploid, diploid, or polyploid.

## V. Nuclear Structures in a Streptomycin-Dependent Strain of *E. coli*

It is known that mutant strains resistant to antibiotics may be obtained, and it has been shown that some streptomycin-resistant mutants are also dependent on streptomycin for growth. When certain of these streptomycin-dependent mutants are grown in normal media, without streptomycin, they produce abnormal shapes, snake-like filaments. Figs. 83 and 83A show an 18-hour culture on agar containing streptomycin; the cells are short rods ("snakes" very rare), with normal-appearing nuclear material. This photograph was taken in a region where "snakes" were especially numerous. Figs. 94-97 show different stages in the formation of "snakes" by this same strain grown on agar without streptomycin (18-hour culture at 37°C.); the "snake" formation begins after a few normal divisions. Fig. 94 shows a long rod with four distinct nuclear elements; in other rods the nuclear substance is in a continuous thread. After 20 hours at 37°C., plus 20 hours at 20°C., very long "snakes" are seen. In most of these the interior is completely filled with nuclear material (Figs. 95-97), but some show regions of swelling where the nuclear substance may be arranged either in an axial thread (Figs. 95 and 95A), in a dense, granular net (Figs. 96, 96A), or in a loose net of granular threads (Figs. 97, 97A). The end of the "snake" ordinarily contains no nuclear material.

I wish to thank Dr. M. Demerec for contributing the strain of *E. coli* (B/r/sd) used in the foregoing observations.

## VI. Lysis of *B. cereus* and *E. coli* by Bacteriophages

When a suspension of bacteriophage is added to a very young culture of a phage-sensitive strain, successive changes are observed. These are essentially the same in *E. coli* and *B. cereus*, except during the last stage, at the time of liberation of phage, and during the first five minutes, when a few chromatic granules form at the periphery of the cell in *E. coli*. *B. cereus*, having larger cells, shows the successive transformations more distinctly. (I wish to thank Dr. I. N. Asheshov for the strain of *B. cereus* and bacteriophage used.) Figs. 86 and 87 show a rapidly growing culture to which a suspension of bacteriophage has been added. The nuclear elements are in the form of transverse rods, often in division; in many places it is possible to observe a very thin transverse membrane between the rods or pairs of rods. Eleven minutes after addition of the bacteriophage, the nuclear material is more abundant and occupies the whole

central part of each cell (Fig. 88), the transverse rods have fused. Twenty-two minutes after addition of the bacteriophage, the whole interior of the cell is filled with nuclear substance, or a substance having the same staining characteristics (Fig. 89). Sixty-three minutes after addition of the bacteriophage, the cells appear the same as in the preceding figure, but considerably enlarged (Fig. 90); they are only slightly increased in length, but are about one-third wider. At 70 to 74 minutes, the first figures of lysis are visible. The chromatic substance disappears, piecemeal but very rapidly, first scalloping the cell border, later leaving only an axial rod (Figs. 91-93), and finally leaving only several granules, which soon disappear. In *E. coli* this stage of cell lysis and bacteriophage liberation is characterized by a lessening of the rigidity of the bacterial membrane, so that first it becomes lumpy, and finally the cell is distended into a large round body, which bursts at the time the bacteriophage is liberated into the medium.

A progressive disappearance of opaque substance from the cell periphery, with simultaneous departure of bacteriophage particles, has been observed by Smiles *et al.* (1948) in electron micrographs of *Staphylococcus aureus* treated with penicillin and bacteriophage.

## VII. SUMMARY

Despite a great number of observations on bacterial cytology, no general agreement has been reached about bacterial structure. Recently, however, there has been such close agreement among the observations of different studies using new methods of staining (Feulgen, Giemsa)— following either acid hydrolysis or enzymatic digestion—that it now seems probable that we are arriving at a knowledge of the true structure of bacterial cells in several species.

Only the simultaneous use of many different methods of observation (on living cells without staining; with vital staining; and with staining of metachromatic granules, lipids, glycogen, and nuclear substance) can enable us to form an adequate conception of the structure of bacterial cells.

The cells of most bacterial species have metachromatic granules and lipid globules; in addition to these, certain species also have some glycogen, and others (sulfur bacteria) have sulfur globules. In every bacterial cell there is also a nuclear element, containing, as do the cell nuclei of every plant and animal species, desoxyribonucleic acid. This element divides at the moment of cellular division, before the formation of the transverse membrane, and has many characteristics of a nucleus. Nevertheless, its intimate structure is not sufficiently well known to permit us, at present, to equate it with the nucleus of higher organisms.

The shape of the nuclear element depends on the shape of the cell. It is a spherical mass in cocci, a central ovoid mass in ovoid cells, a roundish granule in very short rods, and an elongated body, situated lengthwise in the cell, in long rods (axial thread). When reserve substances (lipids, glycogen, metachromatin, sulfur, etc.) are found in the central part of the cell, they push against the nuclear element and deform it, and can also fragment it into several parts, which are perhaps linked by invisible ties.

Division of the nuclear element takes place by simple stretching and separation into two identical parts, without any special internal structure being apparent. A granule lengthens, takes on a dumbbell shape, and then divides into two granules which pull away from each other. An axial thread separates in the middle into two parts. A small rod, transverse to the long axis of the cell, divides lengthwise into two parts which move away from each other in the direction of the long axis of the cell.

When a new culture is made, the cells soon enter a stage of very active multiplication, during which the nuclear elements divide more rapidly than the transverse membranes are formed. This results in cells that have two or four nuclear elements, sometimes more. In rods, each of the potential cells, consisting of one nuclear element with its surrounding cytoplasm, is usually very short and approximately square; the nuclear element in these cells takes the form either of a compact mass or of a short transverse rod compressed between the adjacent substances. When the rhythm of divisions slows down, the cells have time to elongate between nuclear divisions and the nuclear element gradually resumes the shape typical of the species.

The spore of Bacillus species develops from a fragment of nuclear substance which becomes surrounded by homogeneous cytoplasm. This prespore grows slowly to the size of a normal spore, becoming ovoid in shape, with a nuclear element in the form of a short rod. The nuclear element migrates to the periphery of the sporal cytoplasm, often taking (for example, in *B. cereus*) a ring shape, most commonly seen in side view as a curved rod with slightly thickened extremities. In the course of this change, the sporal cytoplasm alters its staining affinities and the spore becomes enclosed in a refractive membrane. During spore formation there is a progressive, not always total, disappearance of the nuclear substance and protoplasm that remained in the sporangium outside the spore. The spore is then liberated.

At germination, the spore swells while the nuclear element approaches the center, enlarges, undergoes various transformations, and then divides. At about this time the young rod emerges from the spore.

Often two or three divisions of the nuclear element are completed before the first transverse membrane is visible. When the resting spore remains inside the remnant of the sporangium membrane, it is sometimes possible to see at germination that one or two granules of chromatic substance are discarded on the exterior surface of the spore coat; these granules are probably remnants of the nuclear substance of the sporangium outside the spore.

Figs. 94 to 97 show the formation of "snakes" in a mutant strain of *Escherichia coli* that needs streptomycin for normal growth. When this strain is cultured in ordinary medium, without streptomycin, the multiplication of the nuclear substance proceeds without the formation of transverse membranes, and this results in very long filaments nearly full of nuclear substance. These filaments have regions of swelling where the nuclear substance fills the whole interior or is in the shape of close-packed granules or a loose granular net.

Figs. 86 to 93 show the process of lysis of a sensitive bacterial culture by a bacteriophage. One observes first a fusion of the nuclear elements inside the cell, then such an increase of the nuclear substance that it fills the whole interior of the cell, and next a marked enlargement of the volume of each cell, which lasts until the time of bacteriophage liberation. At liberation, in *B. cereus*, the chromatic substance disappears little by little from the periphery of the cell; in *E. coli*, the cell swells rapidly into a sphere and bursts.

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Plate I
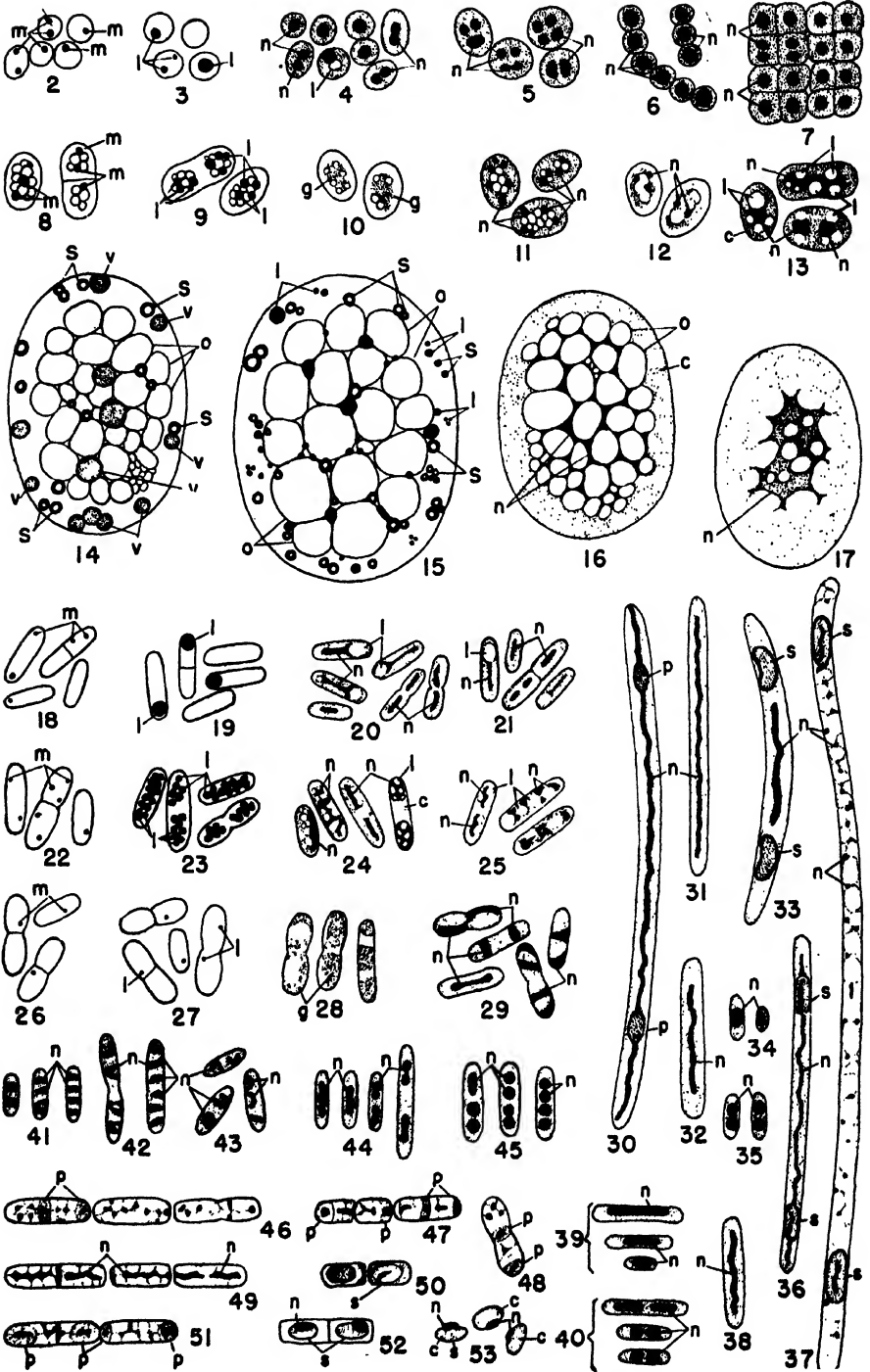
Magnifications: Figs. 2-5, 7-13, 34, 35, 39-53 = 2700 X; Fig. 6 = 5400 X; Figs. 14-17, 30-33, 36, 37 = 900 X; Figs. 18-29, 38 = 1800 X.

$c$ = cytoplasm, $g$ = glycogen, $l$ = lipid globule, $m$ = metachromatic granule, $n$ = nuclear element, $o$ = calcium oxalate, $p$ = prespore, $s$ = spore, $S$ = sulfur globule, $spg$ = sporangium, $v$ = vacuole.

Technique: Giemsa-stained preparations were fixed, without previous drying, with osmium tetroxide vapor for 2-4 minutes, hydrolyzed with 1 $N$ HCl at 60°C. for 10 minutes, then stained with Giemsa solution for 30 minutes.

### FIGS. 2-4. *Micrococcus* sp.

2. 6-day culture. Vital staining of metachromatic granules ($m$) with cresyl blue.

3. 20-hour culture. Lipid globules ($l$) stained with Sudan black.

4. 30-hour culture. Smear fixed with Perenyi and stained with ferric hematoxylin and erythrosin. Nuclear element ($n$) stained black.

FIG. 5. *Micrococcus* sp. Another species; 48-hour culture. Smear fixed with Perenyi and stained with ferric hematoxylin and erythrosin. Nuclear element ($n$) stained black. Two to four nuclear elements are formed before cell division.

FIG. 6. *Leuconostoc mesenteroides* (Cienkowski) Van Tieghem. 48-hour culture. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear element ($n$) black.

FIG. 7. *Sarcina* sp. 19-hour culture. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear element ($n$) black.

### FIGS. 8-13. *Azotobacter agile* Beij. var. *atypica* Kluyver and van den Bout.

Cultivated on agar with 0.1% $K_2HPO_4$, 2% glucose, 2% $CaCO_3$.

8. Vital staining with cresyl blue. Metachromatic granules ($m$) shown in black.

9. Lipid globules stained with Sudan III and drawn black.

10. Glycogen stained with $I_3K$.

11. Smear from a 24-hour culture, fixed with Perenyi and stained with ferric hematoxylin and erythrosin. Nuclear element ($n$) black.

12. Same culture as Fig. 11, smear fixed with 95% alcohol for 20 hours; Feulgen reaction (hydrolysis 10 min. at 60°C., staining 3 hours). Positive reaction in a few granules shown in solid black, and diffuse positive reaction in the stippled area.

13. 15-hour culture on tap water agar and 15-hour subculture on normal medium diluted to one-fourth. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear element, $n$, black; cytoplasm, $c$; and lipid globules, $l$.

### FIGS. 14-17. *Achromatium oxaliferum* Schew. in pond water.

14. Vital staining of vacuoles ($v$) with neutral red. $o$ = calcium oxalate concretions; $S$ = sulfur globules.

15. Staining of lipid globules ($l$) with Sudan black.

16. Smear fixed with Lenhossék, and stained with ferric hematoxylin and erythrosin. Nuclear substance, $n$, in black; cytoplasm, $c$.

17. Young cell obtained after active multiplication. Positive Feulgen reaction in the central nuclear element ($n$), shown by stippling.

Figs. 18-21. *Bacterium* A. 18. 3-day culture. Vital staining of metachromatic granules (*m*) with cresyl blue.

19. 3-day culture. Staining of lipid globules (*l*) with Sudan black.

20. 3-day culture. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin.. Nuclear element (*n*) is sometimes displaced by a lipid globule (*l*).

21. 3-day culture. Smear fixed with 95% alcohol; Feulgen reaction (hydrolysis 10 min., Schiff reagent 5 hours, light green). Same structures as Fig. 20.

Figs. 22-25. .*Bacillus* B.

22. 36-hour culture. Vital staining of metachromatic granules (*m*) with methylene blue.

23. 48-hour culture. Staining of lipid globules (*l*) with Sudan black.

24. 24-hour culture. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear element, *n*; cytoplasm, *c*; lipid globules, *l*. The nuclear substance is between and around the lipid globules.

25. 24-hour culture. Smear treated as in Fig. 21. Same disposition of nuclear substance as in Fig. 24.

Figs. 26-29. *Bacillus* C. 26. 24-hour culture. Vital staining of metachromatic granules (*m*) with methylene blue.

27. 24-hour culture. Staining of lipid globules (*l*) with Sudan black.

28. 24-hour culture. Staining of glycogen (*g*) with I₃K.

29. 24-hour culture. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear element, *n*.

Figs. 30-32, 36, 37. *Bacillus enterothrix* Collin, in intestinal tract of tadpoles. Each cell makes two spores.

30. Sublethal staining of the nuclear element (*n*) as an axial thread, with cresyl blue. The nuclear element of prespores (*p*) is not stained with vital staining.

31. Smear fixed with Perenyi, then stained with ferric hematoxylin and erythrosin. Nuclear axial thread, *n*.

32. Smear fixed with alcohol, then stained with Delafield hematoxylin. Nuclear axial thread, *n*.

36. Smear fixed with chloroform for 30 seconds, then Feulgen reaction. Positive reaction of nuclear axial thread (*n*) inside and outside the two spores (*s*).

37. Same as Fig. 36. Old cell, in which the protoplasm is almost completely disintegrated, a short time before the liberation of the two spores (*s*). Remnants of nuclear substance, *n*.

Fig. 33. *Bacillus camptospora* Collin in intestinal tract of tadpoles. Sublethal staining of the nuclear axial thread (*n*) with cresyl blue. Cells make two spores (*s*). The nuclear element of the spore is not stained with vital staining.

Fig. 34. *Escherichia coli*, strain B/r. 3-hour culture in aerated nutrient broth (from a 24-hour culture in aerated nutrient broth) at 37°C. Smear stained with Giemsa. Nuclear element (*n*) as in 24-hour or older cultures.

Fig. 35. *E. coli*. Same as Fig. 34. Nuclear element (*n*) dividing.

Fig. 38. Bacterial cell in intestine of tadpole. Smear fixed with chloroform, then Feulgen reaction. Nuclear axial thread, *n*.

FIG. 39. *E. coli,* strain B/r/1. 24-hour culture in aerated nutrient broth at 37°C. Giemsa staining. Various aspects of cells.

FIG. 40. Same as Fig. 39; division figures of nuclear elements.

FIGS. 41-43. *E. coli,* strain B/r. 4¾-hour culture in nonaerated nutrient broth at 37°C. Giemsa staining.

41. Three rods having two to four nuclear elements (*n*), one dividing.

42. Two rods. At left, dividing rod with four dividing nuclear elements (V-shaped).

43. Three rods showing various stages of dividing nuclear elements.

FIG. 44. *E. coli,* strain B/r/1. Giemsa staining. Four rods of a 1¾-hour culture in aerated GR medium at 37°C. Various aspects of cells and division of nuclear elements.

FIG. 45. *E. coli,* strain B. 1½-hour culture in aerated nutrient broth at 37°C. Giemsa staining. Three rods that have undergone two divisions of the nuclear element before cell division.

FIGS. 46-53. *B. cereus.* Cultures on sucrose-bean-peptone agar. Giemsa staining. 46. 16-hour culture. Three-rod chain. The two-cell rod at left has two prespores (*p*), in each of which a granule of nuclear substance is surrounded by homogeneous cytoplasm. In the two-cell rod at right the granule of nuclear substance at the extreme right will probably form the nuclear element of a future spore. The transverse membranes separating the cells in the rods are unstained and invisible.

49. From the same culture as Fig. 46. Another aspect of a chain. Nuclear substance disposed in the shape of an axial thread. The rod at right is dividing.

47, 48, 51. One-hour culture made from the 16-hour culture of Fig. 46. In those cells in which sporulation had already started in the old culture, the process continues here. Ten different stages in the process of sporulation. The various prespores (*p*) show the enlargement of the nuclear element and formation of an axial thread and the enlargement of the cytoplasm into an ovoid shape.

50. A few hours later than Figs. 47, 51. The spore is found in the center of the sporangium with some cytoplasm or nuclear substance outside it in the sporangium. The sporal cytoplasm changes its stainability, and stains sky-blue with Giemsa; the nuclear element moves to the periphery of the spore.

52. The peripheral nuclear element has the shape of an oval ring. Nothing is visible in the sporangium outside the spore.

53. Liberated spores. Nuclear element in side view. The spore coat is not stained and not visible; it is situated as seen in Fig. 56.

FIGS. 54, 55. *B. macerans.* 60-hour culture in aerated GR medium at 30°C. Giemsa staining.

54. Top row: Various aspects of cells. Nuclear element, *n*. At left, normal young cells. At right, beginning of formation of spore by swelling; about one-third of the nuclear substance breaks off and becomes round, then ovoid to form the sporal nuclear corpuscle. Bottom row: Unusual arrangement of the nuclear substance, seen in the same culture.

55. Various aspects of almost-ripe spores, with nuclear element at the periphery, and remains of nuclear substance at the poles of the spore.

FIGS. 56-66. *B. mycoides.* Culture on sucrose-bean-peptone agar at 30°C.

56. Resting spores from a 4-month-old culture on agar. Giemsa staining. The protoplasm does not occupy the whole interior of the spore coat. *a*, small exterior granule; *n*, nuclear element.

57. Same as Fig. 56; Feulgen technique. Smear fixed with chloroform; hydrolysis, 10 minutes at 60°C.; Schiff reagent, 5 hours; light green. Nuclear element, *n.*

58. 2-hour subculture from the culture of Fig. 56. Feulgen technique. The nuclear element has moved to the center of the spore, and in some cases has divided once into two rodlets. Spores slightly swelled.

59. Same culture as Fig. 58. Giemsa staining. Various shapes of nuclear element always seen larger than with Feulgen technique.

60. 3-hour culture. Giemsa staining. Various shapes of nuclear element in swelled spores. Except in the spore at right the first nuclear division has been accomplished. Two rods are emerging from their spores.

61. 4-hour culture. Giemsa staining. Rods emerging from spores, many of them having undergone a second or third nuclear division. Various aspects of nuclear element. On two of the rods the spore coat (*s*) can be seen with an exterior purple granule (*a*) and the remnant of the sporangium membrane (*spg*). In the rod at lower right the nuclear element has not divided and therefore appears as an axial nuclear thread; in the rod at the extreme right the axial nuclear substance is dividing.

62. Same culture as Fig. 61. Feulgen technique. In the cell at upper left all the nuclear substance is in the middle of the cell, as is also seen in the cell stained with Giemsa in Fig. 61, center.

63. 5-hour culture. Fixation with chloroform, Giemsa staining. It is often possible to see that the nuclear elements (*n*) are scalloped by and pressed between round globules.

64. Resting living spores (*s*) observed in tryptone solution with cresyl blue. Vital staining of exterior granule (*a*). Spores are inside the remnant of the sporangium membrane (*spg*).

65. The same suspension of spores observed after 30 minutes in tryptone solution and cresyl blue under the microscope. Sublethal—or postvital (?)—staining of nuclear element (*n*).

66. Same spores as Fig. 64 after 1 hour at 37°C. in tryptone solution. Sublethal staining with cresyl blue.

FIG. 67. *B. cereus.* 110 minutes at 30° C. in aerated 2% tryptone solution (culture made from a 15-hour culture under the same conditions). Giemsa staining. Cells always short with nonhomogeneous cytoplasm and globular nuclear elements. Aspect very similar to that seen in Figs. 86, 87.

FIGS. 54, 55.  *B. macerans.*  60-hour culture in aerated GR medium at 30°C. Giemsa staining.

54. Top row: Various aspects of cells. Nuclear element, *n.* At left, normal young cells. At right, beginning of formation of spore by swelling; about one-third of the nuclear substance breaks off and becomes round, then ovoid to form the sporal nuclear corpuscle. Bottom row: Unusual arrangement of the nuclear substance, seen in the same culture.

55. Various aspects of almost-ripe spores, with nuclear element at the periphery, and remains of nuclear substance at the poles of the spore.

FIGS. 56-66.  *B. mycoides.*  Culture on sucrose-bean-peptone agar at 30°C.

56. Resting spores from a 4-month-old culture on agar. Giemsa staining. The protoplasm does not occupy the whole interior of the spore coat. *a,* small exterior granule; *n,* nuclear element.

57. Same as Fig. 56; Feulgen technique. Smear fixed with chloroform; hydrolysis, 10 minutes at 60°C.; Schiff reagent, 5 hours; light green. Nuclear element, *n.*

58. 2-hour subculture from the culture of Fig. 56. Feulgen technique. The nuclear element has moved to the center of the spore, and in some cases has divided once into two rodlets. Spores slightly swelled.

59. Same culture as Fig. 58. Giemsa staining. Various shapes of nuclear element always seen larger than with Feulgen technique.

60. 3-hour culture. Giemsa staining. Various shapes of nuclear element in swelled spores. Except in the spore at right the first nuclear division has been accomplished. Two rods are emerging from their spores.

61. 4-hour culture. Giemsa staining. Rods emerging from spores, many of them having undergone a second or third nuclear division. Various aspects of nuclear element. On two of the rods the spore coat (*s*) can be seen with an exterior purple granule (*a*) and the remnant of the sporangium membrane (*spg*). In the rod at lower right the nuclear element has not divided and therefore appears as an axial nuclear thread; in the rod at the extreme right the axial nuclear substance is dividing.

62. Same culture as Fig. 61. Feulgen technique. In the cell at upper left all the nuclear substance is in the middle of the cell, as is also seen in the cell stained with Giemsa in Fig. 61, center.

63. 5-hour culture. Fixation with chloroform, Giemsa staining. It is often possible to see that the nuclear elements (*n*) are scalloped by and pressed between round globules.
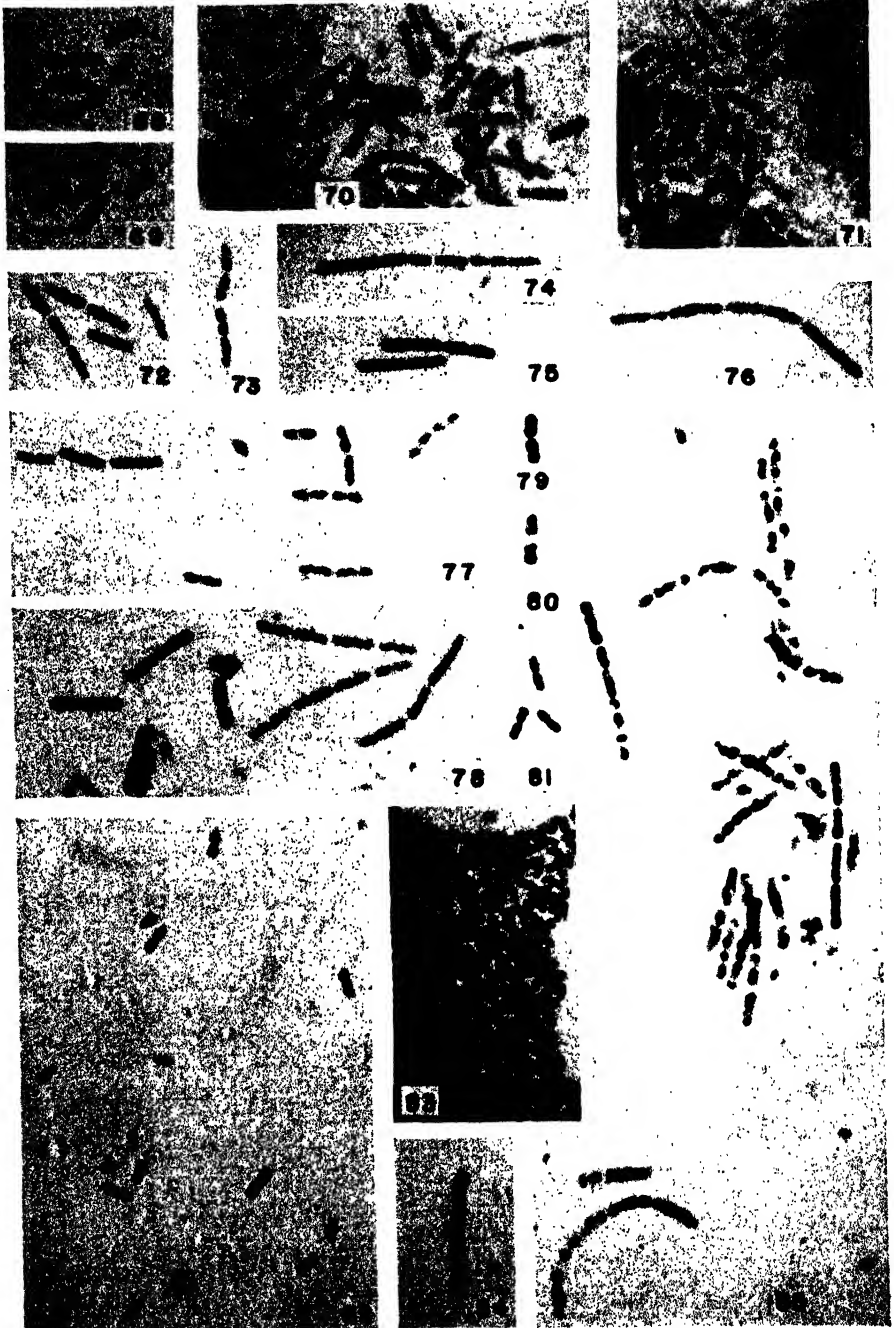
64. Resting living spores (*s*) observed in tryptone solution with cresyl blue. Vital staining of exterior granule (*a*). Spores are inside the remnant of the sporangium membrane (*spg*).

65. The same suspension of spores observed after 30 minutes in tryptone solution and cresyl blue under the microscope. Sublethal—or postvital (?)—staining of nuclear element (*n*).

66. Same spores as Fig. 64 after 1 hour at 37°C. in tryptone solution. Sublethal staining with cresyl blue.

FIG. 67.  *B. cereus.*  110 minutes at 30° C. in aerated 2% tryptone solution (culture made from a 15-hour culture under the same conditions). Giemsa staining. Cells always short with nonhomogeneous cytoplasm and globular nuclear elements. Aspect very similar to that seen in Figs. 86, 87.

BERTHE DELAPORTE

PLATE III

FIGS. 68-71. *B. cereus*. Fixation with osmium tetroxide vapor; staining of lipid globules with Sudan black, and protoplasm with light green.

68-70. 45-minute culture made from a 16-hour culture (see Fig. 71); aerated tryptone broth. Black lipid globules near the middle of cells.

71. 16-hour culture in aerated tryptone broth. Black lipid globules.

FIGS. 72-78, 84, 85. *B. cereus*. Fixation with osmium tetroxide vapor; hydrolysis for 10 minutes with *N* HCl at 60°C. Giemsa staining.

72, 73, 77. Same culture as Figs. 68-70. Nuclear elements in U shapes around lipid globules, or as transverse rods or ovoid granules.

74, 76. 50-minute culture from a 16-hour culture, on bean agar. Long axial nuclear threads especially visible in the two cells at left in Fig. 74. Division, with thread uniting the two principal nuclear masses, seen in Fig. 76 (seventh nuclear element from left).

75. 90-minute culture from a 16-hour culture, on bean agar. In the top chain of cells three division figures (at right) in dumbbell form; in the bottom chain, nuclear granules separated by lipid globules.

78. Same as 74 and 76. Short axial nuclear thread at right; nuclear substance in granules or transverse rods, with adjacent lipid globules. The large black globules are prespores.

FIGS. 79-81. *E. coli*, strain B/r. 4¾-hour culture in nonaerated plain broth. Giemsa staining. Magnifications: Figs. 79 and 80 = 2000 X; Fig. 81 = 1150 X. Nuclear elements as transverse rods.

FIG. 82. *E. coli*, strain B/r. 4½-hour culture in aerated plain broth. Giemsa staining. Nondividing cells with only one globular nuclear element; dividing cells with two nuclear elements. The lower rod in the group of three rods (left) shows median constriction preceding separation; in single rod to the right of this group, the two nuclear elements show the enlargement that characterizes the beginning of division.

FIG. 83. *E. coli*, strain B/r/sd, grown on agar with streptomycin (20-hour culture). Giemsa staining. Cells show short axial nuclear threads in the longer rods and globular nuclear elements in the very short rods. "Snakes" are rare; the area photographed was selected to show several.

FIG. 84. *B. cereus*. Same as Fig. 74. Four prespores and extra sporal nuclear substance in the form of granules or (in the cells at top and bottom) a short axial thread.

FIG. 85. *B. cereus*; 24-hour culture from a 16-hour culture, on bean agar. Various stages in spore formation; beginning (vertical chain at right); middle (lower left); end (top); and free spores (two at lower right).

PLATE III-A
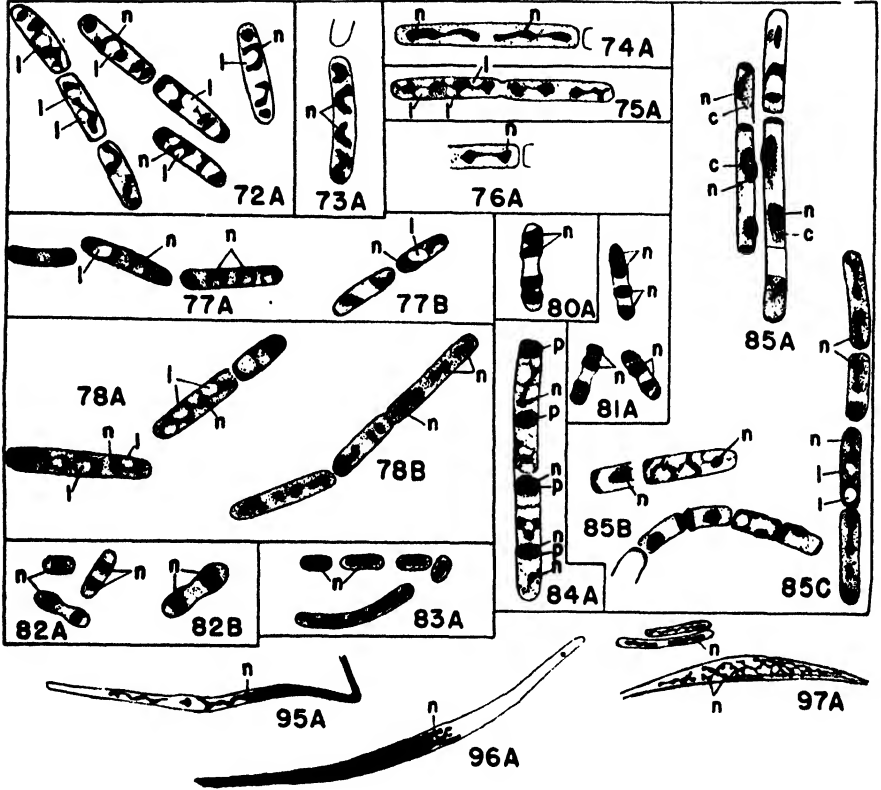
EXPLANATORY DRAWINGS

For explanation of letter symbols, see Pl. I.

FIG. 72A. Photograph 72.

FIG. 73A. Photograph 73, lower rod.

FIG. 74A. Photograph 74, left.

FIG. 75A. Photograph 75, top chain.

FIG. 76A. Photograph 76, cell at middle left.

FIG. 77A. Photograph 77, chain at left.

FIG. 77B. Same photograph, chain at right.

FIG. 78A. Photograph 78, chain and rod at top left.

FIG. 78B. Same, chain at right.

FIGS. 80A-81A. Photographs 80 and 81.

FIG. 82A. Photograph 82, group of three cells near lower left.

FIG. 82B. Same photograph, cell to the right of group shown in 82A.

FIG. 83A. Photograph 83, four cells at top; one "snake" near top.

FIG. 84A. Photograph 84.

FIG. 85A. Photograph 85, two vertical chains at top.

FIG. 85B. Same photograph, two rods and part of chain at lower left.

FIG. 85C. Same photograph, vertical chain at middle right.

FIG. 95A. Photograph 95, the largest "snake"; nuclear substance in form of axial thread near the end.

FIG. 96A. Photograph 96, the largest "snake"; nuclear substance in form of close granules.

FIG. 97A. Photograph 97, two short "snakes" near lower left; and the swollen part of the largest "snake," showing the appearance of the nuclear substance as a loose net of granular threads.

PLATE IV

FIGS. 86-93. *B. cereus.* Lysis by bacteriophage. All photographs at the same magnification. Osmium tetroxide vapor fixation, 3 minutes; hydrolysis, 10 minutes; Giemsa staining; mounted in water.

86, 87. Culture in aerated tryptone broth in the logarithmic phase of growth (three new cultures made every 80-120 minutes) just before addition of bacteriophage. Nuclear elements are thick transverse rods or globular masses. Several division figures appear as two adjacent rods (also as U shapes, Fig. 87); several transverse membranes are visible in the photograph.

88. Eleven minutes after addition of bacteriophage. The nuclear elements have fused inside cells.

89. Twenty-two minutes after addition of bacteriophage. Cells are filled with nuclear substance.

90. Sixty-three minutes after addition of bacteriophage. Same aspect as in preceding figure, but cells are distinctly enlarged.

91-93. Seventy to seventy-four minutes after addition of bacteriophage. Three different examples of lysis by gradual disappearance of chromatic substance from the periphery to the center of cell.

FIGS. 94-97. *E. coli.* Formation of "snakes" by strain B/r/sd-11-8 on plain agar. Osmium tetroxide vapor fixation; hydrolysis, 10 minutes; Giemsa staining; mounted in water.

94. 18-hour culture. Beginning of formation of "snakes"; axial nuclear thread.

95-97. Various aspects of "snakes" in culture kept 20 hours at 37°C. plus 20 hours at 20°C. In many of them, the nuclear substance seems to fill the filament solidly, or in the form of granular threads, for large portions of its length. Some have partial swellings, which contain a nuclear thread (Fig. 95), or closely related nuclear granules (Fig. 96), or a loose net of nuclear threads (Fig. 97).

PLATE V
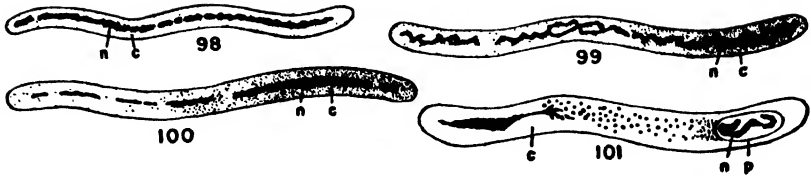


Magnification: 1500 X.

FIGS. 98-101. *Spirillum praeclarum* Collin from intestinal tract of tadpoles. Smears fixed with Perenyi, then stained with iron hematoxylin and erythrosin.

98. Resting cell with nuclear substance (*n*) and cytoplasm (*c*) equally distributed.

99, 100. Accumulation of nuclear substance (*n*) and cytoplasm (*c*) in the region where the spore will be formed.

101. Prespore (*p*) with its nuclear element (*n*). In the sporangium remnants of the nuclear substance (in black) which has partially disappeared.

# Biochemical Genetics of Neurospora

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## CONTENTS

## I. INTRODUCTION

The theory of the gene, in its fundamental aspects, was essentially complete by 1915. We are still engaged in the elaboration of that theory. By establishing the idea that the hereditary determiners are material elements of the cell, the gene theory bridged the gap, in concept at least, between the study of heredity on the one hand and biochemistry on the other. The first biochemical genetical investigation had already been carried out by Garrod (1909) in his famous study of human alcaptonuria,

but the further development of biochemical genetics had to await certain advances in both genetics and biochemistry. Among these were the discovery of the mutagenic action of radiations, the development of the idea of metabolism as a complex system of unit processes, the attack on the chemical nature of enzymes, and the spectacular advances in the field of nutrition.

For the purposes of chemical genetics certain microorganisms have been found to offer some advantages over the classic materials, Drosophila and maize. One of the most useful of these has been the heterothallic ascomycete, *Neurospora crassa*. Neurospora as it is found in nature, the so-called wild-type, is capable of synthesizing all of its cellular constituents except biotin from carbohydrate and inorganic salts. By application of the usual treatments it has been possible to induce gene mutations in the organism which result in the failure of certain biochemical syntheses to take place (Beadle and Tatum, 1941). The resulting mutant strains are unable to grow in the absence of the product whose synthesis has been abolished, or in the absence of appropriate precursors of the substance. By taking advantage of the sexual mode of reproduction, the mutants can be analyzed genetically; at the same time, unlimited quantities of genetically pure material for biochemical study can be obtained by asexual propagation.

The Neurospora investigations have made it quite clear that the gene is at the basis of the synthetic chemical activities of the cell. The biological synthesis of vitamins, amino acids, and nucleic acids is apparently under genic control at every step. Geneticists have long been aware that the genes play an essential part in the economy of the cell, since death of the cell is one of the commonest results of gene mutation. The novelty of the Neurospora approach is that it permits the analysis of a certain fraction of the lethal mutations in terms of cellular biochemistry. This analysis has shown that the ability to carry out the synthesis of the essential ingredients of living matter is inherited in a strictly Mendelian manner. This fact is, in itself, of considerable interest. *A priori*, it might have been supposed (by a non-geneticist) that nature would have contrived some special mechanism to insure the preservation of these vital processes. The fact that no mechanism other than the Mendelian one has revealed itself makes possible a considerable reduction in the number of unknowns in one's concepts of cellular dynamics and evolution. The gene is evidently a fundamental biochemical agent of the cell, and the problems of genetics become to a large extent the problems of biochemistry.

The genetics and biochemistry of Neurospora have been the subject of a number of reviews (Tatum and Beadle, 1942; Beadle, 1945a, b, 1946;

Horowitz *et al.*, 1945a; Bonner, 1946a). These papers adequately summarize earlier work, including the life history of Neurospora and the methods employed for obtaining biochemical mutants. These topics will not be reviewed again here. Instead, it is the intention in the present article to give a picture of the current status of Neurospora research, and to attempt to relate this to the general problem of gene action.

## II. CYTOLOGY AND GENETICS

### 1. *Cytological Observations*

The study of the morphology and behavior of the meiotic and mitotic chromosomes of *N. crassa*, for a long time considered to be hopeless, has been initiated by McClintock (1945) and continued by Singleton (1948). In general, it appears that there is nothing unique or unorthodox in the cytology of Neurospora. Following fusion of two haploid nuclei to form the zygote (the only diploid cell in the life cycle), there occur two meiotic divisions in rapid succession, followed by a mitotic division. Wall formation then takes place, delimiting eight ascospores. Another mitotic division occurs within each ascospore before maturity.

The haploid chromosome number is seven. Previous estimates had been six to nine for *N. crassa* (Lindegren and Rumann, 1938) and six for *N. tetrasperma* (Colson, 1934). The longest chromosome attains a length of approximately 15 μ at pachytene; it is approximately 2.7 times longer than the shortest chromosome. Near the end of the short arm of the second longest chromosome is a nucleolus organizer which develops a nucleolus at each telophase.

Preliminary observations on three translocations were made by McClintock, and on two more by Singleton. Singleton has made tentative suggestions as to the relationship between the cytological chromosomes and the known linkage groups, but the situation is not yet clear.

### 2. *Linkage and Allelic Series. Temperature Mutants.*

Houlahan *et al.* (1949) have summarized some of the linkage data for *N. crassa* which have been obtained in this laboratory and, previously, at Stanford University. Forty-nine loci have been placed in six linkage groups. The data are derived from the analysis of approximately 7000 asci. Biochemical mutants of Neurospora should provide excellent material for a study of the possibility of non-random gene distribution. At present, all that can be said is that if such a distribution exists it does not leap to the eye.

Evidence has been obtained for allelic series at three loci. These are listed in Table 1. Genes at these loci are concerned with the syn-

thesis of adenine, uridine, and inositol, respectively. In each instance, there is, in addition to the normal allele, a mutant allele which abolishes the synthesis over the whole temperature range in which Neurospora is viable (5-42°C.), and one or more alleles which abolish the synthesis over only part of the temperature range. Mutants of the latter type, which are phenotypically mutant at one temperature and phenotypically wild at another temperature, have been called "temperature mutants."

TABLE 1

Probable Allelic Series in Neurospora

| Series | Linkage group | Mutant | Growth factor requirement at 25°C. | 35°C. | Reference |
|---|---|---|---|---|---|
| Adenine | C | 27663 | Adenine | Adenine | Mitchell and |
| | | 70004 | None | Adenine | Houlahan (1946a) |
| Uridine | D | 37301 | Uridine | Uridine | Houlahan and |
| | | 67602 | Small amount uridine | Uridine | Mitchell (1947) |
| | | 37815 | None | Uridine | |
| Inositol | E | 37401 | Inositol | Inositol | Houlahan et al., |
| | | 83201 | None | Inositol | (1949) |

The existence of allelic series of this sort implies that a gene may not only determine whether or not a given reaction can occur, but also at what temperature it can take place.

Temperature alleles are of potential interest in providing material for the study of genetically altered enzyme systems. Usually, temperature mutants are phenotypically normal, or nearly so, at lower temperatures (below 30°C.) and show the mutant character in the range above 30°C. In a few cases this picture is reversed. Several kinds of hypotheses can account for the occurrence of temperature mutants. The most obvious is that in these mutants the gene is altered so that it produces an enzyme with abnormal temperature characteristics, such that the enzyme is inactivated at temperatures within the normal physiological range. Another possibility is that the mutation affects the quantity or quality of the gene product in such a way that it has reduced activity over the whole temperature range. It may be supposed that the reduced activity is sufficient to produce a normal phenotype at one temperature but not at some other temperature where the rates of opposing reactions

overtake it. A third hypothesis is that the gene is totally inactivated, but that the mold can develop an alternative path for the synthesis when grown at certain temperatures. This idea is difficult to reconcile with the allelic series shown in Table 1, however, since on this basis one cannot account for alleles which abolish the synthesis at all temperatures. Such alleles are readily explained on the first two hypotheses as total inactivations of the gene. At present, no choice can be made between these two hypotheses, although the second one seems better able to account for those temperature mutants which exhibit the mutant phenotype in the lower temperature range.

In connection with temperature mutants, mention should be made of a corresponding class of pH mutants. The existence of these was first demonstrated by Stokes *et al.* (1943) who found that a mutant which requires pyridoxine when cultured on the usual medium at pH 5.5 can grow in the absence of the vitamin when the medium is brought to pH 5.8 or higher. Growth under these conditions occurs only if ammonium nitrogen is present in the medium. Other pyridoxineless strains are now known in which the requirement for pyridoxine is not affected by changes of pH. Genetic analysis of the mutants has not proceeded far enough to say whether allelic relationships exist between them.

## 3. Back-Mutation

By virtue of their manner of selection, biochemical mutants of Neurospora produce little or no growth on a medium containing only sugar, inorganic salts, and biotin (minimal medium). If incubated in minimal medium for a sufficiently long time, however, some mutants will eventually begin to grow, and the growth rate may approach that of the wild-type. For want of a better word, this change is often referred to as "adaptation." The term is perhaps unfortunate, since it suggests a connection between this phenomenon and that of adaptive enzyme formation. Actually, there is no reason to assume that adaptive enzymes, as this term is currently understood by microbiologists, play any part in "adaptation," and in a number of cases it is quite certain that they do not.

To date, it has been found that a change from mutant to wild phenotype in Neurospora results from any one of three causes: back-mutation of the original locus, mutation at a different locus (suppressor mutation), and unspecified non-genic changes. Ryan and J. Lederberg (1946) and Ryan (1946) have carried out studies on the spontaneous back-mutation of a leucineless mutant. The demonstration of back-mutation at any locus is of importance in that it constitutes the strongest evidence that the mutation in question is a true gene change and not a deficiency. The

data of Ryan and Lederberg indicate with a high degree of probability that the reversion of leucineless is a true back-mutation. The tests were made by crossing an "adapted" stock to a leucineless strain of the opposite mating type. Leucineless and wild-type progeny segregated, showing that the reversion was genetic. Some of the $F_1$ wild-types were then crossed to another wild-type. If the reversion had been caused by mutation at a locus other than the original leucineless, then leucineless should segregate in the $F_2$. Only wild-types were found in a total of 109 tests for recombination.

Of much interest is the fact that the frequency of reversion of leucineless increases with temperature. It is not yet possible to interpret the temperature effect, however, since the observed frequency of reversion is also a function of the number of cells in the culture and of the leucine concentration. The authors demonstrated that in a heterocaryon (Beadle and Coonradt, 1944) containing leucineless and wild-type nuclei the latter are at a selective disadvantage if leucine is present in the medium and may, in time, be effectively eliminated from growing cultures. Leucine itself is without effect on the growth of wild-type, nor has it been possible to demonstrate an extractable inhibitor of wild-type in leucineless cultures.

Giles and E. Lederberg (1948) have studied the effect of mutagenic agents in inducing reverse mutations in several Neurospora mutants. It was found that the very low spontaneous rate of reversion of an inositolless strain is greatly increased following treatment with ultraviolet; x-rays, nitrogen mustard, and radiophosphorus are also effective. As in the case studied by Ryan and Lederberg, the reversions probably result from back-mutation, since no inositolless progeny were obtained from the cross wild-type × inositolless-reverted.

Less extensive tests carried out by Giles and Lederberg with a riboflavinless strain indicate that it also back-mutates under ultraviolet treatment. A pantothenic acid-requiring mutant, however, was never observed to revert, either spontaneously or following treatment, and may represent a chromosomal deficiency. Preliminary tests carried out on a tryptophaneless strain which is known to revert spontaneously indicated that the spontaneous reversions were non-genic in nature, but induced reversions in the same strain involve a genetic change in some instances.

The determination of the back-mutation rate of biochemical mutants provides a rapid and efficient method for comparing mutagenic activities. When plated out on minimal medium only the cells containing reverted nuclei will grow, and it is therefore easy to test enormous numbers of nuclei. Using a technique similar to that of Giles and Lederberg, the effect of various agents on the back-mutation of an adenineless

strain has been studied by Kölmark and Westergaard (1949), Jensen, *et al.* (1949), and Dickey, *et al.* (1949). Diazomethane and several organic peroxides, as well as x-rays, ultraviolet light, and mustard gas, were found to be mutagenic in this strain. A number of other agents, including phenol and formaldehyde, were inactive.

### 4. *Suppressors*

The first suppressor of a biochemical mutation in Neurospora was discovered in an elegant investigation by Houlahan and Mitchell (1947). A culture of a pyrimidine-requiring mutant (No. 37301) which had previously failed to grow in the absence of uridine or certain related compounds suddenly acquired the ability to grow on minimal medium as if back-mutation had occurred. The culture was crossed to wild-type and the resulting asci analysed. If back-mutation had occurred, two types of asci were to be expected: one containing eight wild-type spores, originating from nuclei carrying the back-mutation, and one containing four wild-type and four pyrimidineless spores, originating from nuclei in which back-mutation had not occurred. (In general, mutations occurring in vegetative cultures of Neurospora give rise to a heterocaryon containing mutant and non-mutant nuclei. A sample of cells taken from such a culture will usually contain both kinds of nuclei).

The expected types of asci were found, but in addition asci containing six wild-type and two mutant spores appeared. To explain these results, it is necessary to assume that a mutation had occurred at a new locus not closely linked to the locus of pyrimidineless, which suppresses the effect of the pyrimidineless mutation. The various ascus types are then readily explained on the basis of recombinations between the two loci. The history of this strain is diagrammed in Fig. 1, where *pyr* represents pyrimidineless and *s* the suppressor of pyrimidineless. Recovery of the four expected genotypes was confirmed by appropriate crosses.

$$
\begin{array}{lll}
& ++ & \text{(Original wild type)} \\
& \downarrow & \\
& pyr\ + & \text{(Pyrimidineless No. 37301)} \\
& \downarrow & \\
++ \quad \times & pyr\ s & \text{(Pyrimidineless-suppressed)} \\
\downarrow & & \\
++ & \text{(Wild type)} & \\
pyr\ s & \text{(Pyrimidineless-suppressed)} & \\
+\ s & \text{(Phenotypically wild)} & \\
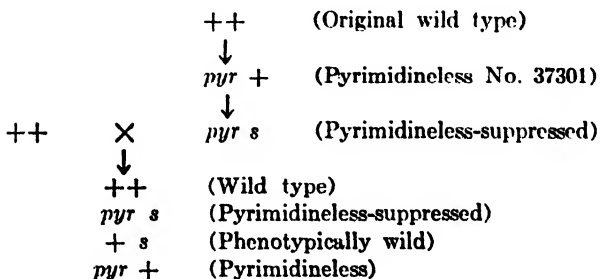pyr\ + & \text{(Pyrimidineless)} &
\end{array}
$$

FIG. 1

History of pyrimidineless No. 37301.

The suppressor gene was found to be without effect on two other pyrimidineless mutants which had previously been shown to be non-allelic with No. 37301. It was effective in suppressing two pyrimidine-less mutants which behave as alleles of No. 37301 (see Section II.2).

Suppressed pyrimidineless grows on minimal medium at about one-half the rate of wild-type. The addition of small amounts of uridine or cytidine brings the growth rate up to normal. Small amounts of arginine, on the other hand, inhibit the growth. The inhibition is over-come by cytidine or lysine. Wild-type carrying the suppressor gene is not affected by arginine. These observations bear a striking resem-blance to the finding of Doermann (1944) that lysineless mutants of Neurospora are inhibited in a competitive manner by arginine. They suggest a connection of some sort between the biological syntheses of pyrimidines, lysine, and arginine, a supposition which has been strength-ened by more recent findings (Houlahan and Mitchell, 1948) which show that certain lysineless mutants accumulate uracil and probably also uridine during growth.

The suppressor of pyrimidineless is of great interest from an evolu-tionary viewpoint, since it appears to represent the acquisition of a new biochemical function by a gene. Similar suppressing actions are well known in Drosophila, but this one seems to offer the best possibilities for biochemical analysis.

Four suppressors of a different sort have been obtained by Emerson (1948) in a mutant characterized by a requirement for sulfanilamide. These will be discussed below (see Section III.8).

## III. BIOCHEMISTRY

### 1. *Introduction*

It has often been pointed out that the production of mutations provides a most delicate and selective method for altering the metabolism of the cell. The gene is a particle of macromolecular dimensions and in a single-gene mutant the initial lesion is therefore of the same dimen-sions. By virtue of the special position which the gene occupies in the cell, this minute alteration may be magnified to a point where it expresses itself in an observable phenotypic change. The delicacy of this method is such that it is possible by its means to isolate and study vital meta-bolic processes in the living organism without disturbing in an observable way any of the other processes of the cell. The significance of this for biochemistry is that it provides the biochemist with a new method for experimenting with the whole organism. The problem of how to re-construct the living cell out of the minces and extracts of the biochemist

has been a vexing one since Buchner's time. Inferences drawn from experiments based on anything less than the whole organism have always been viewed with a slight amount of suspicion by biologists. Do all of the phenomena observed in the test-tube have biological validity, or are some of them experimental artifacts? Or, granting them biological validity, with what relative rates do they proceed in the cell, and how are they integrated into the cellular mechanism?

Biochemists are well aware of these and related problems and have therefore devised techniques for experimenting at all levels of organization from the whole organism down to homogeneous solutions, so that one is not required to remain satisfied with the results obtained by any single method. Experiments on the intact organism are theoretically the least objectionable, but, until recently have also been the least productive of information on the mechanisms of biochemical transformations. The use of isotopic tracers and of single-gene mutations now provide two powerful methods for the study of intermediary metabolism in the whole organism.

The genetic approach to biochemistry not only furnishes a new tool for research, it also provides a new basis for comparative biochemistry. As will develop in what follows, in instance after instance, reaction pathways which are known to be gene-controlled in Neurospora are known or suspected to occur in other species. The occurrence of identical sequences of chemical reactions in the most diverse organisms testifies both to the genetic relationship of living things and to the great age, on the evolutionary time scale, of the biosyntheses of amino acids and vitamins. Elsewhere, the author has taken this point of view in an attempt to explain the evolution of biological syntheses (Horowitz, 1945). It is difficult to escape the conclusion that the same genetic mechanisms— perhaps the same genes—underlie these resemblances. Sturtevant (1948) has recently come to a similar conclusion for genes controlling morphological characters in the genus Drosophila.

## 2. *Arginine and Proline*

The usefulness of Neurospora mutants in a whole-organism experiment is clearly demonstrated in the case of the ornithine cycle. This well-known series of reactions was first proposed by Krebs and Henseleit (1932) on the basis of experiments with surviving liver slices. The existence of the cycle has been doubted by a number of workers, among others by Trowell (1942) whose studies with perfused whole liver do not support the scheme. One may ask whether tissue slice experiments or perfusion experiments give the truer picture. To answer this, recourse must be had to the intact organism. An opportunity for such a test

came when a number of arginine-requiring mutants were obtained in Neurospora. The results of an investigation carried out on these strains (Srb and Horowitz, 1944) clearly support the scheme of Krebs and Henseleit. Of seven different arginineless mutants, four were found to utilize either ornithine, citrulline, or arginine for growth; two utilize citrulline or arginine, but not ornithine; and one strain specifically requires arginine. It was further shown that Neurospora contains arginase, completing the cycle. The finding that the mold also produces urease accounts for the fact that urea is not excreted by this organism.

More recently, Srb (1946) has shown that proline is involved in the reactions leading up to the cycle, since a mutant was found which grows on the addition of proline, ornithine, citrulline, or arginine to the medium. At the same time, another mutant was found to have a specific requirement for proline, so that it appears that the reaction chain is branched, as shown in Fig. 2. The chain has been extended in Penicillium to include glutamic acid. Bonner (1946b) has described a variant in this form which utilizes any one of the following amino acids for growth: glutamic acid, proline, ornithine, citrulline, or arginine. Evidently all of these compounds are readily converted into one another in Penicillium.
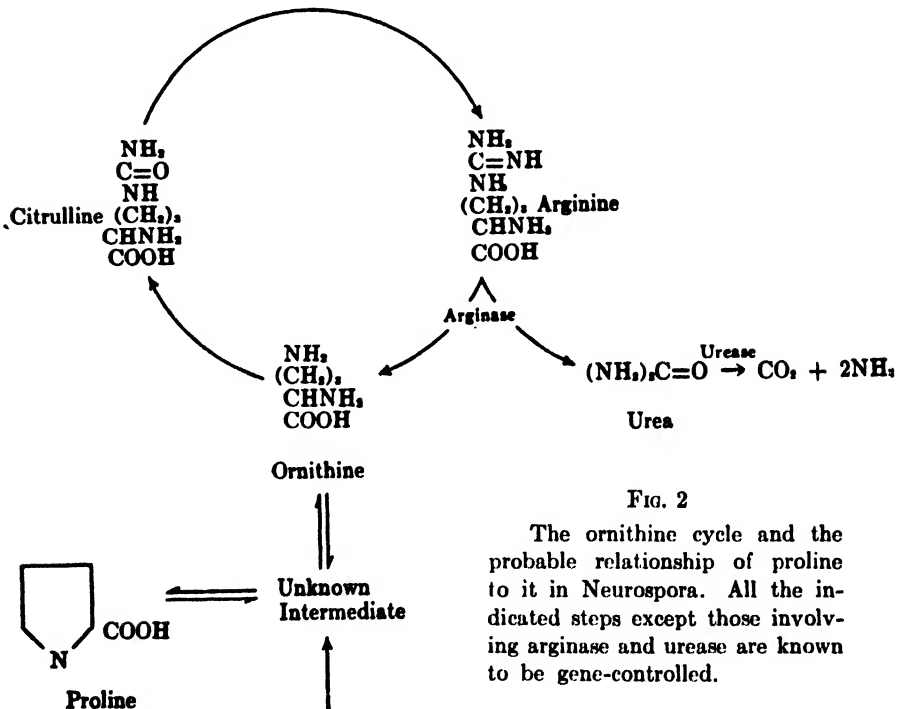


Fig. 2

The ornithine cycle and the probable relationship of proline to it in Neurospora. All the indicated steps except those involving arginase and urease are known to be gene-controlled.

Quite recently, Volcani and Snell (1948) have studied the arginine requirements of a number of different lactic acid bacteria. They find among them strains which exhibit the same types of requirement as Srb and Horowitz found in the Neurospora mutants. Some grow only when arginine is supplied; others can utilize either arginine or citrulline, while still others grow on ornithine, citrulline, or arginine. Volcani and Snell conclude that in the Lactobacilli arginine is synthesized by way of reactions of the Krebs cycle. These findings illustrate in a novel way the principle that all mutations which are produced in the laboratory also occur outside. In this case, mutations which were first produced artificially in one species have been found to be established in nature in a different species. Another example along the same lines is provided in the chick. Klose and Almquist (1940) have found that citrulline, but not ornithine, can replace arginine in the diet of chicks. It is known from the work of Crowdle and Sherwin (1923), however, that the chick can synthesize ornithine, since it excretes large quantities of this amino acid, in the form of dibenzoylornithine, when benzoic acid is added to the diet. In the chick, therefore, the synthesis of arginine is blocked at the ornithine stage, and it corresponds to the mutants of Neurospora which are blocked at the same step.

### 3. *Tryptophane and Nicotinic Acid*

Tryptophane synthesis in Neurospora was first studied by Tatum and Bonner (1943, 1944) and by Tatum *et al.* (1944). It was shown that anthranilic acid and indole are precursors of tryptophane. These investigations were of special interest since they demonstrated for the first time in Neurospora the accumulation of a metabolic intermediate as a result of genetic blocking. It was found that a mutant strain which lacks the ability to convert anthranilic acid to indole and tryptophane (see Fig. 3) excretes anthranilic acid in amounts sufficient to be isolated from the medium. In spite of the fact that sufficient tryptophane for growth of the mutant is supplied in the medium, the intermediate continues to be produced in an automatic reaction and accumulates in the culture because of the absence of an effective mechanism for removing it. Coupled with the fact that anthranilic acid is active in promoting the growth of other tryptophaneless mutants, this constitutes strong evidence that the compound is a natural precursor of tryptophane. Subsequent investigations have shown that precursor accumulation is fairly common among mutants of Neurospora, and a number of these compounds have been isolated and identified.

Tatum and Bonner (1943, 1944) further showed that tryptophane is formed from indole by condensation of the latter with a molecule of
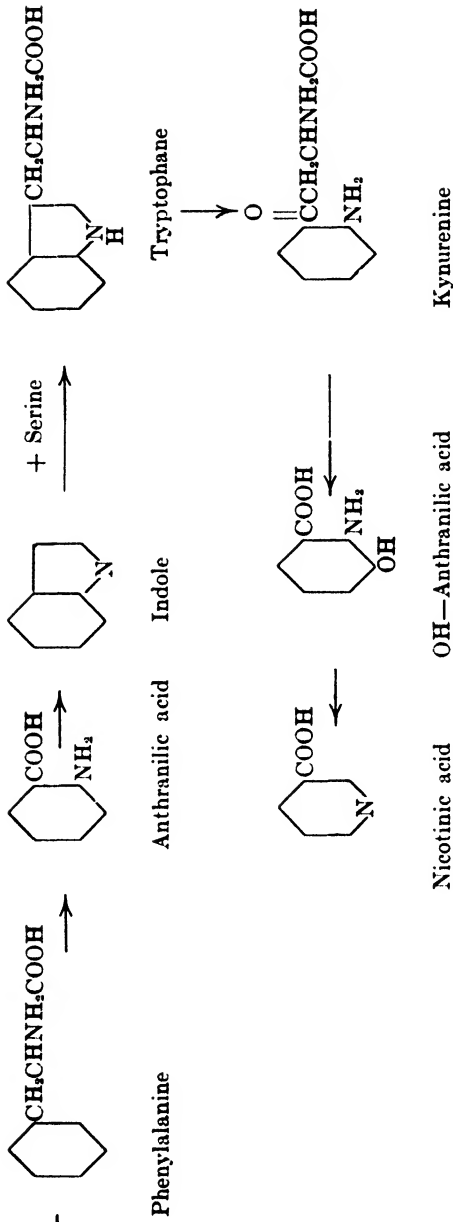
Fig. 3

Relationship of phenylalanine and tryptophane to nicotinic acid synthesis in Neurospora. Evidence that phenylalanine is a precursor of anthranilic acid has recently been obtained by Nyc, Haskins, and Mitchell (1949). Genic control has been established for every step indicated except that from tryptophane to kynurenine.

serine. The enzyme responsible for this reaction was obtained in cell-free extracts from wild-type Neurospora by Umbreit *et al.* (1946). Pyridoxal phosphate is required as coenzyme. At the time this work was done, no mutant lacking the ability to carry out the condensation was known. Such a mutant has recently been obtained by Lein *et al.* (1948), employing a new method for the detection of biochemical mutations. Mitchell and Lein (1948) investigated this mutant for the tryptophane-synthesizing enzyme and found no enzyme activity. The enzyme activity in mutants in which tryptophane synthesis is blocked at other stages approximates that of wild-type. In a further study, Gordon and Mitchell (1950) made the important discovery that the enzyme can be demonstrated in extracts of the mutant following dialysis or precipitation with ammonium sulfate. The mutant enzyme is less active than that from wild-type and is inhibited by boiled extracts of mycelium. This inhibition can be duplicated by various amino acids or by ammonium sulfate. It would appear from these results that the effect of the mutation has been to produce a modified enzyme of lowered specificity which now combines reversibly with various normal constituents of the cell to produce an inactive complex. Further investigation of this interesting system is in progress.

In 1945, Krehl *et al.* discovered a relationship between tryptophane and nicotinic acid in the nutrition of rats. It was found that more nicotinic acid is required by the rat as the tryptophane content of the diet is decreased. From the work of many investigators it now appears that the conversion of tryptophane into nicotinic acid is a general phenomenon in animals. This discovery aroused interest in a group of Neurospora mutants which had been little investigated up to that time. These mutants are characterized by a requirement for either tryptophane or nicotinic acid. The fact that growth occurs on either substance implies that the mold can synthesize one when given the other. A comparison of the quantities of the two substances necessary for maximum growth suggests that the effect of nicotinic acid on tryptophane synthesis is a catalytic one, whereas the function of tryptophane in nicotinic acid synthesis is that of a precursor. These quantities are, for tryptophane, about 125 micromoles per liter and, for nicotinic acid, only 5 micromoles per liter. At the present time, nothing more is known about the role of nicotinic acid in tryptophane synthesis. The pathway by which tryptophane gives rise to nicotinic acid has been clarified to a large extent by Beadle *et al.* (1947) and Mitchell and Nye (1948). It was found that kynurenine and 3-hydroxyanthranilic acid are both active in promoting growth of the mutants, and the evidence indicates that they are intermediates between tryptophane and nicotinic acid, as shown in Fig. 3.

Following this, Bonner (1948) demonstrated that an accumulation product which had previously been isolated from cultures of a nicotinic acid-requiring strain (Bonner and Beadle, 1946) is identical with 3-hydroxyanthranilic acid, constituting proof that this compound is a natural precursor of nicotinic acid. Further details of the reactions are not yet known, except that it was shown in an isotope experiment (Nye *et al.*, 1949) that the carboxyl C of anthranilic acid does not become incorporated into either tryptophane or nicotinic acid.

Recently Bonner and Yanofsky (1949) and Henderson (1949) have independently isolated quinolinic acid (2,3-dicarboxypyridine) from still another nicotinic acid-requiring mutant. This substance serves as a precursor of nicotinic acid for some strains, but because of its low order of activity there is some doubt as to its role in the normal synthesis of nicotinic acid. Quinolinic acid arises from 3-hydroxyanthranilic acid both in Neurospora and in the rat.

In animals, kynurenine has long been known as a product of tryptophane metabolism. It has been identified by Butenandt *et al.* (1940) as the $v^+$-hormone in Drosophila, studied by Ephrussi and Beadle (Ephrussi, 1942). In view of the relationship between kynurenine and nicotinic acid indicated above, it is not surprising that flies carrying the vermilion gene require nicotinic acid in the diet (Tatum, 1939). It would be of interest to know whether wild-type Drosophila requires this vitamin. Mitchell *et al.* (1948) have obtained evidence that 3-hydroxyanthranilic acid can replace nicotinic acid in the diet of rats. In higher plants, this substance occurs as the methylated derivative, damascenine.

### 4. *Methionine, Cysteine, and Threonine*

a. *Cysteine → Methionine.* The class of biochemical mutants most frequently obtained following irradiation of Neurospora is that which is characterized by the inability to synthesize methionine. This class is also the one most often found after treatment with mustard gas (Horowitz *et al.*, 1946; Tatum, 1947; McElroy *et al.*, 1947). This may be related to the fact that methionine synthesis involves the metabolism of sulfur as well as that of carbon, hydrogen, and nitrogen, and this undoubtedly requires the participation of an additional set of genes. This interpretation is not yet established, however, since it is not known how many different loci are represented among the approximately 90 mutant strains of this type. In any case, it has been found that in a large fraction of the mutants the primary metabolic defect is in the reduction of sulfate. (With the exception of a trace of biotin, sulfate is the sole source of sulfur in minimal medium).

It has been established that cysteine is a precursor of methionine in

Neurospora, since approximately half of the methionineless mutants will grow if the methionine in the medium is replaced by cysteine or cystine. It is convenient, for purposes of discussion, to divide the synthesis into two parts, one part dealing with the conversion of the S atom of cysteine into methionine S, and the other dealing with the synthesis of cysteine from the constituents of minimal medium.

The pathway by which cysteine S is transformed into methionine S was investigated by Horowitz (1947). It was found that homocysteine is an intermediate in the reactions and is methylated to give methionine; the source of the methyl group is at present not known (see Section III.8). The mechanism of the transfer of the S atom, from the 3-carbon compound, cysteine, to the 4-carbon compound, homocysteine, was of particular interest on general grounds and because a similar problem was recognized in animal biochemistry, where the biological conversion of methionine to cysteine is well known. The solution to the problem was facilitated by the finding that one of two genetically different mutants, both of which fail to convert cysteine to homocysteine, accumulates a substance which can support the growth of the other mutant. The substance was isolated and identified as the thioether, L-cystathionine (Fig. 4).

The history of this compound is rather interesting. The structure was first provisionally assigned by Küster and Irion (1929) to a substance they had isolated from sulfide-treated wool, but which, on subsequent attempts, they were unable to find again. In 1936, Brand *et al.* suggested that the compound might be an intermediate in the biological conversion of methionine to cystine in man, but since no reliable source of the substance was known there was no way of testing this idea. Again, in 1940, the structure was tentatively assigned by Horn and Jones to an amino acid which they isolated from seleniferous grain in isomorphous combination with the selenium-containing analogue. The experimental investigation of cystathionine became possible in 1941, when the synthesis of DL-cystathionine was accomplished by Brown and du Vigneaud; later, optically active cystathionine and allocystathionine were obtained in the same laboratory (du Vigneaud *et al.*, 1942; Anslow *et al.*, 1946). In a series of papers from du Vigneaud's laboratory (for bibliography, see Horowitz, 1947) it was shown that the behavior of the compound in rat experiments is quite consistent with the role that had been assigned to it by Brand *et al.* To complete the proof, there still remained the actual demonstration that the compound is a normal intermediate in cystine-methionine metabolism. The isolation of cystathionine from a methionineless mutant of Neurospora now leaves little doubt as to the significance of this substance in sulfur metabolism.

The investigation of Horowitz (1947) had shown that the S atom and the 3-carbon portion of the cystathionine molecule originate in cysteine. In a subsequent study by Teas *et al.* (1948) the source of the 4-carbon part of the molecule (and therefore of the carbon skeleton of methionine) was identified. At the same time, it was discovered that methionine and threonine have a common origin in the organism.
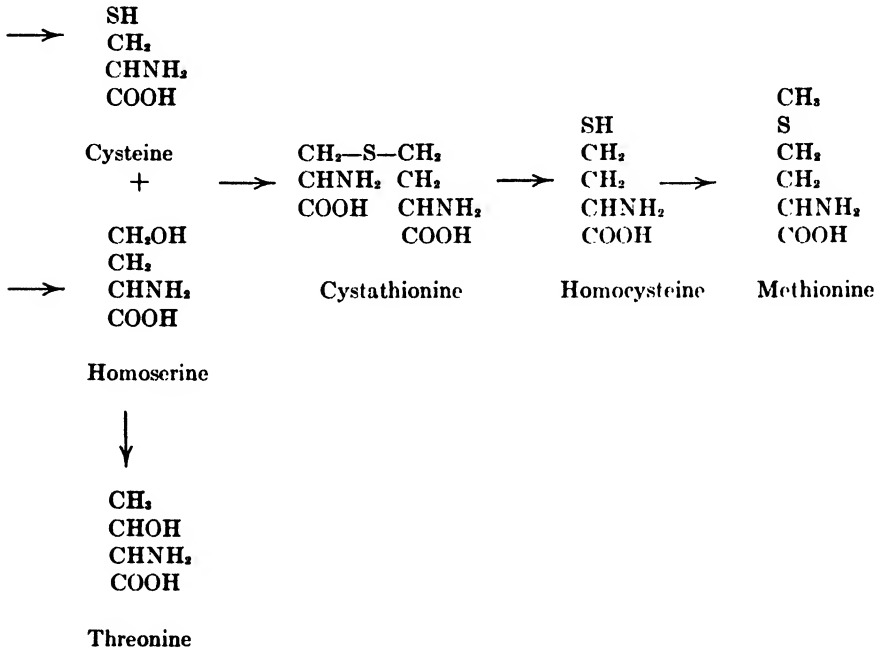
$$
\longrightarrow
\begin{array}{l}
SH \\
CH_2 \\
CHNH_2 \\
COOH
\end{array}
$$

Cysteine

+

$$
\begin{array}{l}
CH_2OH \\
CH_2 \\
\longrightarrow \quad CHNH_2 \\
COOH
\end{array}
$$

Homoserine

Cystathionine

$$
\begin{array}{l}
CH_2-S-CH_2 \\
CHNH_2 \quad CH_2 \\
COOH \quad CHNH_2 \\
\qquad\quad COOH
\end{array}
$$

$$
\longrightarrow
\begin{array}{l}
SH \\
CH_2 \\
CH_2 \\
CHNH_2 \\
COOH
\end{array}
\longrightarrow
\begin{array}{l}
CH_3 \\
S \\
CH_2 \\
CH_2 \\
CHNH_2 \\
COOH
\end{array}
$$

Homocysteine    Methionine

$$
\downarrow
$$

$$
\begin{array}{l}
CH_3 \\
CHOH \\
CHNH_2 \\
COOH
\end{array}
$$

Threonine

FIG. 4

Metabolic relationship between cysteine, threonine, and methionine in Neurospora. Each of the indicated steps is known to be gene-controlled.

This study was based on a mutant which exhibits a double nutritional requirement: both threonine and methionine are needed for growth. Repeated matings to wild-type failed to result in segregation of the two characters, indicating that a single gene change is involved. It therefore appeared that either this gene has two separate functions, contrary to previous experience, or else it governs a single reaction common to both syntheses. Assuming the probable correctness of the second hypothesis, it was found that cysteine synthesis in the mutant proceeds normally, but that the next step, to cystathionine, does not occur. It seemed possible that the mutant was lacking the ability to synthesize the 4-carbon portion of cystathionine. Proof of the hypothesis was obtained when it

was found that the 4-carbon amino acid, homoserine, satisfies both the threonine and the methionine requirement of the mutant. It was concluded that homoserine undergoes two reactions in the organism; in one, it condenses with cysteine to yield cystathionine, and in the other it rearranges to give threonine. The activity of DL-homoserine for the strain equals or exceds that of a mixture of DL-threonine plus DL-methionine, a fact which lends weight to the idea that homoserine is a normal precursor of these two amino acids.

Teas (1947) has carried out further studies on other threonineless mutants and finds that at least two genes are concerned with the conversion of homoserine into threonine. It appears that isoleucine and aminobutyric acid are also involved in the reactions, but the situation is not yet clear. Unpublished data of Fling and Horowitz show that mutant strains which fail to synthesize cystathionine from homoserine and cysteine accumulate threonine as the result of the blocking. This is interpreted to mean that homoserine which would normally be utilized for methionine synthesis is, in these strains, shunted off into excess threonine production.

b. $SO_4^=$ → *Cysteine.* In unpublished experiments of Phinney, Fling, Shen and the author, using mutants blocked at stages preceding cysteine in Fig. 4, it has been found that cysteine synthesis involves
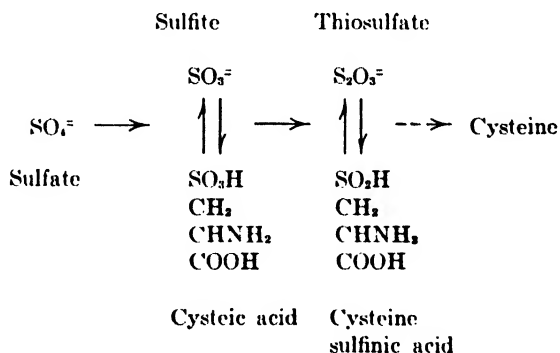


Fig. 5

Suggested pathway of sulfate reduction in Neurospora. Each step (horizontal arrows) is gene-controlled.

the stepwise reduction of sulfate. The latter is reduced to the sulfite level and then to the thiosulfate level in two gene-controlled steps. Both sulfite and thiosulfate can be utilized for cysteine and methionine syn-

thesis by wild-type or by appropriate mutants. Further reduction would be expected to yield elementary sulfur and sulfide. Sulfur has not been tested seriously, but repeated experiments with sulfide have failed to produce convincing evidence that it is used by the mold for cysteine synthesis. It therefore appears that the sulfur atom reacts with an organic compound before attaining the sulfide level of reduction. Organic sulfur compounds in which the sulfur is in a more highly oxidized state than it is in cysteine have therefore been tested by Phinney (abstract, 1948). The preliminary results indicate that cysteic acid may be metabolically equivalent to sulfite, and cysteine sulfinic acid equivalent to thiosulfate in the nutrition of the mutants. This gives the possibility of two alternative paths of sulfate reduction, one organic and the other inorganic. It is unlikely that both mechanisms operate independently, but it is not improbable that the two are in equibrium with one another, as indicated in Fig. 5.

### 5. *Lysine*

Lysineless mutants of Neurospora were first studied by Doermann (1944, 1946). The synthesis of lysine was shown to be controlled by at least three, possibly six, loci. The most peculiar feature of these mutants is their specific inhibition by arginine. Growth is rapidly suppressed when the molecular ratio of arginine to lysine in the medium exceeds 1. Since wild-type is not affected by high concentrations of arginine, it is clear that the phenomenon is in some way related to the mutation. It is now known that inhibitions of this type are not uncommon among biochemical mutants. For example, the suppressor of pyrimidineless discussed above (see Section II.4) is similarly affected by arginine. The homoserineless mutant of Teas *et al.* (see Section III.4-a) is inhibited by a number of amino acids, including L-methionine, for which it at the same time exhibits a requirement. Other examples could be cited, (*v.*, Lein *et al.*, 1948). The interesting point is that the inhibitions are produced by amino acids which occur in all cells, and which, under the same conditions, but little influence the growth of wild-type. Horowitz and Srb (1948) have, however, described a striking inhibition of the growth of wild-type Neurospora by the amino acid canavanine. This amino acid occurs naturally, but seems to be restricted to the jack-bean (*Canavalia ensiformis*) and its close relatives. The inhibition is reversed by arginine, which is a structural analogue of canavanine. Sensitivity to canavanine appears to be genetically determined.

Lysine is unique among amino acids whose fate in the animal organism has been studied, in that it does not participate reversibly in

the general nitrogen exchange (Weissman and Schoenheimer, 1941; Schoenheimer, 1942). A significant advance in the biochemistry of this substance was made by Borsook *et al.* (1948) who found in isotope experiments that lysine is converted to α-aminoadipic acid (Fig. 6) by liver and kidney preparations. This discovery has led to the first important step in the elucidation of the biological synthesis of lysine. Mitchell and Houlahan (1948) tested aminoadipic acid on four different lysineless
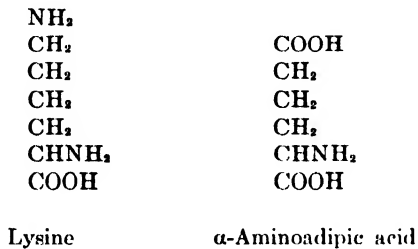
$$
\begin{array}{ll}
NH_2 & \\
CH_2 & COOH \\
CH_2 & CH_2 \\
CH_2 & CH_2 \\
CH_2 & CH_2 \\
CHNH_2 & CHNH_2 \\
COOH & COOH \\
\end{array}
$$

Lysine      α-Aminoadipic acid

FIG. 6

mutants of Neurospora and found that this substance replaces lysine in the nutrition of one of them. In view of the high activity of the compound, it is not unlikely that it represents a normal precursor of lysine. This view is strengthened by the fact that the conversion is blocked in three of the mutants tested. When grown on L-aminoadipic acid the mutant is not inhibited by arginine, but is sensitive to asparagine.

Further differentiation of the lysineless strains is provided by the recent discovery (Houlahan and Mitchell, 1948) that two of them accumulate uracil and possibly also uridine during growth. This finding is in line with other evidence for a metabolic interrelationship between lysine and the pyrimidines (Section II.4), although an interpretation in terms of the reaction pathways involved does not yet seem possible.

## 6. *Isoleucine and Valine*

Bonner *et al.* (1943) described a single-gene mutant of Neurospora which grew on casein hydrolysate but not on a mixture of the known amino acids. It was thought at first that the strain requires an unknown amino acid in casein, but further investigation showed that the activity of casein resides in its content of isoleucine and valine (Fig. 7). These must be supplied to the mutant in a particular ratio—optimally, 70-80% L-valine and 30-20% L-isoleucine. Excess of either amino acid inhibits growth. The double requirement was assumed to be caused by a block in a reaction common to the synthesis of both amino acids. The structural similarity of the two compounds is consistent with this view. A

reinvestigation of the problem has led Bonner (1946c) to revise his original viewpoint. He now considers it more probable that the defect in the isoleucineless-valineless strain is solely in the amination of the keto acid analogue of isoleucine ($\alpha$-keto-$\beta$-methyl-$n$-valeric acid), which
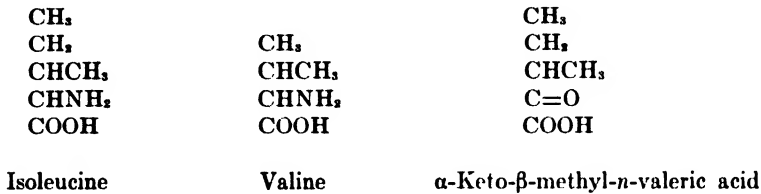
$$
\begin{array}{lll}
\text{CH}_3 & & \text{CH}_3 \\
\text{CH}_2 & \text{CH}_3 & \text{CH}_2 \\
\text{CHCH}_3 & \text{CHCH}_3 & \text{CHCH}_3 \\
\text{CHNH}_2 & \text{CHNH}_2 & \text{C=O} \\
\text{COOH} & \text{COOH} & \text{COOH} \\
\\
\text{Isoleucine} & \text{Valine} & \alpha\text{-Keto-}\beta\text{-methyl-}n\text{-valeric acid}
\end{array}
$$

FIG. 7

he believes is the immediate precursor of isoleucine in the organism. Experiments with the synthetic keto acid show that it inhibits utilization of the keto acid analogue of valine by the mutant. Bonner believes that the requirement for valine is induced by the accumulation of sufficient amounts of the keto analogue of isoleucine in the cells to inhibit the synthesis of valine from its keto acid precursor.

### 7. Choline

Two genes controlling the synthesis of choline are known in Neurospora. One of the mutants accumulates an intermediate which is utilized for choline synthesis by the other. The intermediate has been isolated and identified as monomethylaminoethanol (Horowitz, 1946). There is

$$
\begin{array}{lllll}
& & \text{CH}_3 & (\text{CH}_3)_2 & (\text{CH}_3)_3 \\
\rightarrow & \text{NH}_2 & \text{NH} & \text{N} & \text{N}^+ \\
& \text{CH}_2 \rightarrow & \text{CH}_2 \rightarrow & \text{CH}_2 \rightarrow & \text{CH}_2 \\
& \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\\
& \text{Aminoethanol} & \text{Monomethyl-} & \text{Dimethyl-} & \text{Choline} \\
& & \text{aminoethanol} & \text{aminoethanol} &
\end{array}
$$
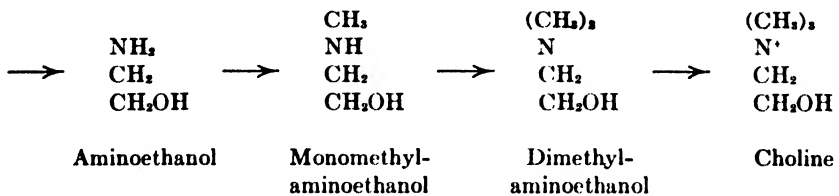
FIG. 8

The probable pathway of choline synthesis in Neurospora. The first two methylations are known to be gene-controlled.

little doubt, on the basis of this finding, that the synthesis of choline proceeds by the stepwise methylation of aminoethanol (Fig. 8). The two genes control the first and second methylations respectively. In support of this conclusion, both strains utilize dimethylaminoethanol for growth, one uses monomethylaminoethanol, while neither uses aminoethanol (**Horowitz** *et al.,* 1945b; Jukes and Dornbush, 1945).

Isotope studies by Stetten (1941) and by du Vigneaud *et al.* (1946) indicate that in the rat choline is probably synthesized by means of a similar series of methylations. The mammal differs from Neurospora, however, in that the former is incapable of synthesizing labile methyl groups and requires a source of them, in the form of choline or methionine, in the diet. Neurospora makes methyl from carbohydrates. There is some evidence that in Neurospora, as in the rat, the methyl groups of choline and methionine are equivalent in metabolism, since methionine has a marked sparing action on choline in both Neurospora mutants (Horowitz and Beadle, 1943; Horowitz *et al.*, 1945b). A methylless mutant of Neurospora, when one is found, will make possible a more general investigation of methyl transfer in this organism.

## 8. p-*Aminobenzoic Acid and Sulfanilamide*

A mutant of Neurospora requiring *p*-aminobenzoic acid (PABA) for growth was described by Tatum and Beadle (1942). It was shown that the antagonism between PABA and sulfanilamide discovered by Woods and Fildes (1940) operates also in Neurospora, both in wild-type and in the mutant strain. A sulfanilamide-resistant mutant of Neurospora was obtained by Emerson and Cushing (1946) by selecting from cultures grown on increasing concentrations of the drug. The strain differs from wild-type by a single gene. Its growth rate is not affected by sulfanilamide in saturated solution. From a cross between this strain and a wild-type there was isolated, from the progeny, a mutant type which failed to grow on solid medium unless sulfanilamide, or a related sulfonamide, was present. It was found that the sulfa-requiring organism was a double mutant carrying the gene for sulfa-tolerance plus another mutation for sulfa-requirement. In outcrosses, the two genes segregated independently.

In a further investigation of the sulfa-requiring mutant, Emerson (1947) found that the strain shows an absolute requirement for sulfonamides when cultured at 35°C., but can grow in their absence at lower temperatures. Even at the lower temperatures, however, a significant stimulation of growth is obtained in the presence of sulfonamides. The sulfa-requiring mutant is not sulfa-resistant. The concentration of sulfanilamide (*ca.* $10^{-3}$ *M*) needed to produce optimal growth of the mutant is less by a factor of 10 than that required to inhibit the growth of wild-type. Higher concentrations of sulfa inhibit growth of the mutant. This is in distinct contrast to the sulfa-resistant strain. In the double mutant carrying the genes for sulfa-resistance and sulfa-requirement, the latter is partially epistatic in that growth is inhibited at concentrations of sulfanilamide approaching saturation. It was further

found, in keeping with its unusual character, that the sulfa-requiring mutant is inhibited by low concentrations of PABA. The inhibition is overcome by the addition of more sulfanilamide to the medium.

The simplest hypothesis to explain these results appeared to be that the sulfa-requiring strain produces an excess of PABA, since PABA is known to inhibit the growth of wild-type in high concentrations. This possibility was ruled out by showing that sulfanilamide does not counter-act the PABA inhibition of wild-type, and, furthermore, that a double mutant carrying the sulfa-requiring gene plus the PABA-less gene of Tatum and Beadle requires both PABA and sulfonamides for growth at 35°C. It was therefore suggested that sulfanilamide might be used as a metabolite by the mutant and that the inhibitory action of PABA in the growth of the strain results from competition between sulfanilamide and PABA for an enzyme surface.

The solution to the problem was obtained by Zalokar (1948) in further experiments with the PABA-less, sulfa-requiring double mutant. In testing folic acid and some of its derivatives, Zalokar found that the double mutant grew on certain of these compounds in the absence of sulfonamides. Further analysis showed that their activity could be explained by small amounts of PABA which they contained as an im-purity. It was found that the double mutant grows in the absence of sulfonamides if very small quantities (less than $5 \times 10^{-8}$ $M$) of PABA are supplied. Higher amounts inhibit growth. The best interpretation of these facts is that the sulfonamide requirement results from chronic PABA intoxication, caused not by overproduction of the growth factor, but by an exceptional sensitivity on the part of the sulfa-requiring strain to the amount normally produced. This presumably results from par-ticipation of excess PABA in the mutant in a deleterious reaction which does not occur in strains lacking the gene for sulfa-requirement. The role of sulfanilamide is to displace PABA from the site of the reaction. The exact nature of the toxic reaction is not yet known, but an important clue has recently been obtained by Zalokar (unpublished experiments). He finds that sulfonamides can be replaced by threonine in the nutrition of the mutant. The implication is that the toxic reaction has the effect of destroying threonine or one of its precursors.

Emerson (1948) has found that the sulfa-requiring mutant fre-quently reverts to a wild phenotype. In each case which has been analyzed, the reversion is due to suppressor mutation at a locus distinct from that which determines the sulfa-requirement. At least four dif-ferent loci can mutate to suppressors. Any mutation which would re-duce the concentration of available PABA in the cells would be expected to have a suppressing effect. Emerson has made a model of this situa-

tion by forming a heterocaryon between sulfa-requiring on the one hand and the double mutant sulfa-requiring, PABA-less on the other. Neither strain by itself grows on minimal medium at 35°C., but the heterocaryon does. This is not the usual kind of heterocaryon (Beadle and Coonradt, 1944) which can be compared physiologically to a double heterozygote in diploid organisms. This heterocaryon is "homozygous" for the gene determining sulfa-requirement, but the expression of the gene is suppressed because the "heterozygous" condition of the gene controlling PABA synthesis limits the production of PABA. Emerson points out the similarity between this case and that of one gene heterosis in maize.

### 9. *Thiamine*

The biosynthesis of thiamine in Neurospora has been studied by Tatum and Bell (1946). Four different single-gene mutants were investigated, three of them in *N. crassa* and the fourth in *N. sitophila*. Evidence from two of the strains indicates that thiamine is synthesized by coupling of the two components, thiazole and pyrimidine (Fig. 9).



Substituted 2,5-dimethyl-4-aminopyrimidine

Methylhydroxyethyl thiazole
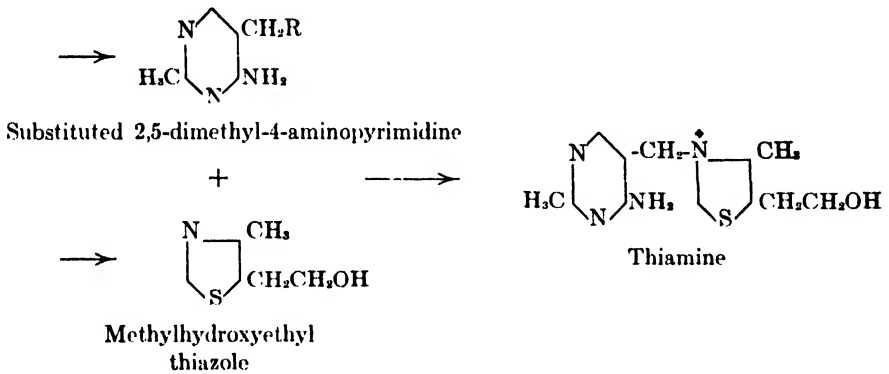
Thiamine

FIG. 9

Biosynthesis of thiamine. The coupling reaction and the synthesis of thiazole are known to be gene-controlled in Neurospora.

One of the strains is unable to carry out the coupling reaction and accumulates both components (determined biologically) during growth. This strain has a specific requirement for thiamine. The other mutant utilizes either thiamine or thiazole for growth, as if synthesis of the latter were blocked. In accordance with expectation, this mutant accumulates a substance which is biologically indistinguishable from vitamin pyrimidine.

The other two mutants, one in *N. crassa*, the other in *N. sitophila*,

are in apparent contradiction to the scheme shown in Fig. 9. These strains require both thiazole and pyrimidine, or else intact thiamine, for growth. The simplest conclusion would be that the synthesis of both components is blocked in these strains. This would imply either that vitamin thiazole and pyrimidine have a common precursor whose synthesis is abolished in the mutants, or else that we are dealing here with genes which control two different reactions. In view of the structural differences between thiazole and pyrimidine it is not very likely that they have a common precursor short of the sugar and inorganic salts from which they are both synthesized. On the other hand, the idea that the genes are bifunctional is objectionable on several grounds. Not only is it contrary to our experience with biochemical mutations in Neurospora, but even assuming that bifunctional genes exist—a proposition which has certainly not been disproved—it is difficult to believe that any such genes would be found in which the two functions bear the singular relation to one another which obtains here. Vitamin thiazole and vitamin pyrimidine are two chemically unrelated compounds whose only known role in the organism is that of combining to produce thiamine. To assume that a single gene controls the synthesis of both of them implies that in bifunctional genes the two functions may be related to one another according to the subsequent fate of the products, regardless of their chemical properties. While it would be foolish to deny the possibility of such relationships, this conclusion is so novel that it cannot be accepted without very convincing evidence.

Actually, there are other reasons for thinking that the hypothesis of bifunctional genes is untenable in the present instance. Both of the mutants in question differ from other thiamineless strains in that the amount of thiamine required for optimal growth is high—by a factor of 5 or more. Furthermore, Tatum and Bell find that both mutants accumulate a substance which is biologically indistinguishable from intact thiamine. As in the case of thiamine, the biological activity of the substance is destroyed by sulfite. Since the substance is apparently inactive for the strains that produce it, Tatum and Bell conclude that it is probably a thiaminelike compound. They suggest that the derangement in the two mutants may be such as to force the synthesis of the compound at the expense of precursors which would normally go into thiamine production. This can account for all the observations if it is assumed that the activity of the thiaminelike substance is much less for the mutants that produce it than for other thiamineless mutants. It is perhaps simpler, however, to assume that the substance is thiamine or perhaps thiamine pyrophosphate, and that the derangement in the mutants is such as to reduce their capacity to retain thiamine at the site

of its action. This could be brought about, for instance, by an increase in the dissociation of a thiamine-protein complex. This, or some similar hypothesis, would explain the available data. In any case, it seems clear that they cannot be accounted for by assuming a block in the syntheses of pyrimidine and thiazole in the two mutants.

## 10. *Riboflavin*

One riboflavin-requiring mutant is known in Neurospora. Its characteristics have been described by Mitchell and Houlahan (1946b). This strain is a temperature mutant (see Section II.2). At temperatures below 25°C. it grows like wild-type on unsupplemented medium. At temperatures above 28°C., however, no growth occurs unless the medium has been supplemented with riboflavin. In the temperature range below 25°C. riboflavin synthesis is normal as shown by bioassay with *Lactobacillus casei*.

## 11. *Pantothenic Acid*

Evidence from a variety of organisms (see Ryan *et al.*, 1945 for bibliography) indicates that the biological synthesis of pantothenic acid involves the coupling of $\beta$-alanine and pantoyl lactone, or derivatives of these compounds. A pantothenic acid-requiring mutant of Neurospora is known which is unable to use these precursors for growth, although it synthesizes them (Tatum and Beadle, 1945). It is concluded that this strain lacks the ability to carry out the coupling. In confirmation of this, Wagner and Guirard (1948) have found that intact, resting mycelium of wild-type, but not of the mutant, produces pantothenic acid when incubated with $\beta$-alanine and pantoyl lactone.

In a more recent study, Wagner (1949) has found that an enzyme system which synthesizes pantothenic acid from the two precursors can be obtained from both wild-type and mutant strains following treatment of the mycelium with acetone. Since the mutant is unable to bring about the synthesis *in vivo*, it is necessary to assume that the enzyme is inactive in the intact cell. Wagner concludes that the mutant possesses an inhibitor of the enzyme. This interesting case thus resembles in some respects that of the tryptophane-synthesizing system discussed previously. It has not been excluded, however, that the effect is due to activation of an inactive enzyme by acetone. A precedent for this exists in the known activating effect of acetone on tyrosinase.

## 12. *Succinic Acid*

A number of mutant strains which require succinic acid or related compounds for growth have been described by Lewis (1948). In addition to succinate, these strains are reported to utilize fumarate, glutamate,

α-ketoglutarate, aspartate, malate, and, to some extent, acetate. Lewis suggests that these results can be interpreted on the assumption that a modified tricarboxylic acid cycle operates in Neurospora, although it is also necessary to assume that the cycle can be blocked at at least one point without producing a lethal effect.

Fincham (1950) has described two probably allelic mutant strains which require for growth any one of at least thirteen amino acids. The most active are glutamate, aspartate, alanine, and ornithine. Keto acids and D-amino acids (which probably give rise to keto acids in the cell) are inactive. This and other evidence indicates that these strains are unable to utilize ammonia for the synthesis of α-amino groups. Growth occurs when amino nitrogen is supplied in the form of one of the active amino acids. The remaining ones are then synthesized presumably by a process of amino transfer.

### 13. *Nucleic Acid Constituents*

a. *Purines.* At least seven different genes in Neurospora are known to control the synthesis of adenine and adenosine (Mitchell and Houlahan, 1946a; Pierce and Loring, 1945). Several of the strains are temperature mutants (Section II.2). All of the strains can utilize hypoxanthine (or its riboside, inosine) in place of adenine, except one; the latter mutant must therefore carry the mutated form of a gene which is necessary for the amination. The deamination of adenosine to inosine is normal in this strain (McElroy and Mitchell, 1946), indicating once again that biological syntheses are not simply biological degradations in reverse. One of the adenineless mutants accumulates a purple pigment during growth. The pigment apparently has no activity for any of the other mutants and is interpreted as a secondary product formed from a labile adenine precursor whose normal reaction pathway is blocked (Fig. 10).

None of the adenineless strains will utilize guanine alone, but at least one of them has been reported to use this purine when it is sup-
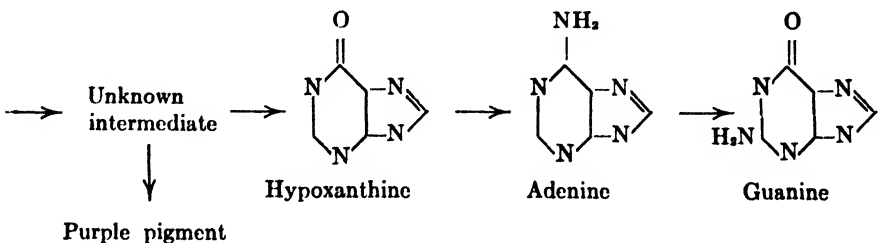


Fɪɢ. 10
Purine relationships in Neurospora.

plied along with adenine (Loring and Fairley, 1948). This finding indicates that the mutant is also deficient in the ability to synthesize guanine, and that adenine is partly converted into guanine in the organism, but not the reverse. This is in line with the results of Brown *et al.*, (1947), obtained by feeding isotopic adenine and guanine to rats. The mechanism of the conversion is unknown.

b. *Pyrimidines.* The mutants of Neurospora which require pyrimidine compounds for growth are a nutritionally complex group. The substance most active in supporting their growth are the nucleosides, uridine and cytidine, and the corresponding nucleotides, uridylic and cytidylic
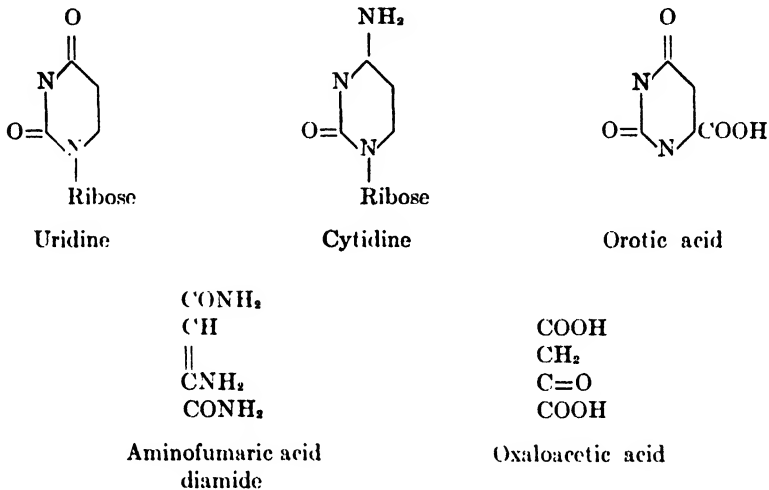


Fig. 11

acids (Loring and Peirce, 1944; Mitchell and Houlahan, 1947). Since the known strains will utilize any one of these compounds, they must be interconvertible in metabolism. There is another group of substances, including uracil, cytosine, aminofumaric acid diamide, orotic acid, and oxaloacetic acid which will also support the growth of some of the strains, but with less than one-tenth the effectiveness of the first group. In view of their relatively low activities, it is questionable whether any of them normally function as intermediates in nucleotide synthesis, although Mitchell and Houlahan (1947) believe that oxaloacetate and aminofumaric acid diamide may do so. Of interest is the fact that one of the mutants accumulates relatively enormous quantities of orotic acid (4-carboxyuracil) during growth (Mitchell *et al.*, 1948). The authors consider it unlikely that orotic acid is an intermediate in uridine synthesis,

but think that it arises as a by-product of a blocked reaction. The accumulation of pyrimidines in cultures of lysineless mutants has already been mentioned (Section III.5).

## IV. The Gene as a Unit of Function

### 1. *The One Gene-One Enzyme Hypothesis*

The results which have been summarized in the preceding pages have shown that, with one or two doubtful exceptions, the analyzed biochemical mutants of Neurospora behave as if the only effect of the mutation is to abolish a single reaction in the synthesis of an essential metabolite. This has led to the hypothesis that a large class of genes exists in which each gene controls the synthesis of, or the activity of, but a single enzyme, a supposition which has come to be known as the "one gene-one enzyme hypothesis." Of 484 biochemical mutants (independent occurrences, but not all genetically different) which have been tested by various workers here and at Stanford University, 405, or 84%, have been reported to grow when a single known chemical substance is added to the minimal medium. The specific requirements of the remaining 16% have in most cases not been worked out, but there is reason to believe that further analysis of these mutants will show that in many instances the effect of the mutation has been to block a single metabolic reaction. These results can be explained only by assuming that the majority of the recovered genes have but one essential function, or, alternatively, that where several functions exist these are always concerned with different steps of the same synthesis. There is no experimental evidence which favors the latter hypothesis, although it is difficult to exclude it in any single instance. It appears considerably less likely, however, in view of the cumulative evidence from series of mutants which shows that the metabolic effect of each mutation is such as to make it possible to assign the gene to one step in a sequence of reactions.

The one gene-one enzyme hypothesis has been of great value as a working hypothesis in the work with Neurospora. In addition, it has provided an adequate representation of the results obtained in other investigations in which it has been possible to analyze gene action at the biochemical level. Among these are the studies which have been made on the synthesis of the brown eye pigment of Drosophila (Ephrussi, 1942), of anthocyanins and related pigments in higher plants (Scott-Moncrieff, 1939), and of melanins in the guinea pig (Wright, 1942). The recent findings of Winge and Roberts (1948) which show that the ability of yeast to ferment maltose adaptively is inherited independently of the ability to ferment galactose is consistent with this idea. Monod (1947)

has concluded from extensive studies of adaptive enzyme formation in bacteria that each such enzyme is determined by a different single gene, withdrawing a previous statement (Lwoff, 1946) to the effect that a single mutational step can affect several different enzymes.

On the other hand, Laughnan (1948) has come to the conclusion from a study of the $A$ series of alleles in maize that certain of the alleles must be assumed to determine two reactions, or, alternatively, that they consist of different, closely-linked genes. It is impossible to decide from the available data whether the assumption of two reactions in this case requires the production of more than one enzyme per allele, since nothing is known about the substrates involved or the reactions which they undergo. The possibility of linked genes at the $A$ locus is of interest in connection with the phenomenon of "pseudo-allelism" in Drosophila, described by Lewis (1945, 1948). "Pseudo-alleles" are genes which are very closely linked and whose phenotypic expressions are similar. If this phenomenon is widespread, then it is quite possible, as Lewis has observed, that complexities which have been ascribed to single genes may in some cases be the consequence of pseudo-allelism.

The one gene-one enzyme hypothesis has not been generally useful as an explanation of the action of genes controlling morphological or other chemically undefined characters, since the only known consequences of the hypothesis are chemical in nature. Conversely, evidence based on such characters can be of little value in testing the theory. The hypothesis has been criticized, for example, on the grounds that in higher organisms single gene mutations frequently result in numerous morphological effects. The fact is that these observations have no bearing on the problem of the number of primary functions per gene, since multiple end-effects are just as readily explained on the hypothesis of one primary function as on the hypothesis of several primary functions per gene. An instance has been cited above (Section III.4-a) in which a double nutritional requirement was shown to result from genetic blocking of a single reaction.

A more pertinent criticism of the one gene-one enzyme idea has been raised by Delbrück (see discussion following the paper of Bonner, 1946a). Delbrück suggests that mutants which support the hypothesis are automatically selected for by the methods used for the detection of biochemical mutations. It is quite clear that not all of the mutations which must occur in the treated cells are recovered, for a variety of reasons (Horowitz *et al.*, 1945). For instance, mutations which will not go through a sexual cycle, or mutations which do not affect essential reactions, will usually not be detected. Further selection occurs on the stock medium (so-called "complete" medium) on which mutants are isolated.

If this medium lacks the specific substance required by a particular class of mutants they will not be recovered. Or if the specific growth factor is present in the medium but is prevented, for reasons of size or charge, from penetrating the cells, the mutants will be lost. Finally, the required substance may be present and diffusible but may be rendered ineffective by the presence of specific inhibitors of the mutants in question (see Section III.5). There is reason to believe that all these factors operate in determining the kinds of mutations that are actually obtained. The question is to what extent they favor the recovery of mutants in which the gene performs but one metabolic function as opposed to mutants in which the gene performs more than one function. In the next section, an attempt will be made to answer this question. Inasmuch as the method employed has appeared only in abstract (Horowitz, 1948), it will be described in detail.

## 2. *The Selection Problem*

In what follows, those gene functions which, when lost through mutation, deprive the organism of the ability to synthesize an essential substance which, for any reason, is unavailable in the "complete" medium, will be referred to as "indispensable functions." The direction of the selection which is exercised by the factors outlined above will depend on the distribution of indispensable functions. If, for example, the unlikely assumption is made that indispensable functions are never associated with multifunctional genes, then selection will favor the recovery of multifunctional genes and will oppose the recovery of unifunctional ones. If, on the other hand, the equally unlikely assumption is made that indispensable functions are never associated with unifunctional genes, then the direction of selection will be reversed. There is no limit to the number of possible distributions, but in the absence of any compelling reason for preferring a particular one it will be assumed that the distribution is random. This means that selection will operate more strongly against multifunctional than against unifunctional genes, since the more functions per gene the greater is the probability that at least one of them will be indispensable. The intensity of selection will depend on the relative frequency of indispensable functions; the higher the frequency, the stronger the selection. In order to evaluate the importance of selection as a factor in determining the kinds of mutants that are recovered, it is therefore necessary to estimate the fraction of all gene functions which are indispensable.

At first glance, this would seem to be an impossible task, since, by definition, mutants which have lost an indispensable function cannot be recovered on either minimal or complete medium. Actually, it may not

be so difficult. A class of mutants has already been described in which the mutant character is manifested only at certain temperatures (Section II.2). These mutants can be recovered and maintained on either complete or minimal medium at a temperature at which they show no growth factor requirement (usually 25°C.), and the nature of the nutritional requirement at a temperature at which the mutant character is exhibited (usually 35°C.) can be determined. If, at the latter temperature, the strain is unable to synthesize a dispensable factor, then it will grow on complete but not on minimal medium at this temperature. If, on the other hand, the effect of the mutation has been to abolish an indispensable function at 35°C., then no growth will occur on either minimal or complete medium at 35°C. These two classes of temperature mutants are in fact known. Of the 26 temperature mutants which have been found, 14 have lost a dispensable function and 12 an indispensable function in the critical temperature range (Table 2).

TABLE 2 *

Temperature mutants in *N. crassa*.

| Class | Number of occurrences | Requirement | Number of occurrences |
|---|---|---|---|
| Dispensable function, known | 10 | Uridine | 3 |
| | | Adenine | 2 |
| | | Riboflavin | 1 |
| | | Inositol | 1 |
| | | Methionine | 1 |
| | | Arginine | 1 |
| | | Lysine | 1 |
| Dispensable function, unknown | 4 | Casein | 2 |
| | | Yeast extract | 2 |
| Indispensable function, unknown | 12 | — | — |

* I am indebted to Mary B. Houlahan for permission to use her unpublished data in making up this table.

If it is assumed that genes controlling indispensable functions are as likely to mutate to temperature alleles as genes with dispensable functions, then the relative frequencies of the two kinds of temperature mutants is a measure of the relative frequencies of the two kinds of gene functions. This assumption seems reasonable in view of the fact that, so far as can be told at present, temperature mutations seem to occur at random with respect to the kinds of syntheses they effect. As can be seen in Table 2, temperature mutations are known which affect the production

of vitamins, amino acids, and nucleic acid constituents. All of the requirements listed, with the exception of riboflavin, occur commonly among non-temperature mutants. On general grounds, there is no reason to think that the rate per gene of mutation to temperature alleles will differ between the two classes, since these classes do not represent natural categories of genes, but are largely the accidents of a particular set of cultural conditions. Thus, for example, a small increase in the arginine concentration of the complete medium would have the effect of shifting all of the known genes concerned with lysine synthesis from the dispensable to the indispensable class.

Available evidence thus suggests that this method is correct in principle, and that it can give a reasonable estimate of the relative frequency of indispensable functions. From the data of Table 2, this frequency is taken to be in the neighborhood of 0.5. Assuming a random distribution of functions, this means that of genes with 1, 2 . . . . $n$ functions, only $\frac{1}{2}$, $\frac{1}{4}$ . . . . . . $(\frac{1}{2})^n$ can be recovered. The value 0.5 is to be regarded as approximate only, since the number of mutants on which it is based is small and since, ideally, the estimate should be based on the number of genetically different mutations, rather than on the total number of occurrences. For the present, however, this value must suffice. It is enough for the present purpose that it indicates that the frequency of indispensable functions is probably not very large or very small.

With this information it becomes possible to calculate a correction to be applied to the frequency of unifunctional genes actually observed. As has been indicated above, a minimum estimate of this frequency from present data is 0.84. It will be assumed that the remaining 16% represent mutations of multifunctional genes. Actually, this assumption biases the data against the one gene-one enzyme hypothesis, since the majority of the second group are probably either mutants with a single requirement which has not yet been determined, or mutants with a complex requirement resulting from loss of a single reaction, or double mutants.

On the assumption of a random distribution of gene functions, genes with 0, 1, 2 . . . . functions would be expected. Since genes with no function would be undetectable they can be treated as non-existent. This is equivalent to saying that every gene has one function to begin with and that a number of additional functions are randomly distributed among them. Given a random distribution, it is obvious that if the majority of genes are unifunctional, as the data suggest, then only a small fraction of the remainder will have more than two functions. A close approximation to the corrected value for the frequency of unifunctional genes can therefore be found as follows:

$$\text{Observed frequency} = \frac{84}{84 + 16} = 0.84$$

$$\text{Corrected frequency} = \frac{2 \times 84}{2 \times 84 + 4 \times 16} = 0.73$$

Essentially the same result is obtained by application of the Poisson distribution, which also takes into account genes with more than two functions. It can be shown that the observed frequency of unifunctional genes is equal to $e^{-c(1-i)}$, where $c$ is the mean number of additional functions per gene and $i$ is the frequency of indispensable functions. Equating this expression to 0.84 and substituting 0.5 for $i$, one finds $c = 0.34$. The fraction of genes with no additional functions (*i.e.*, genes with just one function) is then given by $e^{-c} = 0.71$.

In view of the uncertainties in the data, this figure must be regarded as only a rough approximation to the actual value. Nevertheless, it makes it appear unlikely that selection can account for the observed high frequency of unifunctional genes. It seems unprofitable at the present time to pursue the theoretical aspects of the problem any further—*e.g.*, by assuming non-random distributions of gene functions. It would be more instructive if some clear cases of mutation of multifunctional genes could be obtained for experimental investigation.

### 3. *Gene Action*

Current discussions of the problem of gene action may be said to center around the question of the minimum number of elements which it is necessary to assume in order to account for the observed effects of genes. The theories which have formed the basis of these discussions are of a very speculative nature—only slightly less so, in fact, than they were thirty years ago, or more, when the same or similar theories were first proposed. It is a fairly simple matter, in the present state of our ignorance, to devise schemes of gene action which can formally account for almost any phenomenon. It is another matter, however, to deduce the consequences which necessarily follow from the assumptions, and it is still more difficult to devise experiments which can distinguish between alternative hypotheses.

One of the difficulties is the lack of a sure means of recognizing the primary products of gene action when they are found. There is yet no test which will signal the gene end of the chain as one follows up the casual sequence of events in the cell. The one gene-one enzyme hypothesis has some bearing on this problem. The conclusion that a gene may control the production of but a single enzyme implies that the primary gene product, whatever it may be, is specifically related to just one ele-

ment of the cell—a particular enzyme—and suggests that this element is not many steps removed from the primary product. In fact, the simplest hypothesis which will account for the Neurospora mutants is that the primary product is the enzyme. This hypothesis has not been proven, but it would be considerably strengthened if it could be shown that, besides each gene's controlling one enzyme, each enzyme is controlled by but a single gene. This has not been demonstrated, and, indeed, conclusive proof of such a proposition is beyond the powers of present-day genetics (see also Muller, 1947, for a discussion of this question). Its disproof could, however, be readily accomplished by showing that mutation of any one of a number of genes causes inactivation of a particular enzyme. It should soon be possible to apply this test to individual cases.

In this connection, mention should be made of an even simpler hypothesis of gene action which has recently received indirect support. This is the hypothesis that the gene and the enzyme are identical. McIlwain (1946) has carried out some calculations which make plausible the view that only one or a few enzyme molecules per cell may be necessary to account for the observed rates of certain metabolic reactions in bacteria. The reactions are those which are concerned with the synthesis, interconversion, and breakdown of vitamins and coenzymes. These occur with net rates of the order of micromoles per gram dry weight of organism per hour. Scaled down to the dimensions of a single bacterial cell of dry weight $10^{-13}$ g., such a process implies the reaction of something of the order of 18 molecules per cell per second, a figure well within the range of turnover numbers of known enzymes. The conclusions are valid, however, only if the calculations are based on absolute, not merely net, rates. McIlwain (1947) attempted to estimate from data in the literature the rates of reactions involving cozymase and pantothenic acid and found that the data, such as they were, tended to support the idea that the rates were of the order mentioned. More recently, however, McIlwain and Hughes (1948) have reported on the synthesis and inactivation of cozymase by streptococci. They find that whereas the net rate of synthesis is about 20 μmol/g. dry wt./hr., the rate of inactivation is 400 μmol/g./hr., or higher than had been inferred previously. The rate of inactivation is so high as to make it appear doubtful that, in this system at least, cozymase metabolism can be accounted for on the basis of one enzyme molecule per cell.

The idea that the enzyme is a direct product of gene action is not without difficulties. It implies that enzyme formation proceeds linearly from the gene (Wright, 1941), whereas the growth of individual cells (microorganisms) is apparently exponential (Adolph and Bayne-Jones, 1932; Bayne-Jones and Adolph, 1932). This is not necessarily fatal for

the hypothesis, but it has been suggested by Wright (1941, 1945) that the difficulty would be obviated by supposing that the gene produces as its primary product a genelike entity (plasmagene) which enters the cytoplasm, where it multiplies and determines the production of enzymes or other proteins. The plasmagene hypothesis raises further difficulties, however, since it now becomes necessary to explain why cytoplasmic inheritance, which would be expected under this assumption, is the rare exception and not the rule. The plasmagene hypothesis, without further restrictions, would create, in Rhoades' (1943) happy phrase, "a Frankenstein monster, no longer under the control of its maker." Wright (1941, 1945) has proposed a number of mechanisms by which the plasmagene might be excluded from the germ line.

A claim to have obtained direct evidence for a plasmagene in the form of a self-reproducing enzyme in yeast (Spiegelman *et al.*, 1945) has been withdrawn (Lindegren and Lindegren, 1946), and the inheritance of specific fermentative enzymes in yeast has been explained along conventional lines (Winge and Roberts, 1948). It is maintained, nevertheless, that the kinetics of adaptive enzyme formation are best explained by the plasmagene theory since during the course of the adaptation the enzymatic activity increases in an S-shaped curve, suggesting an autocatalytic reaction (Spiegelman, 1946). The inference that the enzyme is therefore self-reproducing is questionable on several grounds. Monod (1947), for example, has pointed out that an autocatalytic response would be expected in any case, since in all instances reported the adaptive substrate serves as the main energy source for the cell, including that needed for the synthesis of the enzyme itself. Even if this were not the case, however, an S-shaped curve should be obtained if the time required for adaptation varies from cell to cell in a more or less normal distribution; the integrated form of this distribution would resemble an "autocatalytic" growth curve. In general, the use of simple homogeneous reaction kinetics as a basis for deducing events in the living cell must be viewed with scepticism.

There may be other grounds, however, for supposing that some form of the plasmagene hypothesis is preferable to simpler theories. It has been pointed out especially by Wright (1941, 1945) and Sonneborn (1949 and earlier) that the plasmagene idea may provide a solution to the problem of differentation (from which, certainly, the problem of gene action is inseparable). By assuming directed mutation of plasmagenes under the influence of the immediate environment, or by postulating competition among the plasmagenes, the outcome of which is determined by local conditions, it becomes possible to have uniformity of nuclear genes while at the same time allowing for persistent differences in cell

heredities. An escape is thus provided from the too-confining genetic determinism which is often assumed to be unavoidable under simpler theories. It is not quite clear, however, how the extreme precision which characterizes embryonic development can be accounted for, even on the plasmagene hypothesis, without assuming some predisposing factors either in the embryo or in the environment. Given such factors, it is quite possible that systems based solely on nuclear genes could become permanently differentiated. For, as Wright (1945) has observed, an array of interacting cellular constituents may have more than one stable state, and the shift from the previous state to the given one may be brought about by special local conditions. Delbrück (1949) has recently presented a simple model illustrating this principle.

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# The Phenomenon of Position Effect

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## Contents

## I. Introduction and Classification of Position Effects

That the effect of a gene may be dependent upon its position with respect to neighboring genes is now a well-established fact in *Drosophila melanogaster* and one which has recently been demonstrated in a convincing way in *Oenothera blandina* (Catcheside, 1947a). This phenomenon of position effect (Sturtevant, 1925) has long been recognized as a fundamental problem in genetic theory and one which should throw light on the organization of the chromosomes as well as on the primary reactions of specific genes. Notable advances have been made by the accumulation of many examples of position effect in Drosophila and by the discovery of some of the necessary conditions for its detection. At the same time a considerable body of evidence bearing on this subject has yet to be incorporated into a consistent theory. In this review a

## TABLE 1

Loci, normally located in euchromatin, whose normal alleles exhibit a V-type position effect when abnormally set next to heterochromatin. Loci marked by (*) are known only by dominant mutants which are associated with small duplications; variegation of these genes in the examples given is probable but not certain.

| Mutant | Description | Symbol | Locus | Example of Rearrangement | References |
|---|---|---|---|---|---|
| Yellow | Bristle-body-color. | y | X - 0.0 | $y^{3b}$ | Noujdin (1935, 1936, 1944) |
| Achaete | Missing hair and bristle pattern of "ac" type. | ac | - 0.0 | $sc^{8}$ | Noujdin (1935, 1944); Crew and Lamy (1940) |
| *Hairy-wing | Extra hairs and bristles of "ac" and "sc" type. | Hw | - 0.0 | $sc^{8}$ | Alikhanian (1938); Crew and Lamy (1940; Bridges and Brehme (1944) |
| Scute | Missing hair and bristle pattern of "sc" type. | sc | - 0.0 | $sc^{8}$ | Bridges and Brehme (1944) |
| Lethal (1) 7 | Lethal; light eye-color as a mosaic. | 1(1)7 | - 0.3 | $Dp(1;f)X^{e2}$ | Bridges and Brehme (1944) |
| White | Eye-color. | w | - 1.5 | $w^{m4}$ | Muller (1930); Gowen and Gay (1934); Demerec and Slizynska (1937) |
| Roughest | Roughened eye. | rst | - 1.7 | $rst^{3}$ | Grüneberg (1937); Kaufmann (1942); Demerec and Slizynska (1937) |
| Facet | Rough eye; nicked wing. | fa | - 3.0 | $N^{264-57}$ | Demerec (1940, 1941a) |
| Split | Rough eye; extra bristles. | spl | - 3.0 | $w^{258-21}$ | Schultz (1941a) |
| Diminutive | Bristles, body, small. | dm | - 4.6 | $N^{264-52}$ | Demerec (1940, 1941a) |
| Echinus | Enlarged facets. | ec | - 5.5 | $N^{264-52}$ | Demerec (1940, 1941a) |
| Bifid | Fused venation. | bi | - 6.9 | $N^{264-52}$ | Demerec (1940, 1941a) |
| Rugose | Rough eyes. | rg | - 11.0 | $N^{264-85}$ | Demerec (1940) |
| Curlex | Curled wings. | cx | - 13.6 | $N^{264-85}$ | Demerec (1940) |
| Crossveinless | Missing crossveins. | cv | - 13.7 | $N^{264-85}$ | Demerec (1940) |
| Roughex | Small, rough eyes. | rux | - 15.0 | $N^{264-85}$ | Demerec (1940) |
| Vesiculated | Blistered wings. | vs | - 16.3 | $N^{264-85}$ | Demerec (1940) |

| | | | | | |
|---|---|---|---|---|---|
| Forked | Twisted bristles. | f | - 56.7 | $f^{3ns}$ | Belgovsky (1938, 1944, 1946); Noujdin (1946a) |
| *Bar | Small eye. | B | X - 57.0 | $B^{81}$ | Belgovsky (1938); Dubinin and Volotov (1940); Sutton (1943a) |
| Aristaless | Short aristae. | al | 2  0.0 | $al^{V1}$ | Lewis (1945) |
| Asteroid | Small, rough eyes. | ast | - 1.3 | $ast^{V1}$ | Lewis (1945) |
| Brown | Eye-color. | bw | - 104.5 | Pm | Glass (1933); Schultz and Dobzhansky (1934); Dubinin and Heptner (1935) |
| Minus | Bristles, body, small. | mi | - 104.7 | Pm | Schultz and Dobzhansky (1934) |
| Abbreviated | Bristles, body, small. | abb | 2 - 105.5 | Pm | Schultz and Dobzhansky (1934) |
| Hairy | Extra hairs. | h | 3 - 26.5 | T(3;4)684 | Dubinin and Sidorov (1935) |
| Curled | Curled-wing; upturned bristles. | cu | 3 - 50.0 | $T(3;4)D1^{r}$ | Panshin (1935) |

survey of major developments in the field will be presented, together with a discussion of the properties and possible mechanisms of position effect. For much of the early literature on this subject the reader is referred to the review of Dobzhansky (1936).

It has become increasingly evident that there exist at least two distinct types of position effect in Drosophila, and it may be questioned whether they are in fact causally related to one another. In one type, the change in gene action associated with a change in gene position is subject to wide and frequent statistical fluctuations, often among related cells in the same individual, and it thus results in a type of somatic mosaicism. In such cases the associated rearrangement invariably appears to involve the euchromatic and heterochromatic regions of the chromosomes (Schultz, 1936). The phenomenon of somatic instability in gene action arising in association with such rearrangements has been designated "variegation" (Schultz, *loc. cit.*), and changes of this type have been referred to as "eversporting displacements" (Muller, 1930). It will be convenient to refer to this category of effects as the variegated- or V-type position effects. To avoid circumlocution, the expression "variegation of a gene" will be used to express the variegation of a phenotype controlled by that gene. The V-type appear to constitute the bulk of position effects known in Drosophila, and although the cytological picture is lacking, position effects so far detected in Oenothera are probably of this kind. A rather small group of position effects is known in which the change in gene action is of a somatically stable type as with most changes within the gene itself. When a rearrangement is associated with such cases it often involves the wholly euchromatic regions of the chromosomes. This group will be referred to here as the stable- or S-type position effects. Fortunately, several other properties serve to distinguish these two categories of position effect in those cases where a distinction between instability and stability in gene action is not readily made. The V-type position effects are considered first inasmuch as they have been more extensively studied.

## II. Variegation of Genes Located in Euchromatin

### 1. *The Variegated Phenotype*

A large number of genes in Drosophila are now known to exhibit a somatically mosaic phenotype when brought into association with heterochromatin (Table 1.). Although variegation does not appear to be restricted to any particular type of gene (except in so far as its detection depends on an autonomous gene action), relatively few genes have effects which make them suitable for a developmental study of the variegated
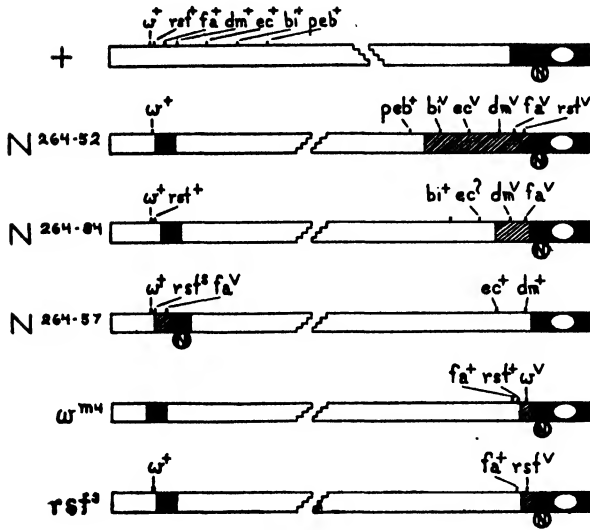
FIG. 1

Examples (diagrammatic) of X-chromosome inversions associated with variegation of genes (see Table 1 and Fig. 4), with reference to the normal chromosome at top of figure. Legend: black = heterochromatin of the X chromosome; unshaded areas = euchromatin; shaded areas = the extent of spreading of the variegated effect; N = nucleolar organizing region; V = variegated; S = stable; + = non-variegated or wild-type.
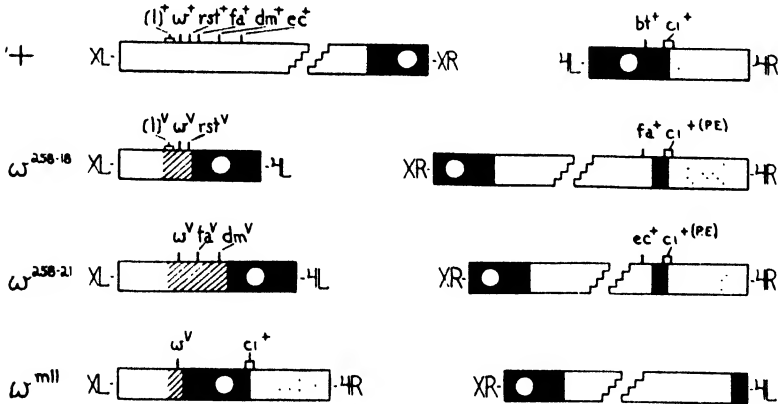


FIG. 2

Examples of translocation between the X and fourth chromosomes detected as white-variegated types (diagrammatic). Legend: black = heterochromatin of X or 4; unshaded = euchromatin of X; stippled = euchromatin of 4; shaded = extent of spreading-effect of the variegation process; (P. E.) = a position effect of the cubitus interruptus gene.

phenotype. Of these, the most widely studied example is the normal allele of the white gene ($w^+$), variegation of which can be detected as a color change over the range from red to white in the 850 or so ommatidia, or "facets," of the compound eye. Examples of rearrangements of this allele having a V-type position effect are shown diagrammatically in Figs. 1 and 2. Although these types are customarily designated as mutant alleles of the white gene, for example, $w^{m4}$, for white-mottled-4, it is convenient to introduce a more generalized notation which expresses at once the presence of a rearrangement (R) and the allele present at the time the rearrangement was produced. Such a terminology, introduced by Stern and Heidenthal (1944) for rearrangements having a position effect of the cubitus interruptus ($ci$) gene in the fourth chromosome (Section III.2), will be extended here to designate rearrangements having a V-type position effect. Thus $R(w^+)$ will be used to designate a rearrangement associated with variegation of the normal allele of the white gene; and R in general has, as in the case of $R(ci^+)$ types, the significance of a euchromatic-heterochromatic rearrangement.

Descriptions of the phenotypes associated with $R(w^+)$ types have been given in some detail by Muller (1930), Gowen and Gay (1934) and Demerec and Slizynska (1937). A specific effect on the white gene is clearly demonstrated by the fact that the heterozygote between $R(w^+)$ and a chromosome bearing the mutant white allele ($w$) has a variegated eye color, whereas $R(w^+)$ / + heterozygotes have normal red eyes. When viable, $R(w^+)$ homozygotes and hemizygotes likewise have a variegated phenotype. The variegation shows an extraordinary range of variability depending on the particular $R(w^+)$ type being considered and the genetic and environmental background (Section II.5). The variegated eye may have a red background color with scattered lighter patches; a white or light background with scattered red or dark facets; and frequently it is of an intermediate color such as cream, or pink, with darker and/or lighter patches present. Facets of intermediate colors have been interpreted on the basis of an all-or-none change by assuming that a mixture of red and white cells are present in the complex of pigment cells which make up an individual facet (Panshin, 1938). Schultz (Morgan et al., 1937) has argued for an all-or-none effect in the case of white-variegation in the Malphigian tubules, where isolated single white cells can be detected in an otherwise yellow tubule. Demerec and Slizynska (1937) assume that gene mutation occurs and that mutation to an intermediate allele of the white series may produce the intermediate background colors. Another special consideration is the light or white background in which a few scattered facets of darker pigmentation may occur (figured by Muller, 1930). These

dark spots have been interpreted as cases in which residual normal cells remain (Schultz, 1941a) and as evidence of reverse mutation (Demerec and Slizynska, 1937); in the latter case, a less restrictive assumption would be that a recovery in gene function may occur, since there is no proof that a process akin to gene mutation is involved (Section II.7). As Gowen and Gay (*loc. cit.*) have found, variegation in an $R(w^+)$ heterozygote having an intermediate allele of the white series in the normal chromosome, does not give rise to facets of a lighter color that those of the heterozygote between that allele and the white mutant.

Variegation of genes affecting the color, structure, or presence of bristles is a more instructive effect than eye-color variegation since it is measurable in terms of specific cells. Noujdin (1938) has noted that (*a*) variegation of the yellow (*y*) gene in an $R(y^+)$ type (the $y^{3P}$ inversion of Patterson) may result in bristle colors ranging from black (wild-type) through dark brown and light brown shades to full yellow; and (*b*) the degree to which a bristle may manifest a forked effect, due to variegation of the forked (*f*) gene in certain $R(f^+)$ types of Belgovsky is also variable. Thus the possibility is open that the variegation process may be subject to variation within a given cell as well as between cells.

Several cases are known in which variegation behaves as a dominant character. Thus variegation, presumably for the facet gene ($fa^+$), results in a variable dominant phenotype of the Notch-type (Muller, 1930). Examples of $R(fa^+)$ alleles of this type are the Notch-variegated types of Demerec (1941a) shown in Fig. 1. Since the Notch phenotype is known to result from a deficiency for the facet gene, variegation in these cases is consistent with the assumption that inactivation of the gene, or its product, occurs in the V-type position effects. Apparent exceptions to this rule are found in certain other dominant variegated-types, notably, in rearrangements of the normal allele of the brown gene ($bw^+$) to heterochromatin. Thus $R(bw^+) / +$ shows mottled brown and red facets, while "allelism" with the brown gene is indicated by the fact that $R(bw^+) / bw$ has an almost homogeneous brown eye color (Glass, 1933; and Schultz and Dobzhansky, 1934). Here, as the latter workers found, there is evidence that a deficiency for the region containing the brown gene does not give a dominant brown phenotype nor does the presence of two doses of the $bw^+$ allele suppress the dominance. Ephrussi and Sutton (1944) have interpreted the dominance as an effect exerted by the rearrangement on the $bw^+$ allele in the normal as well as in the rearranged chromosome. On this basis an $R(bw) / +$ fly should also show dominant brown-variegation as indeed was the case in a rearrangement of the $R(bw)$ type obtained by Moore by x-raying the mutant brown (Glass, 1933). Hinton's discovery of an extreme dominant brown mu-

tant, $bw^D$, which Schultz has interpreted as a one-band duplication in the salivary gland chromosomes (cited in Bridges and Brehme, 1944), indicates that the dominant effect does not require a major chromosomal rearrangement for its production, but rather that it may be a potential property of the $bw^+$ locus or of a neighboring pseudo-allelic locus as in the Star and asteroid mutants (Section IV.1). A hairy-wing effect, probably a V-type position effect, is conspicuously dominant in scute-8 heterozygotes (see Fig. 3). Here again the phenotype is analogous to that of a small duplication (for the 1 B1-2 double band, Fig. 4), namely, hairy-wing, or to that of duplications for the tip of the X chromosome including this locus, but not to deficiencies for this region (Demerec and Hoover, 1939). A model for these dominant effects may be made by assuming that inactivation of one gene leads to an accumulation of the precursor substance normally utilized by that gene and that this excess results in the dominant change. This would reconcile dominant hairy-wing- and dominant brown-variegations with the other V-type position effects as being essentially equivalent to a process of inactivation of the gene or its products. To explain the failure of a deficiency to produce the dominant phenotype would require the assumption that the loss involves also the locus of the gene producing the substance required by the gene in question. A mechanism involving competition of two such linked genes for the same substrate would be equally applicable. Competition between a gene in the normal chromosome and its allele in the rearranged chromosome has been proposed as a model for such dominant effects as that of $R(bw^+)$ by Stern and Heidenthal (1944), such that the rearranged allele retains an ability to compete with the allele in the normal chromosome but owing to its new position is unable to function as efficiently as the latter (Section III.2).

## 2. *Proofs of the Variegated-Type Position Effects*

Dubinin and Sidorov (1935) obtained direct proof of the position effect phenomenon in a study of a translocation between the third and fourth chromosomes associated with a variable change in action of the hairy ($h$) gene in the left arm of chromosome 3. The heterozygote between the hairy mutant and this rearrangement showed a range of one to eleven extra hairs on the scutellum in contrast to "several dozens" in homozygotes for hairy, and none in wild-type. This along with the salivary gland chromosome analysis showing a rearrangement involving the proximal or heterochromatic region of chromosome 4 serves to identify this as a V-type position effect. As the result of crossing over between the locus of hairy and the breakpoint of the rearrangement, a translocated chromosome carrying the mutant hairy gene, that is, $R(h)$,

was derived from R($h^+$) / $h$ females. The insertion of an $h^+$ allele, derived from a normal chromosome, was obtained from R($h$) / $+$ females, as the result of the same type of crossing over. The newly introduced $h^+$ allele was found to acquire the same instability in its somatic action as that found with the $h^+$ allele in the original translocation. Since precisely similar pairing relationships are expected in R($h$) / $+$ and R($+$) / $h$, and since the former was reported to be associated with a wild-type phenotype and the latter with a variable, hairy phenotype, the position effect cannot in this case depend solely on structural heterozygosity. The fact that only R($h^+$) / $h$ departed from normality may be taken to indicate that $h^+$ is altered only when in the rearranged chromosome. Parallel experiments with equivalent results were conducted by Panshin (1935) with a translocation between the third and fourth chromosomes associated with an effect on the normal allele of the curled gene ($cu^+$). Variegation was in evidence in the sense that a population of R($cu^+$) / $cu$ flies showed a wide range of expression of the curled-wing phenotype; in contrast to the constant curled-wing and bristle effect of the $cu$ homozygote. In this case it was reported that 0.8% crossing over was observed between the locus of curled and the breakpoint of the rearrangement; and that five cases of R($cu$) types, representing the insertion of the mutant $cu$ gene into the rearrangement, were recovered. From each of these, R($cu^+$) types, representing the insertion by crossing over of $cu^+$ from a normal chromosome, were obtained. Using a rough grading system Panshin showed that the "new" R($cu^+$) / $cu$ had the same variable curled phenotype as the heterozygotes between the curled mutant and the original translocation.

Grüneberg (1937) reported another type of proof of the position effect as the result of a discovery of a reversal of the rough-eyed phenotype associated with the X chromosome inversion, $rst^3$ (Fig. 1). Genetic analysis and salivary gland chromosome studies (Emmens, 1937) indicated that this change was accompanied by reinversion of the rearrangement, which, as far as was determined, restored the original gene order. A more critical test, the phenotype in the XO male, was not employed (Section II.5). Although Kaufmann (1942) was unable to secure reinversion of $rst^3$ following x-ray treatment of $rst^3$ males, his discovery that induced "reverse mutations" of $rst^3$ are accompanied by new rearrangements (Section II.6) usually returning the roughest gene to a euchromatic position, provides additional evidence that the normal allele, $rst^+$ is still present in the rearranged chromosome.

A strong indirect proof of the V-type position effect can be made out in such cases as the white locus where direct proof is still lacking. Here, over 35 instances of x-ray white-mottled types have been analyzed

by the salivary gland chromosome method as the result of work by Schultz (1936), Sacharov (1936), and particularly by Demerec (1941a) and coworkers. Without exception each of these cases was associated with a rearrangement bringing one of the heterochromatic regions and the white locus into close proximity. Attempts by Griffen and Stone (1940) to explain this association with heterochromatin as the sole result of a relatively high breakage frequency in the heterochromatic and white regions are not supported by (a) Kaufmann's (1946) finding that rearrangements involving the 3 C, or white region more often involved euchromatin than heterochromatin and, more generally, that regions showing relatively high breakage frequencies, such as 3 C, do not recombine preferentially with heterochromatin; nor (b) Demerec's (1941a) finding that rearrangements involving a break near the white locus and another in euchromatin were either unassociated with a change at the white locus or carried a stable mutant allele or an S-type position effect of that gene.

### 3. *The Specificity of the Heterochromatin Association*

Demerec (1941a) has shown that variegation involving the white gene may be caused by the association of $w^+$ with heterochromatin derived from the X chromosome or from any one of the major arms of the autosomes. Heterochromatin from the short arm of the fourth chromosome also appears to be effective in inducing variegation of this gene as first noted by Panshin in white-mottled-11 (Fig. 2). In two cases heterochromatin from the tip of the fourth chromosome was effective, but otherwise breaks were confined to the proximal heterochromatic regions of the chromosome arm involved. Demerec showed that differences exist within the heterochromatic region of a given chromosome arm in its capacity to induce variegation. Thus, not every rearrangement which brings heterochromatin into the immediate vicinity of a gene causes variegation of that gene as may be seen in the Notch variegated types, $N^{264-52}$ and $N^{264-57}$, in which variegation of the diminutive ($dm$) gene occurs in the former, but not the latter where the gene is actually closer to heterochromatin.

Breakages in the heterochromatin of the X chromosome are illustrative since they can be differentiated with reference to (a) a nucleolar organizing region (Kaufmann, 1942, 1944), (b) the so-called "block A" region (Muller *et al.*, 1937), which constitutes a sizable portion of the heterochromatic region of the X chromosome in dividing cells, and (c) a region containing the bobbed gene. This differentiation is shown diagrammatically in Figs. 1 and 3, for the normal chromosome, and for certain inverted sequences associated with V-type position effects. Variega-

tion has been found to occur whether or not the affected gene is next to (a) the centromere (cf., the facet gene in the above-mentioned Notch inversions); (b) the nucleolar organizing region (cf., the white gene in $w^{m4}$ and the scute gene in $sc^8$); and (c) block A or the bobbed gene (cf., the yellow and scute genes in $sc^8$). Although no wholly consistent rules are in evidence, variegation is, as Demerec (1940, 1941a) has discussed, more extreme (a higher proportion of mutant than normal tissue) when the effected
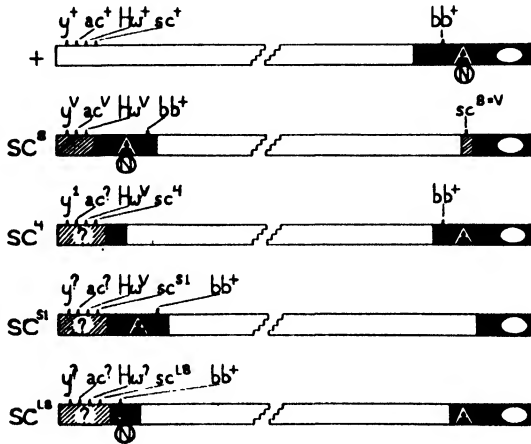


FIG. 3

Examples of X-chromosome inversions associated with variegation of genes in the yellow-scute region with reference to the normal chromosome. (See Fig. 1 for legend; A = Block A; and see Fig. 4 for cytological details). Diagrammatic.

gene is near the centromere; or, at least the variegation process exerts an effect over a greater distance in this case (Section II.4). Panshin (1938) found evidence that the greater the amount of heterochromatin brought next to the white gene the more extreme the variegation (Section II.6). Evidently, highly specific differences also exist within a given heterochromatic region in its capacity to induce variegation, as in the case of $N^{264 \cdot 57}$ (or $rst^3$) vs. $N^{264 \cdot 52}$, cited above. It may be concluded that the establishment of a close association between heterochromatin and a gene normally lying in the wholly euchromatic region is a necessary but not always a sufficient condition for the production of a V-type position effect of that gene.

## 4. *The Spreading Effect*

One of the most remarkable properties of the variegation process is the frequent tendency for several genes in the vicinity of the point of rearrangement of euchromatin and heterochromatin to be affected.

Muller (1930) first noted this effect in a white-mottled type ($w^{m1}$) which had in fact been detected originally as a Notch mutation; it was observed that the extent of white-mottling was in this case directly correlated with the extent of Notch expression. Gowen and Gay (1934) showed that such an effect must be limited since no change in action of genes remote from the white locus occurred, thus ruling out the possibility that a type of unstable translocation was involved (Patterson, 1932b). Demerec (1940, 1941a) has made extensive studies of this process. In the inversion, $N^{264-52}$ (Fig. 1), variegation for 5 genes was observed and was correlated with the rearrangement of these genes to the centromere region of the X chromosome. In this case the effect was observed to spread at least as far as the bifid locus, located in the salivary gland chromosomes at least 50 bands removed from the point of rearrangement.

A unique opportunity for studying this "spreading effect" is presented by certain white-mottled types having an associated change at the roughest locus and/or the split locus. Demerec and Slizynska (1937) studied a white-mottled type, $w^{258-18}$, which was accompanied by variegation of the roughest gene lying immediately to the right of the white locus. Only three general types of facets were found in the eyes of homozygotes or hemizygotes for this R($w^+$) allele; (a) red and smooth (wild-type); (b) red and roughest (roughest variegation); and (c) cream- or cherry-colored and roughest facets (variegation for white and roughest). Schultz (1941a) noted that the R($w^+$) allele, $w^{258-21}$, of Demerec shows similar relationships; in this case the rough eye variegation of the split gene was followed simultaneously with white variegation. The significant feature in both of these studies was the finding that the breakpoint of the rearrangement occurred to the right of the roughest and in the latter case of the split gene, and therefore to the right of white as well. Representing the point of rearrangement to heterochromatin by a period these rearrangements may be symbolically represented as follows: R($w^+$ $rst^+$.) and R($w^+$ $spl^+$.). The effect exerted by heterochromatin on the newly adjoining euchromatic region was thus seen to spread from the point of new arrangement always effecting first the gene closest to that point (Fig. 2).

The concept of the spreading effect may be applied to the data of Raffel and Muller (1940), concerning the three X chromosome inversions, $sc^4$, $sc^{L8}$, and $sc^{S1}$ (Fig. 3). It was found that the left ends of these inversions showed significant and consistent differences from one another with respect to causing a reduction in number of specific bristles and hairs, irrespective of which right end had been combined with them; while the differences exerted by the three right ends, in the presence of

a given left end, were either not significant or were not consistent with respect to any pattern of bristle loss. Bristle reduction was least in the case of the left end of $sc^4$ and greatest in the case of $sc^{I8}$. Two of the sets of specific bristles involved were, as was pointed out, those effected by the achaete ($ac$) gene. This suggests that variegation of $ac^+$ was occurring to different degrees in these three arrangements. The remaining bristle losses by which these inversions could be consistently differentiated were those usually considered to belong to the group affected by the scute gene. Since these inversions had a break in sensibly the same position just to the right of 1B3-4 (Fig. 4) it was concluded that the differences might have to be accounted for by assuming further divisibility of the scute gene, or of genes lying to the right of it. A serious complication however to the analysis of the phenotypes of these inversions is the associated slight hairy-wing effects (noted for $sc^4$ by Alikhanian, 1938; and for $sc^{S1}$ by Crew and Lamy, 1940). Sutton (1943b) made the important discovery that achaete probably represents a separate locus to the left of that of Hairy-wing. Thus the spreading effect in the case of these inversions should cause a superposition of a bristle and hair loss pattern due to variegation of the achaete and scute genes, and an extra bristle and hair pattern due to variegation of the Hairy-wing gene lying between them. It should be noted that even were the changes in actions of the scute gene identical and of the stable type in each of the inversions (as discussed in Section IV.2), the differences can still be interpreted in terms of superposition of achaete and Hairy-wing effects, since extra bristles of the scute as well as the achaete type are known to be effected by the Hairy-wing mutant (Dubinin and Sidorov, 1933).

Gersh and Ephrussi (1946) studied the influence of three white mutations, which were known to be deficiencies for 1, 5, and 13 bands lying immediately to the left of, but not including, the white gene, on the variegation of $w^+$ in $w^{m4}$ (Fig. 1), $w^{258-18}$ (Fig. 2), and $w^{m5}$ (the latter being an X-4 translocation very similar in its breakpoints to $w^{258-18}$). In the case of the latter two $R(w^+)$ types significant reductions in viability occurred when either was opposite a chromosome bearing one of the two longer deficiencies as compared to each opposite a normal chromosome. This result can be interpreted on the basis that variegation of genes lying to the left of white was resulting in recessive lethal or semi-lethal effects. It was observed that the $R(w^+)$ / $Df$, $w$ survivors had darker eyes than their $R(w^+)$ / $w$ sisters, a consistent result if the extreme white-variegated types in the former case are inviable. In $w^{m4}$, which gave no appreciable reduction in viability, variegation if it is spreading at all probably proceeds in the direction of genes which lie to the right of the

white locus and which therefore would be "covered" by their normal alleles in the deficient chromosome.

It is noteworthy that the spreading effect demands a concept of a chromosome linearly differentiated into units with specific developmental effects, as in the classical theory of the gene (*cf.* Goldschmidt, 1946). It may be expected that it will serve as a means of identifying effects of new genes. Variegation of the normal allele of the gene, lethal (1) 7, has already led to the discovery that this "lethal" gene is probably an eye color mutant (Schultz, cited in Bridges and Brehme, 1944).

## 5. *Modifiers of Variegation*

The V-type position effects have been found to be extremely sensitive to a variety of modifying factors. Fortunately some of these factors modify the variegation process as such rather than specific variegated-types and thus constitute useful tools for its study. Thus, Gowen and Gay (1933a, 1934) have shown that variegation of the white gene (in the case of three different $R(w^+)$ types) is suppressed in the presence of an extra Y chromosome, that is, in the XXY female or in the XYY male. Noujdin (1938, 1946a) reported that the addition of heterochromatin of the X, or of the fourth chromosome, or the addition of either arm of the Y chromosome is effective in suppressing variegation (of the yellow gene), although to a less marked extent in each case than the addition of an entire Y chromosome. Schultz (cited by Bridges and Brehme, 1944) found that three Notch variegated-types of Demerec ($N^{264-6}$, $N^{264-9}$, $N^{264-10}$) normally lethal or in one case rarely viable in the male survived in the presence of an extra Y chromosome and no longer show Notch-variegation; curiously such males were sterile.

Schultz (1936) found that the absence of a Y chromosome in the male, the XO condition, caused a marked enhancement of variegation; *i.e.*, an increase in the proportion of mutant tissues; and later (Morgan *et al.*, 1941) noted that a deficiency for heterochromatin of chromosome 2R was almost equally effective in enhancing variegation. Noujdin (1936) found that variegation of the yellow gene in $sc^8$ is increased from less than 1% (per cent of flies having yellow spots) in the XY male to 99% in the XO male.

Gowen and Gay (1933b, 1934) have shown that high temperature leads to a suppression of variegation and low temperature to an enhancement of the process. It was found however that the Y chromosome effect dominated the temperature effect, in the sense that a white-mottled type which showed extreme white variegation at 18°C., was wild type at this temperature if an extra Y chromosome were present. Chen (1948) found that cold treatment (16-17°C.) applied to various stages of the larval

and pupal development of the R($w^+$) strains, $w^{258\text{-}18}$ and $w^{m5}$, was effective in decreasing the amount of eye pigment only when applied in the early pupal period. It is possible however that a more effective period for temperature modification of variegated types exists in the egg stage (see discussion by Schultz, 1941a; and Noujdin, 1945).

Schultz (1941a) has noted several cases in which a rearrangement between the euchromatic and heterochromatic regions acted as a modifier of variegation of another such rearrangement in the same nucleus. There is then the possibility that a V-type rearrangement may act, *per se*, as a modifier of the variegation process, thereby further complicating the analysis of V-type position effects. Although structural heterozygosity is not a necessary condition for the production of the V-type position effect, it becomes important to know whether the structural state can act as a modifier of variegation. An experimental study of this point was made by Gersh and Ephrussi (1946) in the experiments already referred to (Section II.4), in which the influence of deficiencies near the white gene on the phenotype of R($w^+$) heterozygotes was measured. It was found that the same deficiency appeared to modify the phenotype of different R($w^+$) types in different directions. Compared to the control R($w^+$) / $w$ sisters, the eye color averaged darker in the case of $w^{m5}$ / $Df$, $w$ and $w^{258\text{-}18}$ / $Df$, $w$ and lighter in the case of $w^{m4}$ / $Df$, $w$, with either the 5-band or 13-band deficiencies; in the case of the 1-band deficiency, $w^{258\text{-}45}$, R ($w^+$) / $Df$, $w$ was lighter in color than R($w^+$) / $w$ for the three R($w^+$) types studied. When allowance is made, however, for a differential loss of the more extreme white-variegated flies on the basis that variegation of lethal genes was occurring (as discussed in Section II.4), the results can be taken to indicate that the deficiencies resulted in an enhancement of variegation. It is possible that these deficiencies were associated with a change in action of the white gene more extreme than that in the chromosome bearing the mutant white allele and, therefore, that the deficiencies themselves were not influencing the variegation process. Although Gersh and Ephrussi showed that this was not evident from tests comparing $Df$, $w$ with $w$ opposite the apricot allele, $w^a$, the reviewer has found (unpublished) that the deficiency, $w^{258\text{-}45}$, gave a lighter eye color when opposite the eosin allele ($w^e$) than did $w$ (*cf.*, results similar to the latter case with known white deficiencies—Mohr, 1919).

In individuals homozygous for a rearrangement having a V-type position effect, it would be anticipated that less mutant tissue would be present than in individuals which are heterozygous for the rearrangement and which have an extreme mutant allele of the gene in question in the normal chromosome; that is, in the former case, the chance that

an individual cell would have both normal alleles of that gene impaired in function would depend on two separate events, one of which is sufficient for its detection in the heterozygote. Such a result would be expected quite apart from Ephrussi and Sutton's (1944) consideration that the homozygote should be less extreme than the heterozygote on grounds of greater stress on the gene in the latter case imposed by pairing difficulties. There is little reason to believe that the above relation holds in Drosophila, at least for the genes which normally lie in euchromatin; it may, however, apply in Oenothera (Section V). Demerec and Slizynska (1937) report that the $R(w^+)$ homozygote had lighter eyes than $R(w^+) / w$, in the case of $w^{258-18}$; and Schultz (1941a) has remarked that "the homozygotes always show more variegation than the heterozygote." Possibly, somatic pairing in the case of the homozygous rearrangement brings about a much closer association of heterochromatin and the affected pair of alleles, than is possible in the case of the heterozygote, where pairing between the heterochromatic regions in the normal and rearranged chromosome is impaired. Noujdin (1935, 1944) has interpreted results of studies of variegation of the yellow and achaete genes associated with the $sc^8$ inversion in terms of maternal and paternal effects. In this work, the state of structural heterozygosity or homozygosity is assumed to modify not only the degree of mosaicism within the individual but to impose semipermanent changes on the chromosomes transmitted by them. Noujdin (1946b), however, reported that these remarkable properties had disappeared after some generations in some lines of scute-8, which suggests that modifier genes may have played an important role in the earlier experiments. Subjective errors may also have been high since only the number of flies having spots (yellow or achaete) was recorded.

The existence of a diversity of modifier genes (exclusive of Y chromosome effects) which enhance or suppress the variegated phenotype has been inferred from the fact that light and dark lines of mottled-eye types may be readily sorted out by selection (e.g., Demerec and Slizynska, 1937); in other cases (Gowen and Gay, 1934) selection for such lines was ineffective indicating that permanent changes within the affected gene are not at work in the former cases. Schultz (Morgan et al., 1937) reported an autosomal modifier having "a dominant maternal effect for the suppression of variegation." Demerec and Slizynska (1937) found a spontaneous, inherited change, which caused the background in an $R(w^+)$ line, $w^{258-18}$, to appear white instead of the usual cream or cherry color.

Such a diversity of modifying factors as that outlined above may well have obscured significant relationships within, and clearly compli-

cate the analysis of, the V-type position effects. In this respect, it should be noted that special precautions, such as those taken in the experiments of Raffel and Muller and those of Gersh and Ephrussi cited above, are needed to minimize the influence of such modifiers in comparative studies of the V-type rearrangements.

### 6. *Reversals of the V-Type Position Effects*

One experimental approach to the study of the V-type position effect has consisted in the induction of further changes within given mottled types by x-ray treatment. One study of this kind was Panshin's (1938) analysis of the R($w^+$) type, $w^{m11}$, (Fig. 2) involving an X-4 translocation in which the white locus was transferred to heterochromatin of the short arm of the fourth chromosome. A total of eight induced "complete reversals" of the variegated phenotype was analyzed cytologically. Seven rearrangements which had transferred the white gene to a euchromatic position, and one case transferring it to the distal heterochromatin of the Y chromosome, were effective in restoring the normal action of the gene. A similar result was obtained in an analysis of thirteen partial reversions of $w^{m11}$; in these cases, however, rearrangements which replaced the heterochromatin of the right arm of chromosome four with euchromatin were effective in reducing the amount of variegation of the white gene in the other arm. On the other hand new derivatives of $w^{m11}$ which were detected as extreme white-variegated types were found to be caused by the presence of additional heterochromatin near the white gene. Griffen and Stone (1940) subjected the translocation, white-mottled-5 (Fig. 2), to further x-ray treatment. Unfortunately, only the results obtained from an analysis of the partial or complete reversions of the white-mottled phenotype were recorded. These results were in agreement with those of Panshin in indicating that such reversions are due to a transfer of the white gene to a new euchromatic position. It is likely that the partial reversions obtained by Panshin and these workers are the result of carrying over some of the heterochromatin from the original attachment to the fourth chromosome to the euchromatic region. As Kaufmann (1942) has pointed out it is also possible that such partial reversions reflect an influence of interstitial heterchromatin on the normal allele of the white gene. If such be the case it is likely that such regions have a very feeble capacity of inducing variegation and such cases would likely have been overlooked in experiments in which the original mottled type is detected. The cytological properties of seventeen x-ray induced partial or complete reversals of roughest variegation associated with the $rst^3$ inversion (Fig. 1) were studied by Kaufmann (1942). In

these cases, transfer of the $rst^+$ gene to a new euchromatic region was the general rule.

Studies of induced changes in variegated types are complicated by the possibility that the x-ray treatment has caused the production of more or less non-specific suppressors and enhancers of variegation. Schultz (Morgan *et al.*, 1937) has pointed out that some of the new x-ray induced derivatives of an $R(bw^+)$ type obtained by Dubinin (1936) may have been of this nature, especially those derivatives in which new rearrangements had occurred at some distance from the brown locus and had acted as modifiers of the dominant brown phenotype.

The frequency with which reversions of variegated types arise is expected to be high if the sole requirement is the removal of the gene from its new heterochromatic association to any non-heterochromatic region. In the case of $w^{m5}$, such conditions are probably essentially similar to those required in the case of position effects of the $ci^+$ gene (Section III.2), (cf., the position or $ci^+$ in the normal fourth chromosome, with the position of $w^+$ in $w^{258\text{-}18}$, which is similar to $w^{m5}$ except that in the former $w^+$ probably immediately adjoins heterochromatin of the fourth chromosome, Fig. 2). Griffen and Stone (1940) cite data of Mickey indicating 0.3% "true reversals" of $w^{m5}$ among 7,015 tested gametes at a dosage of 4,000 r units. The frequency with which a weakened dominance of $ci^+$ is obtained following x-ray treatment at this dosage may be taken as being within the range of 0.3% (Muller, 1930) and 1% (Stern *et al.*, 1946), intermediate values having been found by Khwostova and Gavrilova (1935, 1938). Kaufmann (1942) estimated a frequency of 0.4% reversals of roughest variegation in $rst^3$ (4,000 r units). The high frequency with which reversions of variegated types is obtained provides additional indirect evidence that such rearrangements still carry the normal allele of the gene in question as expected on the position effect hypothesis. The question of whether the mutability of such wild-type alleles is increased when they are adjacent to heterochromatin is discussed in the next section.

## 7. *The Question of Germinal Stability*

Muller (1930), Gowen and Gay (1934) and Demerec (1941a) have noted that $R(w^+)$ types maintain the inheritance of the somatically unstable phenotype with no evidence that stable reversions or stable mutant changes in the rearranged $w^+$ allele occur spontaneously. In the progeny of males carrying the $sc^8$ inversion (Fig. 3) a high spontaneous rate of lethal changes resembling simultaneous mutation of the yellow and achaete genes occurs. Sidorov (1940, 1941a) found that the rate of these changes was 0.019% among 250,366 tested gametes and demon-

strated that these types arise as the result of crossing over between the X and Y chromosomes. It was established that such crossovers involved the distal heterochromatin contained in the scute-8 inversion and the short arm of the Y chromosome (pairing in reverse order). The lethal yellow-achaete changes represented X chromosomes in which the tip of the chromosome had been replaced by the tip of the short arm of the Y chromosome. On this scheme a complementary crossover type carrying the tip of the X chromosome attached to the proximal part of the short arm of the Y chromosome is expected, and was detected by a special test. The addition of such a Y chromosome and the complementary, deficient X chromosome restores the normal balance, and was found to give a variegated phenotype identical with that of the original $sc^8$ chromosome and a normal Y chromosome. Crew and Lamy (1940) also obtained a crossover chromosome having the tip of $sc^{81}$ (Fig. 3) attached to the Y chromosome in the above manner and found that such an attachment did not alter the variegation of genes in that region. Such exceptions, then, have served to prove the rule; namely, that variegation is a process which is confined to the somatic cells of an organism. The same rule seems to apply in the case of position effects in Oenothera (Section V).

The high mutability of the y and ac genes in the $sc^8$ inversion when x-rayed (Patterson, 1932a; Sidorov, 1936; Belgovsky, 1939; and Muller, 1940) is in part due to the deficient types of crossovers with the Y chromosome, occurring spontaneously, as pointed out by Sidorov (1941a). Among the non-lethal changes in these loci which were induced, it was proposed, see Muller (loc. cit.), that minute rearrangements of these genes had occurred. Possibly, rearrangements involving the heterochromatin adjoining these genes in the inverted chromosome contributed to some of these by producing extreme variegation of these genes.

## 8. Cytological Aspects of Variegation

There is general agreement (Schultz and Caspersson, 1939; Prokofyeva-Belgovskaya, 1939; Cole and Sutton, 1941) that the euchromatic regions of the salivary gland chromosomes tend to lose their capacity to stain as sharply banded regions when they are brought into close proximity to heterochromatin as in the V-type rearrangements, just as the distinction between euchromatin and heterochromatin in the normal chromosomes is not clearly defined (see divisions 19 and 20 in Fig. 4). Prokofyeva-Belgovskaya suggested that this represents a weakening of the conjugational properties of the chromonemata of the euchromatic regions as a result of proximity to the loosely organized heterochromatin. An analysis of this effect was made by Caspersson and Schultz (1938)

and by Cole and Sutton (1941), who have measured the relative amounts of ultraviolet absorbing substances in specific euchromatic bands brought next to heterochromatin. In these studies the $R(w^+)$ type, $w^{258-21}$ (Fig. 2), was investigated. The breakpoint in the X chromosome in this translocation was found to lie between the bands 3E5 and 3E6
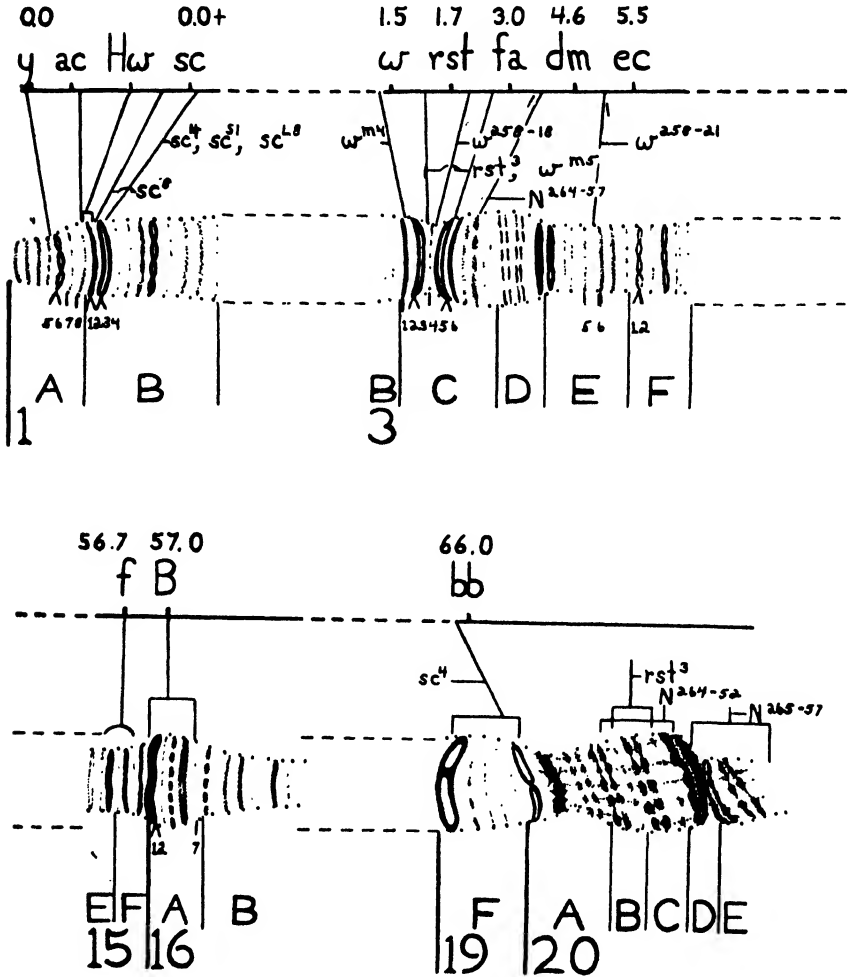


Fig. 4

Genetic and cytological correspondences in four regions of the X chromosome which have been widely studied for position effects. Details and references are contained in the work of Bridges and Brehme (1944) and in the text. The drawing of the salivary gland chromosome is based on Bridges' (1938) revised map of the X-chromosome.

(Fig. 4) and Demerec (1942) found that variegation of the genes, *w*, *rst*, *fa* and *dm*, but not *ec* occurs. From measurements of the double band 3F1-2, Schultz and Caspersson concluded that an increase in the nucleic acid content of this band appeared when it was adjacent to heterochromatin in the rearranged chromosome as compared to the amount found in the same band of a normal chromosome present in the same nucleus. On the other hand, Cole and Sutton (1941), using a similar type of technique, were unable to demonstrate consistent differences in the density of absorbing substances in the bands 3E1-2 and 3C2-3; the latter two bands being associated with the white gene which was known to show variegation in this rearrangement. The later discovery (Demerec *et al.*, 1942) that the locus of echinus is associated with the 3F1-2 band, suggests, since no variegation for this gene was reported, that the differences in intensity of absorption of this band have no direct bearing on the variegation process as it is detected genetically by means of changes in gene activity. Prokofyeva-Belgovskaya (1939) observed that the chromonemata or interband regions which ordinarily do not take the Feulgen stain in the euchromatic regions begin to stain when such regions are brought next to heterochromatin, in a manner similar to that of the interband regions of normal heterochromatin (see section 20 in Fig. 4). More recently (1945, 1947), she has reported that the degree to which a euchromatic region resembles heterochromatin in the case of the V-type rearrangements depends on whether the rearrangement is homozygous or heterozygous and on the previous history of the chromosome, that is, whether or not it was derived from a male parent or the female parent. It was claimed that complete confirmation of the results of Noujdin (1944) on the paternal and maternal effects and the state of structural heterozygosity or homozygosity on variegated types was obtained (see Section II.5).

## III. The Behavior of Genes Located in or near Heterochromatin

### 1. *Variegation of the Light Gene*

A rearrangement which involves euchromatin and heterochromatin may in some cases be associated with a change in action of a gene lying in or near the heterochromatic region concerned. In one case, that of the light gene ($lt^+$) in the left arm of the second chromosome it is clear that the change may be of the variegated type (Schultz and Dobzhansky, 1934). Although several rearrangements have been recorded as having a light variegated effect and a correlated break in the heterochromatin of the left arm of the second chromosome (Schultz, 1936), little is known about the necessary conditions which call forth the posi-

tion effect in this case. It has however been noted that variegation of
the light gene in such cases is suppressed in the XO male and enhanced,
that is, more mutant tissue is present, in individuals with extra Y chromo-
somes (Schultz, *loc. cit.*). The latter then constitutes the inverse of the
relationship found for variegation of genes located in the wholly euchro-
matic regions. These results suggest that the V-type position effects
may be approached in two ways which in a sense complement each
other; on the one hand, there is the antagonistic effect of heterochro-
matin on the action of genes normally lying remote from it, and at the
same time, there is an indication that some genes depend for their normal
functioning on proximity to heterochromatin. The cubitus interruptus
gene (*ci*) which lies in the neighborhood of, if not within, the proximal
heterochromatin of the fourth chromosome appears to represent another
example of the latter phenomenon as discussed below; here, the pheno-
type of the mutant *ci* is highly variable and confined largely to a single
wing vein, so that the detection of a variegated-phenotype in the case
of position effects of this gene is scarcely possible.

## 2. *The Dubinin Effect*

Position effect of the cubitus interruptus gene, sometimes referred
to as the "Dubinin effect," was first discovered by Dubinin and Sidorov
(1934a, 1934b) who found that ten among nineteen translocations in-
volving the fourth chromosome showed a weakened dominance of $ci^+$
when they were tested against a chromosome bearing the recessive, *ci*
mutant. Such $R(ci^+)$ types, or "position alleles" (following the termi-
nology of Stern and Heidenthal, 1944) were remarkable in that the
hemizygotes, which were obtained for four of the original ten cases, and
the homozygotes viable in two cases possessed normal venation in con-
trast to the gaps which appeared in the fourth vein of $R(+) / ci$ and
*ci / ci* genotypes. Moreover, eight combinations made up by combining
two different $R(ci^+)$ types also gave normal venation. Two examples
of R $(ci^+)$ types, $w^{258-18}$ and $w^{258-21}$, are shown in Fig. 2. The validity
of the position effect hypothesis was best attested to by Khwostova's
(1939) extensive study by the salivary gland chromosome method of
**196** x-ray induced *ci* changes. Of these 193 were associated with chromo-
somal rearrangements and for the most part only rearrangements of the
euchromatic-heterochromatic type gave the Dubinin effect; in all cases
a break had occurred in the proximal heterochromatic region of chromo-
some four. These results had already been foreshadowed by the extensive
genetic analysis of $R(ci^+)$ types by Panshin (1935). Only two types
of exceptions to the eu-heterochromatic relation were observed: (*a*) re-
arrangements involving the fourth chromosome and the distal as opposed

to the proximal heterochromatic regions of the X or Y chromosomes, gave the Dubinin effect (*cf.*, Panshin's (1938) finding of a similar anomaly in the case of reversals of white variegation—Section II.6) and (*b*) those involving the heterochromatic regions or the adjoining euchromatic regions of the autosomes gave the effect only when such regions were removed through inversion from the centromere region. Dubinin *et al.*, (1935) showed that the break in the proximal heterochromatin of the fourth chromosome need not be identical in different $R(ci^+)$ types, it being to the left of the bent gene in one case and to the right of this locus in another. It was evident that the position effect spread through the locus of bent but no detectable effect on the bent gene was observed. Stern *et al.* (1946) also found that the breakpoint in two $R(ci^+)$ types had occurred to the left of the cubitus interruptus gene.

Khwostova's plot of the observed breaks in the euchromatic region of 193 $R(ci^+)$ types revealed no evidence for excessive grouping of breaks in any given region, and such grouping as was observed appears to be of the same type expected if some regions show a somewhat higher breakage frequency per unit salivary gland chromosome length than others (Bauer *et al.*, 1938). Her results did however reveal a possible significant departure from randomness in that breaks in the euchromatic regions immediately adjoining the centromere regions of the other autosomes were not found. It may be assumed that any rearrangement involving the removal of the $ci^+$ gene from heterochromatin of the fourth chromosome and its transfer to any euchromatic region, not immediately adjoining the proximal heterochromatin, will show a weakened dominance of $ci^+$.

If the position effect of the $ci$ gene were due solely to structural heterozygosity then it would follow that $R(+)$ / $ci$ and $R(ci)$ / $+$ would be identical in phenotype when R is the same rearrangement in each case. Although such a comparison has not yet been technically feasible, Stern *et al.* (1946) have obtained indirect evidence that it can not play a major role in evoking the Dubinin effect just as it played little or no role in the V-type position effects of the hairy and curled genes (Section II.2). After treating wild type and $ci$ males with the same dosage of x-rays and mating separately to $ci$ and to wild-type females respectively, it would be anticipated, were $R(+)$ / $ci$ and $R(ci)$ / $+$ in general identical in phenotype, that individuals with vein interruptions of the $ci$ type would arise with equal frequencies in the two experiments, other factors being equal. From the mating of $ci$ females and treated wild-type males (4,000 r units), 39 individuals having gaps in the fourth vein were obtained among a total of 4,358 progeny reared at 26°C.; using

the same x-ray dosage and temperature conditions, they found that the reciprocal mating gave no individuals of this type in a comparable number of progeny (4,639). In the latter as well as in the former experiment, several individuals were obtained having a slight $ci$ phenotype, consisting in a thinning of the fourth vein. Earlier experiments (Stern and Heidenthal, 1944) had shown that rearrangements involving the $ci$ mutant, $R(ci)$, detected on the basis that they gave a more extreme $ci$ phenotype opposite $ci$ than did the $ci$ homozygotes, give a slight $ci$ phenotype in the heterozygote with a normal allele and that this dominance is enhanced at 18°C. (see also Sidorov, 1941b). Although this result might suggest that the action of the $ci^+$ allele in the normal chromosome had been directly interfered with, it is known that a much more striking dominant $ci$ effect can be given by either of two dominant mutations, $ci^W$ (Wallace) and $ci^D$, the latter known to be apparently normal cytologically (Bridges, 1935b).

For an adequate appreciation of the nature of position effect of the $ci$ gene it becomes particularly necessary to consider the properties of the mutant alleles of this gene when they lie in their normal position. Such a study has been given by Stern (1943) and Stern and Schaeffer (1943a, 1943b). It was determined that three doses of the $ci$ mutant gene (the triplo-IV condition) lead to more nearly normal venation than do two doses (diplo-IV), which in turn were more nearly normal than one dose (either as the haplo-IV condition or in tests opposite a deficiency, Minute-4, for the $ci$ gene). The mutant, $ci^W$, on the other hand was nearly normal in one dose and very extreme in venation abnormality in two doses. These and other relationships with the $R(+)$ and $R(ci)$ types will be summarized here by the use of series expressing the relative degree of venation disturbance. Thus, when $ci$ and $ci^W$ are compared with each other by testing each opposite a chromosome bearing $ci$ (or $ci^W$), then the following seriation results with reference to $ci^+$ (where the sign, $>$, means has more nearly normal venation than):

$$+ \; > \; ci \; > \; ci^W \tag{1}$$

If, however, the same set of alleles is tested against a deficiency for the $ci$ gene the position of $ci$ and $ci^W$ in the series was significantly reversed as follows (where the sign, $\geqq$, means equivalent to or more nearly normal than):

$$+ \; \geqq \; ci^W \; > \; ci. \tag{2}$$

The same contradiction expressed in series one and two was later (Stern *et al.*, 1946) found to hold for $ci$ and those $R(+)$ alleles which were especially selected because they gave greater vein defects in the het-

erozygote with $ci$ than did $ci$ homozygotes. The seriation opposite $ci$ was, therefore, by definition:

$$+ > ci > R(+) \qquad (3)$$

In tests opposite a deficiency the same three alleles gave the following series:

$$+ \gtreqless R(+) > ci \qquad (4)$$

In complete confirmation of the above results of Dubinin and Sidorov, the four $R(+)$ types appeared wild-type in the hemizygote. Series **4** also held true when the test chromosome was the same $R(+)$ allele; again three of the four $R(+)$ types gave normal venation in the homozygote; in the exceptional case designated, in Stern's notation, $R^2(+)$, the homozygote was very similar to the $ci$ homozygote. This exception was notable in another respect; namely, it had involved a break in the left arm of the fourth chromosome. In the light of Panshin's (1938) discovery that sixteen rearrangements having breaks in the short arm of the fourth chromosome did not show a Dubinin effect, it is possible that the $R^2(+)$ allele had in fact an associated mutant allele of $ci$ induced by the x-ray treatment at the same time as the rearrangement; *i.e.*, its effect appears to conform to that of the $R(ci)$ type, described below.

Finally, similar relationships were obtained with $R(ci)$ alleles, selected because they gave the following seriation with respect to $ci$ in tests opposite $ci$:

$$+ > ci > R(ci) \qquad (5)$$

More recently (Stern, 1948b) it was reported that $R(ci)$ types in general give normal or nearly normal venation in the hemizygote, so that the following series holds in tests opposite a deficiency:

$$+ \gtreqless R(ci) > ci \qquad (6)$$

It had earlier been shown (Stern and Heidenthal, **1944**) that series **6** also holds if the test is made opposite an $R(+)$ type.

Series **1** through **6** summarize some of the complex relationships observed with position alleles of the $ci$ gene. Whatever plays a role in causing the shift in position of $ci$ and the position allele in series **3** and **4**, or **5** and **6**, may well be related to the factor or factors responsible for a similar shift observed for $ci$ and $ci^w$ as between series **1** and **2**. In a sense, the $R(+)$ alleles of series **3**, act more like a deficiency for the gene than like either $ci$ or $ci^w$; possibly the $ci$ phenotype results from an accumulation of one substance relative to another produced in an adjoining region (or locus) such that the partial inactivation of the

whole region, which might occur in R(+), would fail to give the $ci$ phenotype (see discussion of other genes having dominant mutant alleles in Section II.1). The R($ci$) alleles, by comparison with the R(+) alleles more closely resemble the $ci^w$ allele in that ($a$) they are slightly dominant to the normal allele, ($b$) the hemizygote approaches normality, ($c$) and the homozygote is as extreme or more extreme (Stern, 1948b) than the $ci$ homozygote. But series 1 and 2 indicate that the transition between a $ci$ and $ci^w$ type of change would involve a discontinuity. Such a discontinuity is observed, however, among different R($ci$) alleles: those which give the least dominance of the $ci$ phenotype (closest to $ci$) opposite a normal chromosome bearing $ci^+$ give the greatest amount of vein interruption when opposite an R($ci^+$) allele (Stern and Heidenthal, 1944). Further clues may be obtained when results of tests of the R($ci$) and R(+) types with $ci^w$ or $ci^D$ alleles are reported.

Some of the observed relationships between the mutant alleles and position alleles of the $ci$ gene could be explained (Stern $et\ al.$, 1946) by assuming that the gene possesses two, more or less independently varying attributes, ($a$) its "combining power," c, for a substrate (S), and ($b$) an "efficiency," e, with which S is converted into a gene product (P). Since the effectiveness of a particular allele in its production of P must be represented by the product, c·e, the hypothesis proved insufficient to account alone for any one of the three discrepancies which are expressed by the above series. In the case of series 3 and 4, many facts could be brought into line if it was assumed that ($a$) the $ci^+$ allele in the rearranged chromosome has a reduced amount of substrate available in its new position, and ($b$) competition between alleles in homologous chromosomes occurs in R(+) / $ci$ such that $ci$ has a priority, by virtue of its normal position, over the R(+) allele for S. In the R(+) homozygote or hemizygote this competition would no longer be present and the $ci^+$ allele could still lead to normal venation even in the presence of reduced amount of S, because of its relatively high combining power and efficiency in comparison to the $ci$ allele. Additional assumptions would have to be made however to fit in the later results expressed by series 5 and 6 in which the $ci$ allele in the R($ci$) hemizygote appears to function in a more nearly normal fashion than when it is in its regular position. Stern (1948a) also obtained evidence which strongly indicated that competition between alleles for a common substrate would have to occur independently of whether or not the alleles are close together as in the structural homozygote, or are far removed, as in one R(+) allele where a minute region of the fourth chromosome carrying the $ci^+$ allele was transferred and inserted to another chromosome.

Stern (1948b) suggested that qualitatively different phenomena may

be involved in some of the R($ci$) types. In several independent occurrences of these, the rearrangement had a break within a narrow region of the right arm of the second chromosome and at the same time these cases were stated to be exceptional in having a break to the right of the $ci$ gene instead of to the left which appears to be the rule for the R($+$) alleles and presumably other R($ci$) alleles. Whether this result indicates that a specific type of association with the $ci$ gene has occurred with respect to genes located in this region of the second chromosome, or whether the effect is due to a position effect on genes in that region which enhances or simulates the $ci$ phenotype is not clear. Although the position effect interpretation can, in most cases, be considered valid for the known R($+$) rearrangements giving the Dubinin-effect, the evidence suggests that the R($ci$) types represent a complex group of changes not all of which necessarily involve position effects of the $ci$ gene itself.

If the Dubinin effect is considered as another example of variegation then the behavior of the R($+$) types might be explained by assuming that in the homozygote the addition of a variegation pattern for the $ci$ character would tend to give wild-type because the probability that the alleles in both chromosomes would be effected simultaneously would be low; to explain the fact that the hemizygote is wild-type it might be assumed that the deficiency for heterochromatin of the fourth chromosome in such individuals would by analogy with the effect of the loss of the Y chromosome on the variegation of the light gene (Section III.1) cause a suppression of the variegation. Panshin (1936) and Khwostova (1939) have both reported that R($+$) alleles are more extreme in the XXY female than in the XX female; the published data indicate that this effect is rather slight and not always consistent. Results of tests in the XO male have not appeared. The variegation hypothesis cannot be regarded as very satisfactory and would not explain the finding that the R($ci$) hemizygote has normal or nearly normal venation.

## IV. The Stable-Type Position Effects

### 1. Position Effect without Chromosomal Abberration

Position effects associated with a stable change in gene action are rare by comparison with the number of examples of V-type position effects. Indeed there are no established cases in which a stable change arising in association with a chromosomal rearrangement has been proven to be due to position effect rather than to intragenic mutation. Proofs of the stable type position effects have been obtained, however, in other types of phenomena: (a) The behavior of different dosages of the Bar region as studied with the Bar duplication (Section IV.3) and (b) the

apparent allelism exhibited by the closely linked genes, Star ($S$) and asteroid ($ast$). In the latter case (Lewis, 1945), a quite different phenotype is obtained depending on whether the two mutants are in the same chromosome and their normal alleles in the other, that is, $S\ ast\ /\ +\ +$ as compared to the equivalent genotype having the mutants in opposite chromosomes, namely, $S\ +\ /\ +\ ast$. This example of the position effect did not depend on the presence of a chromosomal rearrangement since each of these mutants appeared normal in the salivary gland chromosomes. In the former case the phenotype was equivalent to that of $S\ /\ +$ in having a slightly smaller than normal rough eye; while in the latter case extreme reduction in the size and roughness of the eye occurred in all individuals. A similar comparison involving another asteroid allele, $ast^4$, also showed a marked difference in phenotype between the equivalent genotypes. Finally a comparison of the two equivalent genotypes, $S\ ast\ /\ +\ ast^4$ and $S\ ast^4\ /\ +\ ast$ indicated that the former produces a larger eye than the latter. From females of the type, $S\ ast\ /\ +\ +$, an asteroid mutant allele was recovered as a crossover on three occasions and possessed the same properties as the original asteroid mutant. A cytogenetic analysis of these mutants showed that they were correlated with a doublet structure (see Fig. 5; other examples: 1B3-4, 19F1-2, in Fig. 4) near the end of the left arm of the second chromosome. It was therefore suggested that these exceedingly closely linked loci might represent an instance of a duplication, now
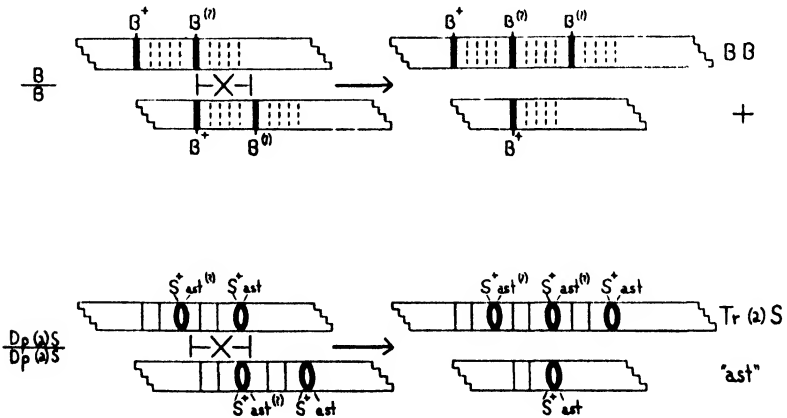


Fig. 5

Diagrammatic representation of the origin of normal chromosome and triplication types from contiguous, direct duplications of the Bar (B) and Star (Dp-S) type. Uncertainty as to the presence of a stable mutation as opposed to position effect of a gene is indicated by (?). See text for description.

established in the species, of a single ancestral gene. Another case of linked genes, namely, bithorax and bithoraxoid, exhibiting a position effect of this sort has been noted (Lewis, 1948). The evidence suggests that the two genes involved in each of these pseudo-allelic series are similar but not identical in function. Two simple models suggest themselves: (a) the two genes are competing for the same substrate (S), (b) each gene controls a separate step in a reaction series of the following type: $S \rightarrow A \rightarrow B$. Results of studies with the bithorax and bithoraxoid mutants (unpublished) support the latter model. It is suggested that the position effect may in some cases be brought about by the direct dependence of one gene upon the reaction product of its neighbor gene in the same chromosome and that these products are produced in such limited amounts, or diffuse so slowly, that each chromosome behaves more or less independently of its homolog with respect to these reactions. The possibility that duplicate genes may often diverge by a process whereby one comes to control a reaction successive (or antecedent on the Horowitz (1945) hypothesis) to that controlled by the old, receives support from an independent consideration of enzyme specificities. Thus in a reversible reaction series of the type: $A \rightleftharpoons B \rightleftharpoons C$, the enzymes which control these two steps may in many cases have to be structurally very similar to one another if they are to share a common specificity for the intermediate product, B. Such a specificity might only be possible in the case of enzymes produced by genes which were once identical.

Bridges' (1935a) interpretation of the doublet structures in the salivary gland chromosomes as one-band duplications and their widespread occurrence throughout the chromosomes, suggests that there may be many pairs of closely linked, functionally related, genes in this organism. Wherever their functioning is dependent upon close proximity a basis is provided for detecting position effects of the S-type. Rearrangements, especially those associated with stable, as opposed to variegated-type, changes in gene action may in some cases be position effects on genes of the above type.

## 2. *Possible Examples of the S-type Position Effects*

A considerable number of chromosomal rearrangements in Drosophila have been found to be associated with stable as opposed to variegated changes in gene action. As a rule, such rearrangements involve the wholly euchromatic regions of the chromosomes and the associated stable change exhibits quite different properties from that of variegation of genes accompanying the eu-heterochromatic rearrangements. Demerec (1941a, 1941b) and his coworkers have described many such cases in-

volving stable changes at such loci as *w*, *rst*, *N*, and *ct*. The locus of
the affected gene was usually adjacent or only a few bands removed
from the point of rearrangement. It was noted, therefore, that the sensi-
tive region of the gene change was much narrower in these cases than
in the V-type position effects. Significantly, there was no evidence of
the spreading effect (Section II.4); that is, an extreme change usually
resembling inactivation of the gene occurs when the gene is one or several
bands removed from the point of rearrangement, while the intervening
loci remain unaffected.

A common example of the above phenomenon is the high frequency
with which chromosomal rearrangements of x-ray origin are found to be
associated with lethal changes near the point of breakage. Although
these have often been assumed to be the result of position effect, Lea and
Catcheside (1945) found that the existing data relating x-ray dosage
to the frequency of production of sex-linked recessive lethals do not
require such an assumption, it being sufficient to assume that in a certain
proportion of rearrangements a lethal gene mutation is induced at the
same time (estimated roughly to be about three in eight breaks). They
estimated that the maximum proportion of lethals of the position effect
type, which could be reconciled with existing data (Timoféeff-Ressovksy,
1939) relating dosage to sex-linked lethal frequency, would not be likely
to exceed 17%. It should be noted that some lethals of the position
effect type are expected in the case of X-chromosome rearrangements
having extreme variegation effects, but even the proportion of these
may be low; examples are several of the Notch-variegated types which
act as lethals in the XY male, but which survive in the XYY male
(Section II.5), indicating that no permanent change within the gene has
occurred. Kaufmann (1947) found that when the frequency of rear-
rangements following x-ray treatment at 4,000 r units was increased by
pretreatment with infrared radiation, the proportion of lethals associated
with rearrangements diminished with respect to the proportion in the
control, which was treated at the same dosage but without the infrared
pretreatment. This was the result expected on the basis that few posi-
tion effect lethals occur, but the results were not statistically significant.
On the basis of extensive cytological analyses of x-ray induced sex-linked
lethals and their rate of production over a wide range of doses, Dubinin
*et al.* (1941) have also concluded that lethals arise largely as the result
of a primary effect of the x-rays.

It is likely that the frequency with which lethal or "visible" gene
changes occur in Drosophila in association with chromosomal rearrange-
ments is too high to be accounted for on the basis of chance coincidence
of two independent processes or "hits." Demerec and Fano (1941) have

concluded that a "single-event" process may account for the production of most of the short (less than 15-band) deficiencies of x-ray origin. It is therefore reasonable to assume that mutation may sometimes be induced simultaneously with chromosomal breakage in genes several bands removed from, as well as those adjacent to, the breakpoint; just as Hoover's (1938) cytological analysis of a selected group of eighteen breakage points revealed the presence of small deficiencies at these points in five cases. An example derived from the V-type rearrangements is the inversion, $N^{264-57}$ (Fig. 1), in which the stable change at the roughest locus, several bands removed from the point of rearrangement, may be viewed as an intragenic mutation, if not loss, of this gene (Demerec, 1941a).

There is some possibility that certain genes, such as, scute, cut, Star, and asteroid, exhibit a stable-type position effect when rearrangements occur directly adjacent to the gene. In the case of the latter pair of closely lined genes, there was some reason to suppose that breaks did not actually occur between them but that breaks to the right of the asteroid locus when induced in a normal chromosome, were effective in producing an inactivation of both genes; or curiously, if *ast* was x-rayed, reverse (non-lethal) mutations of asteroid were found to be associated also with a break immediately to the right of these genes (Lewis, 1945). Breaks and new reunions which occur immediately to the right of the band 1B3-4 (Fig. 4) are associated with a whole series of scute changes (see discussion by Muller, 1941); Sutton (1943b) found additional cases of such changes, however, in which the break near scute had occurred to the left of this doublet, and even in some cases to the left of 1B1-2. Unfortunately, it is very difficult to obtain an unselected sample of breakage points close to specific loci. Sutton, however, in the above work obtained several rearrangements having breaks just to the left of 1B3-4 or 1B1-2 detected as yellow changes, which did not have associated effects on the scute gene; similarly, breaks just to the left of and one to the right of the Star-asteroid loci did not have associated changes in these genes, in a total of three cases analyzed (Lewis, *loc. cit.*).

One means of testing the position effect hypothesis in the case of stable changes accompanying rearrangements is to try to induce reversals of that change on the assumption that the occurrence of a new rearrangement of the gene in question might restore its normal action. Raffel and Muller (1940) did not obtain reversals of the scute-4 phenotype, associated with the $sc^4$ inversion (Fig. 3), among "approximately 50,000" male offspring from a mating of attached-X females and treated (3,000 r units) $sc^4$ males. Goldat (1936) obtained no reverse mutations of the scute-7 change, associated with a rearrangement of the wholly

euchromatic type (having a break just to the right of scute, as in $sc^4$), among 46,842 daughters from a mating of scute females (which were also yellow and achaete-3) and treated (4,000 r units) $sc^7$ males, where the occurrence of any reversals associated with recessive lethals could have been detected. Reversals of the dominant phenotypic effect of certain changes associated with chromosomal aberrations, such as, Dichaete and Lyra (Dubovsky and Kelstein, 1938), or Glazed (Griffen and Stone, 1939) may not be cogent, since here it is likely that partial or complete inactivation of the gene concerned would also lead to such a reversal; that is, such cases may still have been lethal or abnormal in the homozygous condition. An apparent reversal of the Punch phenotype, a dominant non-variegated eye color change, associated with a rearrangement, was lethal when homozygous and abnormal in combination with Punch, as shown by Oliver (1941).

Another somewhat more probable example of the S-type position effect than those discussed above, is the finding that x-ray induced Bar-like (small-eye) phenotypes (or induced reversals of such phenotypes in the case of Bar itself) are often accompanied by a rearrangement having a break very near the 16 A1-2 bands (Figs. 4 and 5). In these cases, however, the situation appears much more complex and difficult to analyze as discussed in Section IV.3.

A special application of the problem of the origin of the stable change in action associated with rearrangements is that of deficiency-bearing chromosomes, in which such a change may occur in a gene close to, but not actually included in, the deficient segment. Such a deficiency then becomes totally unsuitable as a means of identifying the cytological location of that gene with any precision. Examples of such cases were found by Sutton (1943b) in the case of the yellow and scute genes. These cases are puzzling if one assumes that the locus of the gene has to be within the deficient segment, for one would then have to conclude, as Goldschmidt (1944) has done in this case, that within a whole region near the tip of the X chromosome the 'locus' of yellow could be practically anywhere. The accurate location of genes in the chromosomes clearly demands much more refined methods, namely, the use of only those rearrangements which do *not* have associated stable changes in the action of the gene or genes in question. It was by the use of such rearrangements that Sutton (1943b) was able to obtain an unequivocal placing of the yellow and achaete genes in the region from 1A5 to 1A8, inclusive (See Fig. 4).

### 3. *Position Effect and Intrachromosomal Duplications*

As the result of the independent cytological studies of Bridges (1936) and Muller *et al.* (1936), it is now known that the original case of position effect found by Sturtevant (1925) depends on the way in which four doses of a given region of the X chromosome are distributed with respect to homologous chromosomes (rather than two, as originally supposed). Thus, the Bar "mutation," *B*, was found to be a duplication in tandem, or contiguous, direct order for the bands of division 16A of the salivary gland X-chromosome, and the unequal crossover product (see Fig. 5) known as double-Bar (*BB*) was shown by Bridges to be a serial triplication for this region. It will be recalled in this case that the position effect was in evidence by the fact that *B* / *B* females had significantly larger eyes than those of the equivalent genotype, *B B* / +; more recently, Chevais (1943) has shown that this difference is maintained when individuals of these two genotypes are fed on the so-called B+ substance, which results in significant increases in the number of eye facets in each case. The fact that attached-X females of these two genotypes likewise can be distinguished by the difference in their facet number suggests that the Y chromosome has little influence, if any, on this position effect (L. V. Morgan, 1931). Although the position effect hypothesis may be considered established in this case, the nature of the change which brings about the Bar phenotype is by no means clear. Rapoport (1936, 1940a) synthesized higher derivatives of the Bar duplication and obtained a five-fold repetition of the Bar region, called quadruple-Bar, $B^q$ or *BBBB*, from homozygous *BB* females. A comparison of *BB* / *BB* with *BBBB* / + females indicated that the latter probably had significantly smaller eyes than the former. Eye size diminished with increasing doses of the Bar region until only a few facets remained in the *BBBB* male.

Results of studies with an enhancer of Bar, symbol, *E-B*, have been reported by Bonnier *et al.* (1943) and Bonnier *et al.* (1947). The locus of this change appeared to be either near the right end of the right section of the duplication or somewhat beyond. The mutant acted as a recessive lethal change. A position effect comparison was possible in the case of females of the following genotypes: *B* + / + *E-B* and *B E-B* / ++; the former, which were similar to *B* / *B* in phenotype, had consistently larger eyes than the latter.

It may be seen from a diagram of the Bar duplication (Fig. 5) that a new rearrangement of genes has been accomplished at the point where the end of the first or left section of 16 A joins the beginning of the second section. Griffen (1941, and Bridges and Brehme, 1944) noted from a

cytogenetic analysis of a rearrangement which had separated the two sections at approximately this same point, the Bar-Stone translocation, that the extreme dominant Bar effect was associated only with the second or right section. A Bar effect was induced, however, in what had constituted the original left section, by further x-ray treatment and in this case a new association of the 16 A1-2 doublet contained therein had occurred. Slight dominance of Bar-like effects which did not depend upon the presence of a duplication for the Bar region have also been found in two other cases to have involved a break very close to 16 A1-2; *i.e.*, just to the left in the case of $B^{203-48}$, analyzed by Sutton (1943a); and, according to Bridges (Morgan *et al.*, 1936), between 16 A1 and 2 in the case of "Baroid," studied by Dobzhansky (1932, 1936). These results then point to the conclusion that there are at least two separate components of the Bar phenotype to be considered, (*a*) *a stable change in the action of a gene or genes lying next to the point of rearrangement, possibly within the 16 A1-2 doublet at that point, and* (*b*) *an influence somehow exerted by the close juxtaposition of two sets of identical loci.*

Dubinin and Volotov (1936, 1940) and Sutton (*loc. cit.*) found that the change which produces the reversal of the dominant Bar phenotype (or that of double-Bar) can also arise without cytologically detectable change in the duplication (or triplication) or elsewhere. A genetic analysis of one such case by Dubinin and Volotov (1940) revealed that it resembled $B / B$ when opposite $B$, even though the homozygous females and males had normal eyes. From females homozygous for this apparent reversal of Bar, Bar-like sons were derived. These proved to be associated with crossing over (in the forked-Beadex region) and proved to be triplications for the Bar region, as in $BB$; their frequency was 0.04% (among 16,364 sons) which corresponds to that obtained by Sturtevant (1928) for what are now recognized as triplication types. Their method of analysis did not permit a decision between the possibility that this change was a dominant suppressor mutant, linked, perhaps closely, to the Bar duplication, or a change near the point of rearrangement in the Bar duplication. Rapoport (1941) found the analysis of x-ray induced modifications of the Bar phenotype, was complicated by the induction of suppressor and enhancer mutants at other loci.

In all of the above studies in which reversals of the dominant Bar phenotypes appeared, it was found as expected on the basis of a known Bar deficiency that loss of the 16A region does not give a Bar effect nor does it enhance or suppress Bar, that is $B / Df$-$B$ resembled $B / +$ (Bridges, 1917). Again the problem as to the nature of a gene change which can produce such results arises (Section II.1); if the bands 16A1-2 are considered as representing two closely linked genes with similar

effects (Section IV.1) the problem becomes a very complex one, indeed, in the Bar case.

Demerec and Hoover (1939) found that the Hairy-wing mutant was associated with a tandem duplication of the bands 1B1-2, at the left tip of the X chromosome (Fig. 4), and suggested that the associated phenotype was the result of a position effect as in the case of the Bar duplication. Spontaneous reversions of Hairy-wing (or triplications) following unequal crossing over are not expected with an appreciable frequency in this case, assuming the duplication to be of the direct type (*i.e.*, ABAB), because of the excessively low crossing over in this region of the X chromosome; Rapoport (1940b) reported none among over 33,000 offspring from homozygous Hw females. Rapoport obtained two reversals of the dominant Hw effect following x-ray treatment (3,000 r units) among 9,005 tested gametes; these proved to be lethal and were evidently deficiencies. Schultz (Morgan *et al.*, 1941) found that the dominant, Confluens mutant was associated with a tandem duplication of a section involving the Notch locus, and assumed a position effect was at work.

A cytogenetic analysis of the Star-duplication (Fig. 5), which includes the Star and asteroid genes and which arose from homozygous females showed that the mutant allele in the right section of the duplication had remained unchanged whereas the asteroid gene in the left section behaved as though it had reverted to wild type (Lewis, 1941, 1945). Extensive tests of the possibilities that the left section still carried a mutant asteroid allele were negative, however. In a whole series of position effect comparisons which were made possible by introducing various combinations of alleles of the Star and asteroid genes into the duplication there was no evidence for the existence of a position effect extending from the loci in one section of the duplication to those in the other. A comparison of the homozygous duplication versus a triplication of this region opposite a normal chromosome gave identical (but practically wild-type) phenotypes in each case. A comparison of the homozygous triplication (having slight venation and facet abnormalities) with the equivalent genotype carrying a quintuplication for this region in one chromosome and a normal complement of genes in the other chromosome likewise failed to show evidence of a position effect.

## V. Position Effect in Oenothera blandina

An important advance in position effect studies came with the discovery and proof of position effect in *Oenothera blandina* (Catcheside, 1939, 1947a). A remarkably close parallel exists between this case and the V-type position effects of Drosophila. The position effect was

detected in a rearrangement, symbol, A, involving the 3.4 and 11.12 chromo-
somes. Variegation was observed for the dominant alleles, $P^r$ (red sepal-
color) or $P^s$ (light red sepals, recessive to $P^r$ but incompletely dominant
to the lower alleles, $P$ and $p$, causing green sepal-color) of the $P$ gene,
and for the $S$ ($S$, causing yellow petal-color; $s$, sulfur-colored petals)
gene located in arm 3. It was found that R($P^s$) / $P^r$ had deep red sepal-
color in the flower buds as with $P^r$ / $P^r$ and $P^r$ / $P^s$ genotypes, while
R($P^r$) / $P^s$ had variegated sepals containing deep red patches and green
areas intermingled. Since the homozygous $P^s$ plant has light red sepal-
color (with narrow green stripes) the direction of change in $P^r$ is evi-
dently towards one of the lower alleles, $P$ or $p$. Variegation also
appeared in plants of the type, R($P^s$) / $P^s$, these having variegated
sepals with light red tissue like that of $P^s$ / $P^s$ and green tissue like that
of $P^s$ / $p$, therefore, no change of $P^s$ towards $P^r$ was in evidence.

It was shown that $P^r$, or $P^s$, loses its unstable action when extracted
from the rearrangement by crossing over; and a return of these alleles to
the rearranged chromosome resulted in a complete restoration of the
variegated behavior. A total of 58 transfers of these alleles in and out of
the A interchange was detected. In a single homozygous R($P^r$) plant
obtained, sepal-color variegation was much less extreme than in R($P^r$)
/ $P^s$, and was such as "would be expected if two $P^r$ variegations were
superposed" (Catcheside, 1947a) (contrast with Drosophila examples,
Section II.5).

Variegation of the $S$ allele could be detected when the normal
chromosome carried the $s$ allele. Thus plants of the type R($S$) / $s$ had
yellow- and sulfur-colored petals, while introductions of $s$ into the inter-
change to give R($s$) / $S$ plants led to uniformly yellow-colored petals.
Variegation of at least the $S$ gene was found to be influenced widely by
environmental conditions.

Rarely, variegation with respect to the $P$ locus led to large patches
of green tissue comprising whole branches of the plant; within some cases
of this kind an occasional bud was variegated or even wholly red. How-
ever, progeny derived from green buds of the wholly green branches
were again variegated. Evidence of a "spreading effect" (Section II.4)
was obtained in a case in which genetic analysis had indicated the
presence of a duplication for the $P$ and $S$ genes in the interchange chromo-
some (Catcheside, 1947b). In such a chromosome it was found that the
variegation process affected only the $P$ and $S$ loci lying nearer to the
point of rearrangement, thus ruling out the possibility that the variegated
pattern represented losses of the distal portion of the translocated arm
by an unstable breakage mechanism. Although cytological proof was
lacking, it seemed likely that the break in the A interchange had oc-

curred in the proximal arm of chromosome three, presumably within heterochromatin (see Marquardt, 1937).

## VI. Interpretations of the Position Effect Phenomenon

There exist two general hypotheses regarding the mechanism of the position effect. The first involves the assumption that it is primarily related to immediate or early gene products; e.g., Sturtevant (1925), Offerman (1935), and Stern and co-workers (see Section III.2); on this basis the gene itself is assumed to remain unaltered. Ephrussi and Sutton (1944) have called this the kinetic hypothesis. The latter workers and Muller (1941, 1947) have argued for a structural hypothesis, which is based on the assumption that the gene itself is altered, but in a way which is readily reversible and which may be analogous to structural, as opposed to chemical, changes in large protein molecules. Either of these hypotheses has two fundamental aspects of the problem to consider: (a) intrachromosomal position effects due, e.g., to the association of a gene with heterochromatin as in the V-type position effects, whereby the effect can be manifested in the total absence of a homologous chromosome, as in the XO male, and (b) the possibility that position effects may occur in which an influence extends from one chromosome to its homolog. In the latter consideration, advocates of the structural hypothesis assume that the forces of somatic pairing operating in an individual heterozygous for a chromosomal rearrangement exert a stress, imposed by pairing difficulties, on the genes located near the points of rearrangement; while on a kinetic hypothesis it would be assumed that such forces acting in a structural homozygote would facilitate interactions (e.g., competition) between gene products produced in one chromosome and those produced in the homolog by bringing homologous loci into close proximity, and that these interactions break down in the structural heterozygote. This consideration, however, can be disposed of as a primary factor in the production of the known and established types of position effect in either Drosophila or Oenothera. That is, the position effect has been demonstrated in several cases to be manifested as between two identical structural heterozygotes, e.g., in R(+) / h and R(h) / +, where R was the same rearrangement in each case and the phenotypes corresponded to a variegated-hairy effect and to wild-type, respectively (Section II.2), or similar examples in Oenothera, (Section V), where somatic pairing itself would be more or less unlikely as Catcheside (1947a) has discussed.

There is the distinct possibility that "interhomolog" position effect is sometimes superposed on the intrachromosomal type. Gersh and Ephrussi (1944) performed experiments to test the possibility of a modi-

fying influence of structural heterozygosity on the manifestation of a variegated phenotype. As already discussed (Section II.5) the evidence is not yet conclusive that a specific modification of the position effect was induced in this case. The possibility of such an action remains an attractive one, since it lends itself very well to experimental test. It has been noted that some evidence suggests that, in the case of the V-type position effects, the modifying influence may be to cause the structural homozygote or hemizygote to be *more* extreme than that predicted for the structural heterozygote (Section II.5); evidently structural homozygosity of the heterochromatic regions might be a more potent factor in modifying the adjoining euchromatic gene, than any stresses exerted on the latter by pairing forces in the structural heterozygote.

In the case of the intrachromosomal position effect, which must be regarded as the fundamental one, a decision between the kinetic and structural hypothesis is far more difficult. In the case of the V-type position effects, the spreading of a structural disturbance initiated within the heterochromatic region by stresses from non-homologous pairing forces (*e.g.*, Ephrussi and Sutton, *loc. cit.*), or the spreading, *along the chromosome,* of some substance possibly nucleic acid, produced in the heterochromatic region and interfering with gene reproduction (Schultz, 1941b) represent alternative but sufficiently non-specific processes to accord with the finding that variegation effects are exerted upon genes without regard to their specific functions (Section II.1 and II.4). In the case of the S-type position effects, far more specific associations of genes appear to be required. In such cases the concept of localized interactions between gene products may constitute a better working hypothesis than the structural one (Section IV.1).

The V-type position effects have established a division of the genetic material into two major components, which happen to correspond, perhaps not accidentally, with euchromatin and heterochromatin, as seen in the salivary gland chromosomes. This type of position effect may well be very widespread in other animals and in plants, especially judging by the example of position effect in Oenothera. On the other hand, the evidence from the Drosophila and Oenothera studies indicates that a great number of conditions may have to be met before it is possible to detect the phenomenon. It is possible to conceive of many situations in which the V-type position effect would be present but not detectable; *e.g.*, in the case of Drosophila itself, its detection is often made impossible in the presence of extra Y chromosomes, presumably meaning an excess of heterochromatin with respect to euchromatin.

The S-type position effects suggest that the chromosomes are organized not wholly at random with respect to the specific roles which the

genes are to play in development; but rather that there occur, perhaps in most organisms, instances of adjacent genes which are related in function probably as the result of an origin by duplications of a single gene (Section IV.1). When such genes are dependent for their normal functioning on close proximity, the possibility of detecting a position effect arises; otherwise proximity probably reflects only a more or less recent origin of such genes. One of the practical consequences of such a relation is that the separate genes which exhibit a positional dependence may act like multiple alleles of a single gene, in a phenotypic sense. Examination of apparent multiple allelic series, or closely linked genes, from this standpoint are under way in many cases and may reveal the existence of the S-type position effect in other organisms (e.g., Dunn and Caspari, 1945; and Laughnan, 1949).

There have been isolated reports of the occurrence of very slight phenotypic changes, which might be slight V- or S-type position effects, associated with rearrangements (Brink, 1932; Jones, 1944; Roberts, 1942, in the case of maize; and see, e.g., Goldschmidt et al., 1939, in the case of Drosophila). Here, however, it would be all but impossible to exclude the possibility that differences within modifying genes were responsible for the apparent position effect.

## VII. Summary

Position effects in Drosophila can be divided into two rather distinct categories: (a) the variegated- or V-type, associated with a mosaic phenotype, and (b) the stable- or S-type, in which a phenotypic change occurs which is similar to, if not identical with, that of a stable intragenic mutation. The V-type appear to be detectable whenever a gene normally lying in euchromatin is brought next to heterochromatin, provided certain other conditions, not fully understood, obtain. They are subject to an almost bewildering number and variety of modifying factors. It is clear that the "variegation of a gene" is not initiated by structural heterozygosity but is an intrachromosomal phenomenon somehow brought about by an influence of heterochromatin, and possibly only specific portions of such material, on genes abnormally set next to it. The fact that the change in gene action does not occur in every somatic cell, the fact that no germinal instability has been detected, and the ready reversibility of the change all suggest that the gene itself has not been altered in any permanent way; finally, it has been possible to prove this in several instances. Variegation of a gene lying in or near the heterochromatic region may occur when it is abnormally set next to euchromatin; whether position effects of the cubitus interruptus gene are of this type is not clear.

The S-type position effects do not require a chromosomal rearrangement as a necessary basis for their detection; when one is present, it often involves the wholly euchromatic regions of the chromosomes. The S-type, when associated with x-ray induced rearrangements, is open to confusion with the phenomenon whereby x-rays appear to cause chromosome breakage and intragenic mutation in a gene close by as the result of a single "hit." The valid cases of the S-type position effect probably depend for their existence on the presence of specific functional relationships between neighboring genes; rearrangements which separate such genes, the artificial production of such genes as the result of an intrachromosomal duplication of the Bar-type, and intragenic mutation within one of such genes, provide means whereby a position effect of the S-type may be detected. The position effect will be present however only in those cases in which proximity of such genes is a requirement for their normal functioning.

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# The Theory of Genetical Recombination

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## CONTENTS

## I. INTRODUCTION

One of the aims of genetical research is to determine in various organisms the topography of the genetical material, and to represent the mutable genes on each chromosome as occupying positions on a linear map. The order and spacing of the genes has in the main to be inferred from the amounts of recombination exhibited by sets of genes when appropriately designed backcross experiments are carried out, though this process is, occasionally, aided by cytological studies and the artificial production of rearrangements.

With linked loci which are closely spaced the recombination value $y$ may itself be used as a conventional measure of distance (*i.e.* as a *metric*) in which to map the chromosome. But, in order to extend the map to wider intervals, it is necessary to introduce a more sophisticated convention. The *map distance* $x$ between two loci is defined as the average number of points of exchange per strand which is formed between the loci in the course of a large number of meioses. This quantity is

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not directly observable by purely genetical methods, and is not equal to the recombination fraction y, which is the probability of occurrence of an odd number of exchange points on the interval between the loci. The inequality of x and y results from two causes, the occurrence of multiple crossing-over, and the operation of *interference*. With no interference, then, as pointed out by Haldane (1919), x and y are related by the formula

$$y = \tfrac{1}{2} (1 - e^{-2x}).$$

Following other early work by Haldane (1919), Kosambi (1944) has proposed the formula

$$y = \tfrac{1}{2} \tanh 2x,$$

as an approximate representation of the connection between x and y, subsuming the effects, both of multiple crossing-over, and of interference of the order of intensity encountered in many organisms. If this relation applies, the recombination fraction over the sum of two contiguous intervals with fractions $y_1$, and $y_2$ is given by

$$y_{1+2} = \frac{y_1 + y_2}{1 + 4y_1y_2}.$$

Where data are available for three loci, this latter relation has in many cases been verified to hold to a fair degree of approximation, and so represents a considerable advance.

The position is, however, left unsatisfactory in three respects. In the first place, the Kosambi formula sets an unpassable upper limit of 50% to recombination over any segment, and requires that y be a steadily increasing function of map length. But recombinations in excess of 50% have been occasionally reported, and recently (Wright, 1947) some good data of this kind have been obtained for the sex chromosome in the house mouse. Values of the order of 56% were observed (the excess over 50% being statistically significant) and, more strikingly, two genes known to be widely separated (by about 35cm) were found to show almost equal recombinations (56.07%, 56.73%) with the sex determining portion, which is known not to be situated between them.

Secondly, Kosambi's formula, containing no reference to an origin for x, predicates that the properties of the chromosome, in respect to interference, are uniform along its length. However, there is some evidence of variation with distance from the centromere. Thirdly, the Kosambi relation by itself does not provide a complete theory of gametic frequencies for linked loci, since with four genes or more the frequencies

of the modes of gamete formation cannot be specified fully by its means. What is required, therefore, is a general formulation, in which may be specified, in terms of n parameters, the probability $p_{r_1 r_2} \ldots r_{n-1}$ of there being exactly $r_1, r_2, \ldots r_{n-1}$ points of exchange formed per strand in the intervals $(1,2)$, $(2,3)$, $\ldots$ , $(n-1,n)$ between any number n of linked loci.

The question of a general mathematical description of the process of recombination, has until the present been neglected. An exception is the work of Jennings (1923) which, postulating a serial mechanism of formation of exchange points, anticipates later cytological findings (Mather, 1936, 1937) and recent mathematical work (Fisher *et al.* 1947; Owen, 1949). It could hardly have been expected, however, that any such formulation would have been attempted until insight into the underlying mechanism of crossing-over had been gained as a result of combined cytological and genetical study. An elaborate formal analysis rarely seems promising of reward which does not relate to an actual mechanical or physical theory of the process considered.

The purpose of this article is to sketch recent mathematical developments, and the cytological evidence on which its main ideas are founded, and to give a survey, arranged partly in historical and partly in logical order, of the development of the theoretical concepts involved in the study of linkage. Attention will be confined to normal situations in diploids, as the theory of linkage in polysomic inheritance is relatively far less advanced. The whole subject of *estimation* of recombination values will be omitted, for though of extreme practical importance it is not directly relevant.

## II. The Development of the Theory of Linkage

### 1. *The Linear Arrangement of Linked Factors*

The first occasion on which particular Mendelian factors other than sex were recognized as being associated with a particular chromosome of a species, was in 1910, when Morgan (1910a) showed that several factors in *Drosophila melanogaster,* including that one for red or white eye color, accompanied the X chromosome in its segregation. This discovery was soon followed by that of incomplete linkage of factors in the sex chromosomes of female Drosophila (Morgan, 1910b). By 1914 the other three linkage groups, unassociated with sex, had been observed in Drosophila. (Morgan and Lynch, 1912; Sturtevant, 1913a; Muller, 1914). It was also evident that the data of Bateson and Punnett for the sweet pea related to the same kind of phenomenon.

Janssen's chiasmatype theory (1909), put forward on the basis of

cytological observations, came to hand to support the view that the separation and recombination of factors in a linkage group was due to an interchange of chromosomal material, and in 1911 Morgan was able to enunciate the theory of recombination by *crossing-over*.

Morgan's suggestion that the frequency of recombination of two genes must be, to a certain extent at least, an index of the distance apart of the genes on the chromosome, appeared as a reasonable corollary of the chiasmatype theory. The idea and method of treatment of the data which finally won general acceptance for the theory of linear arrangement and recombination were provided by Sturtevant; also in 1911. (Muller, 1916; Sturtevant, 1913b, 1915). If the genes of a linkage group are in linear order, and the recombination fraction shown by any pair of them is, to a first approximation, proportional to their separation, then the recombination fractions will have the property of being additive. That is to say; with three loci, 1, 2 and 3, we shall have

$$y_{13} = y_{12} + y_{23} \quad \text{or} \quad y_{13} = y_{13} \sim y_{23}$$

according as the locus 3 lies beyond or between the loci 1 and 2. The additive relation has been amply verified in *D. melanogaster* and other organisms whenever the recombination values concerned are small. The failure of the additive property for large values of recombination constitutes no objection to the hypothesis of linear arrangement and crossing-over, but merely shows that the recombination fraction is not in general the most suitable metric in which to exhibit the organization of the linkage group, although it has a legitimate primacy in any system for analyzing linkage, since it is an observable quantity and not an inferential one.

## 2. *Map Length as an Additive Metric*

a. *Coincidence and Multiple Crossing-over.* The reasons which make the recombination value y non-additive for large separations of the loci concerned were recognized early on by the Drosophilists as being twofold; namely, multiple crossing-over and interference. In the first place Sturtevant 1913a, 1915, 1914) pointed out that coincidence could occur on segments of sufficient length. That is to say, two cross-over points have a finite probability of being formed. More generally, on long segments, multiple cross-overs, sets of 2,3,4, . . . points of exchange, could occur with diminishing but finite probabilities. The recombination fraction for two loci A and B, defined as the probability of separation of A and B at meiosis, would therefore be equal, not to the probability of a single point of exchange being formed on the segment AB of the strand, but to the probability that the total number of cross-overs on AB should

be *odd*. Thus, if the probabilities of exactly 0,1,2,3, . . . cross-overs occurring in AB are respectively equal to $p_0$, $p_1$, $p_2$, $p_3$, . . ., then the recombination y over AB is the sum

$$y = p_1 + p_3 + p_5 + \dots.$$

Suppose now that the formation of exchange points takes place quite at random; that is to say, the appearance of one such point is statistically independent of the appearance of any other. Consider AB, BC, two contiguous intervals, with respective systems of cross-over probabilities $p_0$, $p_1$, $p_2$, . . . ., and $p'_0$, $p'_1$, $p'_2$, . . . , and having recombinations $y_1 = p_1 + p_3 + p_5 + \dots$ and $y_2 = p'_1 + p'_3 + p'_5 + \dots$. If AB, BC are short segments, then $p_1 \gg p_3$, $p_5$, . . and $p'_1 \gg p'_3$, $p'_5$, . . . and to a high degree of approximation $y_1 = p_1$ and $y_2 = p_2$. If $y_{1+2}$ denote the recombination over the combined interval AC = AB + BC, then $y_{1+2}$ is equal to

$$p_1(1 - p'_1) + p'_1(1 - p_1) \fallingdotseq p_1 + p'_1$$

together with terms of higher order. Thus to the same order of approximation

$$y_{1+2} = y_1 + y_2$$

and recombination is an additive function of interval, provided that all the factors considered are near to one another.

If AB, BC are not small, then y must be calculated exactly as the probability of an odd number of cross-overs in AC. An odd number of cross-overs in AC can occur as one of the mutually exclusive events (a) an odd number in AB, conjunctive with an even number in BC or (b) an odd number in BC, conjunctive with an even number in AB. The probability of an odd number in AB is $y_1$, and that of an even number in BC is $1 - y_2$, and these events are, on the hypothesis of random crossing-over, independent. Hence the probability of event (a) is $y_1(1 - y_2)$ while that of (b) is, similarly, $y_2(1 - y_1)$. Finally we have

$$y_{1+2} = y_1(1 - y_2) + y_2(1 - y_1),$$

giving

$$y_{1+2} = y_1 + y_2 - 2y_1y_2$$

This relation is sometimes known as Trow's formula (1913), and figured in the early and now forgotten controversy between the adherents of linear order and the supporters of other hypotheses such as the reduplication theory.

With random crossing-over, Trow's formula is universally true for all pairs of contiguous segments, and states the addition theorem for

recombination as a function of interval. It shows that, under conditions of independent crossing over, y, possessing a definite (even though non-linear) addition theorem, is a valid metric for describing the chromosome. It is desirable, however, to exhibit results in a way satisfactory to geometrical intuition which requires a completely additive metric. This will be achieved if there exists a transforming function $M(y)$, with the property that the quantity x defined by $x = M(y)$ is an additive function of interval. If $M(y)$ exists, then x is a suitable metric for mapping the linkage group, and an analysis of genetic results will consist in applying the transformation $x = M(y)$ to the values of y estimated from the genetical data found for the various segments. The function $M(y)$ may appropriately be called the *mapping function*. It lacks uniqueness, as so far defined, to the extent of a multiplicative constant, but definiteness can be provided by imposing an auxiliary condition. Clearly the best choice of such a condition, is to impose the restriction that for small values of y, x must be approximately equal to y and not merely proportional to it.

In conditions of random crossing-over, such a mapping function may readily be constructed, and is the transformation

$$x = M(y) = -\tfrac{1}{2} \log_e(1 - 2y),$$

which has an unique inverse transformation given by

$$y = m(x) = \tfrac{1}{2}(1 - e^{-2x}).$$

Substituting in Trow's formula the values $y_i = \tfrac{1}{2}(1 - e^{-2x_i})$, $(i = 1,2)$ we get:

$$m(x_{1+2}) = y_{1+2} = y_1 + y_2 - 2y_1y_2$$
$$= \tfrac{1}{2}(1 - e^{-2(x_1+x_2)}) = m(x_1 + x_2)$$

Hence $x_{1+2} = x_1 + x_2$.

For small y, x and y are approximately equal, so that we recover the additive relation $y_{1+2} = y_1 + y_2$.

The Drosophila workers did not introduce the map distance x by the formal process of deduction carried through here, but brought it in more naturally, defining it by a second important property which it has; namely, that of being the mathematical expectation of the number of cross-overs occuring on an interval. This property of x may readily be seen to follow from its additive nature and its equality to y in small segments. For any short interval, x and y are both approximately equal to the probability of a single cross-over occurring in the interval, and are also, therefore, equal (nearly) to the statistical expectation of the number of cross-overs observed in the given short segment. Any general

interval AB may be dissected into some large number N of very short intervals $AA_1, A_1A_2, \ldots\ldots, A_{N-1}B$, so that

$$x_{AB} = x_{AA_1} + x_{A_1A_2} + \ldots + x_{A_{N-1}B}$$
$$= E(r_{AA_1}) + E(r_{A_1A_2}) + \ldots + E(r_{A_{N-1}B})$$
$$= E(r_{AA_1} + r_{A_1A_2} + \ldots r_{A_{N-1}B}),$$

since the mathematical expectation of the sum of a number of random variables is the sum of their separate expectations (whether they are statistically independent or not). Thus $x_{AB} = E(r_{AB})$; and, for any interval, x is the expected number of cross-overs. The additive metric x has thus a very natural significance as the number of cross-overs in a segment averaged over a large number of meiotic cells, and, historically, was defined in this way in terms of its natural meaning, which makes it an index of the total amount of crossing-over likely to occur in a segment. The essential virtue of map distance x lies in its possession of the double property of being both additive and essentially positive. That is, for any three loci O,A,B whose order on the chromosome is the same as the order as here written, we have $x_{OB} > x_{OA}$. For this reason the x map is a true map in the sense that it accurately reproduces the order of the genes on the chromosome. But clearly this is the total extent of the geometrical information which the map conveys, as there is no reason to suppose that the map distances bear any precise relation to physical length of the strand, whether a compact length as in one of its coiled states or an intrinsic length along a spiral. The geometrical information in the map is exhausted by that conveyed about order, although, of course, the map provides a record, in an organized form, of recombination data, and, enabling the approximate prediction of other recombination values, is also a repository of valuable information of a non-geometrical kind.

No doubt the map length preserves a rough proportionality to cyto-logical length, this being evidenced by the fact that the relative map lengths of the chromosomes of a set, as in *D.melanogaster*, appear roughly proportional to the relative physical lengths of the chromosomes when cytologically examined. This seems to hold for both the meiotic and the giant salivary gland cells. It is probable that the kind of physical length observed differs in these two cases. The relation of map length to physical or cytological length is not constant, but subject to various influences such as temperature or the presence of particular genes modifying the amount of crossing-over. An extreme case is the almost complete absence of crossing-over in the males of Drosophila. The sole invariant derived from linkage data is thus gene order. Even this state-

ment, though very generally applicable, is relative since it supposes that abnormal situations, such as inversions, are excluded from consideration.

The mapping function $y = m(x) = \frac{1}{2}(1 - e^{-2x})$ was introduced above in a slightly mystical fashion as a kind of god from the machine to transform Trow's formula into a linear relation in x. Haldane (1919) appears first to have noted that, in the absence of interference, the relation between map length and recombination is of this form, his proof being on the following lines.

If exchanges take place as a system of completely random and statistically independent events, then the probability, $p_r$, of exactly r points of exchange being formed on a given interval AB of the strand is the *rth* term of a Poisson series, and, therefore, equals

$$p_r = e^{-t} \frac{t^r}{\lfloor r} \; ; \; (r = 0,1,2, \ldots)$$

where t is a parameter, $t = t_{AB}$, characterising the segment AB.

Since the recombination over AB is the probability of an odd number of exchange points being formed between the loci A and B, it is given by the expressions

$$y = y_{AB} = \sum_{\bullet}^{\infty} p_{2r+1} = p_1 + p_3 + p_5 + \ldots$$

$$= \frac{1}{2}\{p_0 + p_1 + p_2 + p_3 + \ldots - (p_0 - p_1 + p_2 - p_3 + \ldots)\}$$

$$= \frac{1}{2}\{1 - \sum_{r=0}^{\infty} (-)^r p_r\} . \tag{1}$$

Because $\sum_{\bullet}^{\infty} p_r = p_0 + p_1 + p_2 + p_3 + \ldots = 1,$

the probabilities $p_r$ for $r = 0, 1, 2, \ldots$ corresponding to an exhaustive set of mutually exclusive possibilities.

The map distance between the loci A and B is equal to the expectation of r and given by

$$x = x_{AB} = E(r) = \sum_{r=0}^{\infty} r p_r \div \sum_{r=0}^{\infty} p_r \tag{2}$$

*i.e.*, $\sum_{r=0}^{\infty} r p_r$, since $\sum_{0}^{\infty} p_r = 1 .$

Generating functions are a great convenience in all work of this kind and if the generating function $P(\lambda)$ is defined by

$$P(\lambda) = P_{AB}(\lambda) = \sum_{r=0}^{\infty} p_r \lambda^r$$

then $p_r$ is the coefficient of $\lambda^r$ in $P(\lambda)$, and for x and y we have, by (1) and (2), the relations

$$\begin{cases} x = \sum_0^\infty rp_r = [\sum_1^\infty (r\lambda^{r-1})\ p_r]_{\lambda=1} = \left[\dfrac{d}{d\lambda} P(\lambda)\right]_{\lambda = 1} \\ y = \tfrac{1}{2}[1 - P(-1)] \end{cases} \quad (3)$$

which will be seen to be generally true in all circumstances whatever the particular values of the probabilities $p_r$ may be, whether terms of the Poisson series or not.

In the present case,

$$P(\lambda) = \sum_{r=0}^\infty p_r\lambda^r = \sum_{r=0}^\infty e^{-t}\ \frac{t^r\lambda^r}{\lfloor r} = e^{-t} \sum_0^\infty \frac{(t\lambda)^r}{\lfloor r}$$

$$= e^{-t} \cdot e^{t\lambda} = e^{(\lambda-1)t}.$$

Therefore $\quad P(-1) = e^{-2t}$ and $\dfrac{d}{d\lambda} P(\lambda) = t.e^{(\lambda-1)t}$

so that, by (3),

$$\begin{cases} x = t \\ y = \tfrac{1}{2}(1 - e^{-2t\cdot}). \end{cases} \quad (4)$$

These equations are jointly equivalent to the Haldane relation, $y = \tfrac{1}{2}(1 - e^{-2x})$ but the parametric form (4) is more perspicuous. If, for instance, we introduce any other variable u connected unambiguously with t by a continuous, monotone and differentiable functional relation $t = \phi(u)$, then equations (3, 4) become

$$\begin{cases} x = \phi(u) \\ y = \tfrac{1}{2}(1 - e^{-\phi(u)}) \end{cases} \quad (5)$$

The second of equations (4) shows that the number t associated with a given interval, and introduced originally as the parameter of the Poisson probability distribution of the number of exchange points on that interval, is a suitable metric for mapping the strand, since in the event it comes out equal to the map length defined as the expected number of exchange points (t being the mean of the Poisson distribution). But the transformed system (5) brings into prominence the fact, previously implied, that the map distance is not an unique metric for the representation of the chromosome but one of an infinite number (even if we require the metrics to be additive). We are thus brought in a natural way to anticipate the leading idea of present-day researches, which is to attempt, on the basis of suitable approximations and assumptions, to express the quantities of interest as functions of values of some metric t which may not necessarily be identical with x.

Any function of x, t = $\psi(x)$, which is monotone-increasing and differentiable constitutes a suitable metric. Physical or cytological length·s is itself such a function unless, as is sometimes the case, there are intervals on the strand which are inactive with respect to crossing-over. If such intervals exist then x = x(s) is a step function of s, which is non-decreasing but may be constant over some s intervals, namely those on which crossing-over does not occur.

If x = $\phi(t)$, the inverse relationship being t = $\psi(x)$, where $\phi$ and $\psi$ are both monotonic and differentiable functions, the order of the factors is the same in the x map as in the t map. The density of crossing over at a point in the x map is $\dfrac{dx}{dt} = \phi'(t) = \dfrac{1}{\psi'(x)}$ times the density at the corresponding point in the t map.

Strictly speaking, every metric, including x, must be "quantised" since the genes are not infinitely close together, but ranged at small but definite intervals. The number being so great and the spacing being so small, however, it is hardly necessary to work with a discrete metric. (Compare the work of Jennings and of Winge which are discussed in Section II.4).

Before going on to consider interference, it is profitable, with a view to later developments, to give a further proof of the Haldane formula, using the method of generating functions which requires no previous knowledge of the theory of the Poisson distribution.

If t is any suitable metric, then, with random crossing-over, let $P(\lambda, t)$ be the generating function of probabilities for a segment of metrical length t. Then, for two contiguous segments, of metrical lengths $t_1$ and $t_2$, we have on account of independence

$$P(\lambda, t_1 + t_2) = P(\lambda, t_1) \cdot P(\lambda, t_2)$$

where $P(\lambda, t_1 + t_2)$ refers to the combined interval.

Therefore $\log_e P(\lambda, t_1 + t_2) - \log_e P(\lambda, t_1) = \log_e P(\lambda, t_2)$

and $\dfrac{d}{dt_1} \log_e P(\lambda, t_1) = \lim_{t_2 \to 0} \dfrac{1}{t_2} \{\log_e P(\lambda, t_1 + t_2) - \log_e P(\lambda, t_1)\}$

$$= \lim_{t_2 \to 0} \dfrac{1}{t_2} \log_e P(\lambda, t_2). \qquad (6)$$

If $p_0, p_1, p_2, \ldots$ are the probabilities for the interval $t_2$, then when $t_2$ is very small $p_1 \gg p_2, p_3, \ldots$ and therefore

$$p_0 \approx 1 - p_1 \quad \text{and} \quad P(\lambda, t_2) \approx 1 + (\lambda - 1)p_1$$

Also $p_1$, being the probability of a single cross-over in a short segment length $t_2$ situated at $t_1$, will be given very nearly by an expression of the form $t_2 \cdot g$ where g depends on $t_1$ only and is therefore some function $g = g(t_1)$. Hence (6) gives

$$\frac{d}{dt_1} \log_e P(\lambda, t_1) = \lim_{t_2 \to 0} \frac{1}{t_2} \log_e \{1 + (\lambda - 1)t_2 \cdot g(t_1)\}$$

$$= (\lambda - 1) \cdot g(t_1)$$

Therefore $\log_e P(\lambda, t) = (\lambda - 1) \int_0^t g(u) du,$

since $\qquad P(\lambda, o) = 1.$

Hence $\qquad P(\lambda, t) = e^{(\lambda - 1)\phi(t)}$

where $\qquad \phi(t) = \int_0^t g(u) du.$

It will be noted that with random crossing-over then as a consequence of Trow's formula, or equally of the Haldane relation $y = \frac{1}{2}(1 - e^{-2x})$, every recombination value y is less than $\frac{1}{2}$, and that as x the length of a segment is increased y tends to the value $\frac{1}{2}$, which is the figure for independent segregation. Further, it is easy to see from either formula that y is a steadily increasing function of segment length and cannot show oscillations.

b. *Interference.* The second feature complicating the problem of mapping was noted by Muller in 1916 and given by him the name of *interference.* Interference is said to operate whenever the cross-overs fail to occur completely at random: *i.e.* when they are not statistically independent events. In general the evidence, both of linkage crosses and of cytology, shows that interference is present in the chromosomes of most or all organisms, and is positive. That is to say, the occurrence of one cross-over tends to inhibit and not to favor the occurrence of a second in its neighborhood. More precisely stated, the probability of two cross-overs occurring in a segment is less than the product of the probabilities of occurrence of each cross-over separately.

Consider two contiguous segments, AB,BC. If there were no interference but random crossing-over, the probability of recombination taking place simultaneously over both AB and BC would be equal to $y_1 y_2$. Let z denote the actual value, in any given instance, of this probability. Then, with zero interference $z/y_1 y_2 = 1$, and with positive interference $z/y_1 y_2 < 1$. The presence of interference may therefore be tested by performing a three point cross. If n is the number of organisms bred, and $n_1$, $n_2$, $n_{12}$ are respectively the numbers of individuals showing recombination in the first segment with or without recombination in

the second segment, the number of individuals showing recombination in the second segment with or without recombination in the first segment, and the number of individuals showing recombination in both segments, the ratio $C' = \dfrac{nn_{12}}{n_1 n_2}$ is the appropriate estimate of the index $C = z/y_1 y_2$. The ratio $C'$ has, since Muller's first work, been called the coefficient of *coincidence*. More properly this name should be applied to the index C of which it is the estimate. The quantity C is here defined with reference to two contiguous intervals, *i.e.*, three loci. It may equally well be defined with reference to two non-contiguous intervals (*i.e.*, four loci) by means of the same formula $z/y_1 y_2$, and thus used to investigate interference over longer ranges, but this application will not be discussed here. It may be mentioned however that in this case the correct estimate from observations is $\dfrac{nn_{12}}{n_1 n_2}$ , where $n_i$ is the total number of individuals showing recombination in the segment i and nowhere or anywhere else and $n_{12}$ is the total number of individuals showing recombination in both of the segments 1 and 2 and nowhere or anywhere else. Other formulae for estimation of coincidence for non-contiguous segments proposed by Weinstein (1918), Graubard (1932), and Schweitzer (1934), have been critically examined by Stevens (1936) and shown to lead to incorrect inferences. The fallacy in each case has been connected with a failure to distinguish between a parameter and the statistic employed to estimate it.

The extent to which C deviates from unity is an index of the intensity of interference. In Drosophila, C has been found not to be a constant, but to vary, not only from chromosome to chromosome, but to be dependent on the lengths of the segments involved and on their positions on the chromosome. The nature of the variation will be considered later in Section III. Here we may note that the existence of interference means that the theory of the recombination fraction and the map length is not valid in the simple form in which it was discussed above. Firstly, it may be seen however that x, if defined as the mathematical expectation of the number of cross-overs within an interval, retains its property of approximating to the recombination fraction whenever the interval is short. x is additive for all intervals, and therefore retains its validity as a metric for mapping. y remains additive for all short intervals, since all numbers of multiple cross-overs and also their covariances with unit cross-over are negligible for such segments. Trow's formula fails however, as does the form for the mapping function.

If $P(\lambda) = \sum\limits_{r=0}^{\infty} \lambda^r p_r$ is the generating function of probabilities for any given segment, we have as before the formulae

$$x = \sum\limits_{0}^{\infty} r p_r = \left[\frac{d}{d\lambda}P(\lambda)\right]_{\lambda=1} = E(r).$$

It is easily verified that, with any degree of interference, the quantity z for two contiguous intervals is given accurately by

$$z = \tfrac{1}{2}(y_1 + y_2 - y_{1+2}).$$

For if $w_1$ is the probability that there will be recombination over AB and none over BC, and if $w_2$ is the probability that there will be recombination over BC and none over AB, then

$$y_1 = w_1 + z$$

$$y_2 = w_2 + z$$

$$y_{1+2} = w_1 + w_2$$

and therefore $\qquad y_1 + y_2 - y_{1+2} = 2z$

Hence in place of Trow's formula we have, since $C = z/y_1 y_2$,

$$y_{1+2} = y_1 + y_2 - 2C\,y_1 y_2 \qquad (7)$$

Previously with interference assumed to be zero C had the theoretical value unity. In any real case C has to be regarded as a function of the lengths and the position of the segments which has to be determined empirically. The form for the mapping function is no longer $-\tfrac{1}{2}\log_e(1-2y)$. It is easy to see that it is not merely a question of a pure change of form of the mapping function. There is evidence that the mode of functional dependence of C on $y_1$, $y_2$ itself varies with the position of B upon the chromosome. A complete solution of the mapping problem therefore involves the determination [for all admissible values of $x_1$] of the functional relation $x = M(x_1, y)$. Here x is the map distance OP, and $x_1$ the map distance AP, and y is the recombination over AP, O being some locus arbitrarily chosen as an origin for x. The form of such a complete mapping function has never been the object of theoretical enquiry. Haldane and Kosambi have each attempted the provision of approximate forms which assume uniformity of interference along the chromosome. Their work is described in Section II.3 following. Recent researches (Fisher *et al.* 1947; Owen 1949) have attempted the theoretical problem in a slightly modified form, expressing (under certain assumptions) x and

y measured over a metrical interval $(t_1, t_2)$ as functions $x(t_1, t_2)$ and $y(t_1, t_2)$. This approach is described in Section IV.

The reduction of the Drosophila data to the form of a map, as carried out by the Drosophilists, is in essence a question of determining, for each chromosome and various selected loci $x_1$, numerical forms for the functional relation $x = M(x_1, y)$. The correct forms are found from the system of y values by a numerical process of success of successive approximation. The results are exhibited in sets of curves which show for a sequence of selected 'anchoring' genes the amounts to be subtracted from the map distance measured from each 'anchor' to convert this map distance into recombination with the anchor. A detailed account of the method of reduction of the data to this form is given by Bridges and Morgan in "The Third Chromosome Group of Mutant Characters of Drosophila melanogaster" (1923).

### 3. *Theories of the Mapping Function*

Though in general an uniform mapping function $x = M(y)$ will not exist, and an exact function $x = M(x_1, y)$ is required for all relevant values of $x_1$, it is possible to find uniform mappings which will represent conditions to a certain degree of approximation over the whole or a large part of the chromosome. This problem is in fact the same as finding an approximate addition theorem for y, which in turn is a question of finding one single functional relation

$$y_{1+2} = F(y_1, y_2)$$

that will cover the whole range of y values (assumed for present purposes to be from 0 to $\frac{1}{2}$) in a reasonably satisfactory manner.

Haldane (1919) argues as follows: The general formula (7) namely $y_{1+2} = y_1 + y_2 - 2Cy_1y_2$ becomes $y_{1+2} = y_{1+2}$ for short segments. For long segments it becomes $y_{1+2} = y_1 + y_2 - 2y_1,y_2$, for then $y_1$, $y_2$ and $y_{1+2}$ each approximate to the value $\frac{1}{2}$. This suggests that the approximation $C = \frac{1}{2}$ be applied to segments of intermediate length. (It would hold accurately if, for every double cross-over that should have taken place on the hypothesis of zero interference, a single cross-over occurred.) Haldane analyzing three-point data of Morgan and Bridges (1916) for the X chromosome of Drosophila, suggested that its range of applicability is defined by $y_1 + y_2 \approx \frac{1}{2}$, though this suggestion has not been examined for other data. Haldane next considers the construction of an approximate uniform mapping function. If $m(x)$ is the inverse mapping function, then, making the substitution $y = m(x)$ in (7) we get

$$m(x_1 + x_2) = m(x_1) + m(x_2) - 2C\,m(x_1)\,m(x_2)$$

and, writing $x_1 = x$, $x_2 = h$, this is

$$\frac{m(x + h) - m(x)}{h} = \frac{m(h)}{h}\{1 - 2C\,m(x)\}$$

As $h \to 0, \dfrac{h}{m(h)} \to 1$ and $\dfrac{m(x + h) - m(x)}{h} \to m'(x) = \dfrac{dy}{dx}$,

if this derivative is assumed to exist at all points.

Therefore $m'(x) = 1 - 2C_0\,m(x)$ where $C_0$ denotes $\lim\limits_{h \to 0} C$.

$C_0$ has been called by Haldane the "marginal coincidence," since it refers to a finite interval together with a very short adjacent segment.

We have the differential equation

$$\frac{dy}{dx} = 1 - 2C_0 y \quad \text{or} \quad \frac{dx}{dy} = \frac{1}{1 - 2C_0 y}$$

Hence the x and y of any segment are connected by

$$x = \int_0^y \frac{dv}{1 - 2C_0 v} \tag{8}$$

since $x = 0$ when $y = 0$. Here $C_0$ is the marginal coincidence of that segment commencing at the point $x = 0$ and of such extent that its recombination is equal to v where $o \leqslant v \leqslant y$.

For short intervals we therefore have the approximations

$$x = y + 2\int_0^y C_0\,v\,dv$$

$$y = x - 2\int_0^x C_0\,v\,dv$$

If the marginal coincidence were constant we would have for all intervals

$$x = M(y) = -\frac{1}{2C_0}\log_e (1 - 2C_0 y),$$

$$y = m(x) = \frac{1}{2C_0}\{1 - e^{-2C_0 x}\}.$$

For small x, this gives $x = y$,

and for large values (since $C \to 1$ and therefore $C_0 \to 1$),

$$x = -\tfrac{1}{2}\log_e (1 - 2y) \quad y = \tfrac{1}{2}(-e^{-2x}),$$

corresponding to Trow's formula. (This analysis involving the deriva-

tion and solution of a differential equation will be seen to be closely related to that employed at the end of Section II.2a).

Haldane plots the curves  $y = x$  (9)
and  $y = \frac{1}{2}(1 - e^{-2x})$  (10).

It is found that all empirical mapping curves lie between these boundaries, which correspond to the extremes of complete and zero interference. Haldane, therefore, instead of inserting an empirical function $C_0 = C_0(v)$ in (8) and then integrating, as could be done, fits to the data for the X chromosome an empirical curve lying between (9) and (10). The method of Anderson and Rhoades (1931), also applied to the X chromosome, is a procedure of essentially the same kind. They introduce as a measure of interference the quotient

$$Q = \frac{y - \frac{1}{2}(1 - e^{-2x})}{x - \frac{1}{2}(1 - e^{-2x})}$$

which vanishes for zero interference and is unity when interference is complete. Empirical values of Q are calculated at various loci from a Drosophila map. From a curve $Q = Q(x)$ fitted to these values it would be possible to get a mapping function

$$y = x \cdot Q(x) + \frac{1}{2}(1 - e^{-2x})\{Q(x) - 1\}$$

valid to a certain degree of approximation. This was not actually done by Anderson and Rhoades. Unless a simple approximate form of the function $Q(x)$ can be found there would be no great point in carrying out the process, for a complex empirical mapping function would have no superiority to the original Drosophila map which itself represents the result of an empirical mapping process.

Haldane's empirical curve got by direct fitting is

$$x = 0.7y - 0.15 \log_e (1 - 2y)$$  (11)

for which

$$\frac{dy}{dx} = 1 - \frac{0.6y}{1 - 1.4y},$$

so that his result is equivalent to assuming $C_0 = \dfrac{0.3}{1 - 1.4y}$

in his differential equation. (11) fits the data fairly well and is successful to the same degree with other data, but the associated form of $C_0$, namely $\dfrac{0.3}{1 - 1.4y}$ is theoretically unsatisfactory. Though it behaves properly for long segments, tending to unity, it implies an excessively high coincidence for short segments, tending to $0 \cdot 3$ as $y \to 0$. De Win-

ton and Haldane (1935) treating their own linkage data on *Primula sinensis* removed this objection by imposing the condition $C_0/y \to$ a constant as $y \to 0$. They took $C_0 = \dfrac{2\,cy}{(1 + 2(c - 1)y)}$, and got

$$x = \int_0^y \frac{dv}{1 - 2C_0v} = \frac{1}{2c(c+1)} \log_e (1 + 2cy) - \frac{c}{2(c+1)} \log_e (1 - 2y).$$

Choosing 2 as an appropriate value of c in *Primula sinensis*, they obtain

$$x = \tfrac{1}{12} \log_e (1 + 4y) - \tfrac{1}{3} \log_e (1 - 2y). \tag{12}$$

Kosambi (1944) made the criticism that this form for $C_0$ is unnecessarily complicated, and without special virtue. For instance taking $C_0 = y/(1 - y)$, Kosambi obtains the integral

$$x = \tfrac{2}{3} \log_e (1 + 4y) - \tfrac{1}{6} \log_e (1 - 2y)$$

which is slightly superior to (12) giving somewhat better consistency for the values of x when applied to the three point Primula data. Consistency is the sole criterion (other than mathematical simplicity) in preferring one mapping function to another, since there is no intrinsic unit of map length, and that formula is the most suitable for which distances are additive to within the limits of significance.

At the same time Kosambi used Haldane's differential equation $\dfrac{dy}{dx} = 1 - 2C_0y$, to derive a very useful approximate mapping function,

$$x = \tfrac{1}{2} \tanh^{-1} 2y.$$

He takes the unknown function $C_0$ to depend in some way on x and to increase steadily. The simplest of such functions is one linear in x and y, and the simplest linear function taking the values 0 and 1 for the extremes of y (0 and $\tfrac{1}{2}$) is 2y.

For this case $\dfrac{dy}{dx} = 1 - 4y^2$

giving $$x = \tfrac{1}{4} \tanh^{-1} 2y = \tfrac{1}{4} \log_e \frac{1 + 2y}{1 - 2y}$$

$$= 25 \log_e \frac{1 + 2y}{1 - 2y} \text{ cm}$$

and $y = \tfrac{1}{4} \tanh 2x$.

Values of map length for given y can be derived by the aid of tables of common or of natural logarithms, or read off directly from the table of Fisher and Yates (1948) for the transformation of the correlation coefficient, taking $2y = r$, $2x = z$. The great advantage of Kosambi's

mapping function is that the corresponding addition theorem for y, is very simple, being

$$y_{1+2} = \frac{y_1 + y_2}{1 + 4y_1y_2}. \tag{13}$$

It has been verified to give a good fit in many cases where three or four point data are available, and lately has been usefully employed by Bhat (1948) in an analysis of Punnett's sweet pea data, and of his own data on *Oryza sativa* (Bhat, 1950). Kosambi finds it to give as good a fit to Haldane's 1919 data as does the curve (11).

Kosambi's formula (13) suggests the introduction of a second co-efficient comparable to the coincidence ratio C, and of similar value as an index of the intensity of interference. K will be called the *Kosambi coefficient* if

$$K = \frac{y_1 + y_2 - y_{1+2}}{4y_1y_2y_{1+2}}.$$

K may be written as $2C/y_{1+2}$ and also as

$$K = 1 + \frac{1 + 4y_1y_2}{4y_1y_2y_{1+2}}\left\{\frac{y_1 + y_2}{1 + 4y_1y_2} - y_{1+2}\right\}$$

Whenever the Kosambi formula (13) is exactly satisfied, K is equal to unity. The Kosambi relation is satisfied precisely if and only if a certain intensity of interference operates uniformly over the length of the chromosome. If, for convenience, interference of this intensity is said to be at the Kosambi level, then a computed estimate of K which is significantly less than unity indicates the operation of interference in excess of the Kosambi level, while K greater than unity shows the interference to be less than the Kosambi level. For interference at the Kosambi level $C = 2y_{1+2}$, and the marginal coincidence is 2y.

## 4. *Interference and the Possibility of Recombination in Excess of 50%*

In the absence of interference, the relation $y = \frac{1}{2}(1 - e^{-2x})$, (or equally Trow's formula) shows that y is always less than 50% and increases steadily towards a limit of 50% (the value for independent segregation) as the length of the segment is indefinitely increased. Since interference modifies the mapping relation $y = m(x)$, there is no longer any theoretical warrant for believing that it is limited in all cases to values less than 50%. Indeed, if interference was complete so that the occurrence of a single cross-over inhibited any further cross-over anywhere on the chromosome, obviously $x = y$, so that the increase of y would keep pace with that of x. Observation suggests, however, that interference is always incomplete, and vanishes over sufficiently long

intervals, so that on a very long chromosome, two loci remote from one another, would exhibit extremely loose linkage and therefore to all intents and purposes would segregate independently showing a recombination of 50% with one another. The possibility is left open for less remote pairs of factors to show recombinations greater than this limiting value. These points were investigated by Jennings (1923) and by Winge (1935). Both writers employed a discrete metric when setting up a model of the chromosome. Jennings' work is especially interesting, since he employed the idea of the serial formation of chiasmata, developed later by Mather on the basis of combined cytological and genetical studies, and employed (independently of Jennings' work) by Fisher *et al.* (1947) and by Owen (1949).

Jennings regards the strand as series of joints, each joint having the same prior probability b of breaking, and $1 - b$ of not breaking. For two genes A,B separated by n joints let $p_r$ be the probability that exactly r of the n joints will break, and write $P(\lambda, n)$ for the generating function $\sum_{r=0}^{\infty} \lambda^r p_r$.

When there is no interference, breaking at one joint is independent of breaking at all other joints. Consequently the generating functions $P(\lambda, n - 1)$, $P(\lambda, 1)$, $P(\lambda, n)$ for the respective intervals $AAn_{n-1}$, $A_{n-1}B$ and $AB$ are connected by

$$P(\lambda, n) = P(\lambda, n - 1) \cdot P(\lambda, 1)$$

Therefore
$$P(\lambda, n) = \{P(\lambda, 1)\}^n = \{1 + (\lambda - 1)b\}^n$$

since
$$P(\lambda, 1) = (1 - b) + \lambda b = 1 + (\lambda - 1)b$$

Hence $P(-1, n) = (1 - 2b)^n$ and $y_{AB} = \frac{1}{2}\{1 - P(-1, n)\}$
$= \frac{1}{2}\{1 - (1 - 2b)^n\}$

Map distance is $x = x_{AB} = nb = \left[\dfrac{d}{d\lambda} P(\lambda, n)\right]_{\lambda=1} =$

$$\frac{b}{\log_e (1 - 2b)} \log_e (1 - 2y).$$

$$P(\lambda, n) = \{(1 - b) + \lambda b\}^n \quad \text{implies}$$

$$p_r = \binom{r^n}{} (1 - b)^{n-r} b^r = \frac{\lfloor n}{\lfloor n - r \rfloor r} (1 - b)^{n-r} b^r,$$

and, in particular, $p_0 = (1 - b)^n$.

If the intervals between the joints are very small so that b is a small number we have, in effect, Haldane's formulae for a continuous metric:

$$P(\lambda,n) \simeq e^{(\lambda-1)nb}; \quad p_r = e^{-nb}(nb)^r/\lfloor r$$

$$y \simeq \tfrac{1}{2}\{1 - e^{-2nb}\}$$

$$x = nb \simeq -\tfrac{1}{2}\log_e (1 - 2y)$$

Also
$$p_0 \simeq e^{-nb} = \sqrt{1 - 2y}$$

$$p_r = \frac{p_0(\log_e p_0)^r}{\lfloor r} = [ -\tfrac{1}{2}\log_e (1 - 2y)]^r \frac{\sqrt{1 - 2y}}{\lfloor r}$$

When there is complete interference, Jennings puts b for the probability of any break when no other break has occurred. The probability of no-break over AB is then $(1 - b)^n$ and therefore the proportion of chromosomes showing a single break is $1 - (1 - b)^n$, which is also the recombination fraction y.

Thus $y = p_1 = 1 - (1 - b)^n$.

Hence for different values of n, y takes values from 0 to 1, and recombinations greater than 50% are shown by all sufficiently long segments.

Jennings considers a model where partial interference obtains. He assumes that the breaks occur in serial order of time, progressing from one extremity of the chromosome to the other. Thus in the numbered series of joints 1,2,3,4, . . . , the fate of number 1 is first determined, then that of 2, that of 3 and so on. He notes that determination proceeding serially in both directions from a common point (in a modern context, from the centromere) would give the same results. He assumes for the interference that when a certain point undergoes a break, then its first $k - 1$ successors undergo no break.

With these assumptions he calculates the probability of exactly r breaks occurring within AB. The formulae are rather complicated and will not be given here. His conclusions are (*a*) For interference of the character he has considered and extending over a map distance of 30cm or less, y is a steadily increasing function of segment length, and never exceeds 50%. (*b*) When interference extends over a greater interval than 30cm, y is an oscillatory function of interval length, and has maximum values greater than 50%. If the interference distance is sufficiently great any value less than 100% may be achieved.

These results necessarily give qualitative information only, since the assumption that interference is complete up to a certain point after which it ceases entirely to act is much too crude and artificial. It is certainly the case that, in general, interference which is complete for short distances, tapers smoothly (even if not monotonically) to zero with increasing range (Muller, 1916; Weinstein, 1918).

Winge also considered a model with breaking at joints. If $b_1$,

$b_2$, $b_3$ . . . are the probabilities of breaking at a series of consecutive joints 1, 2, 3, . . . . such that joint 'i' separates genes $A_i, A_{i+1}$, then the recombination value $y_{A_1 A_{N+1}}$ is, when there is zero interference, given by

$$(1 - 2y_{A_1 A_{N+1}}) = (1 - 2b_1) (1 - 2b_2), \ldots (1 - 2b_N)$$

which is the generalized Trow's formula for N intervals.
Hence

$$y_{A_1 A_{N+1}} = \Sigma b_1 - 2\Sigma b_1 b_2 + 2^2\Sigma b_1 b_2 b_3 - \ldots + (-2)^{N-1} b_1 b_2 \ldots b_N . \ (14)$$

To take account of interference Winge assumes that a cross-over occurring in one joint reduces by a prescribed amount the frequency of breaks in its first, second, third nearest neighbor, etc., and in formula (14) appropriately reduces each product term $b_1 b_b \ldots b_k$. For instance, he takes a model in which the interference is 80% between nearest neighbors, 40% between next nearest, 20% between second next neighbors and so on, the interference falling by a factor of 2 at each stage. By 80% interference, it is meant that a break in the one joint reduces to 0.2 of its *a priori* value the probability of a break in the other joint. For this model, a maximum recombination value of 50.75% is attained for genes at a separation of about 120-130cm.

For a model in which interference rises and falls in a rhythmic manner according to the scheme 80%, 40%, 20%, 0%, 20%, 40%, 80%, 40% and so on, the highest value of y is 55.2%, attained on a segment 110-120cm in length. In each case y falls steadily to 50% as the segment length is further increased.

This result is interesting when considered in conjunction with those of Jennings. Interference of sufficient intensity but operating in different ways is seen to give rise to recombinations in excess of 50%. Winge's model is, however, like that of Jennings, too crude to have other than suggestive value.

### III. INTERFERENCE: THE CYTOLOGICAL AND GENETICAL EVIDENCE

#### 1. *Introductory Remarks*

The recombination of genetical factors is the result of the breakage and rejoining with crossing-over of the chromatids of paired chromosomes in meiosis, and is associated therefore with the formation of chiasmata. The evidence on which this conclusion is based, is strong and plentiful, and is summarized in Mather's review, "Crossing Over" (1938) to which the reader may be referred for a discussion of the cytology of recombination, and for many references to cytological literature which will be omitted here. Another useful survey of the

question is Darlington's article "The Time, Place and Action of Crossing-over" (1935).

In Section II, a certain deliberate vagueness of nomenclature and description was maintained, as it was desired to develop the subject initially within a formal genetic framework without reference to the cytological mechanism. Crossing-over was therefore discussed with the minimum of cytological reference, as if in fact it took place between whole chromosomes in the way envisaged by the original chiasmatype theory of Janssens. The terms *strand* and *chromosome* were used (inaccurately) as synonyms. For some of the purposes of the geneticist, this is a completely adequate viewpoint, since he is usually concerned only with happenings on a single strand. It is hardly possible however to construct a general mathematical theory of recombination without calling on cytological findings to guide us in our choice of initial assumptions or axioms.

## 2. *Four Strand Crossing-over*

Genetically it has been shown in Drosophila, Zea, and Habrobracon that crossing-over must occur at the four strand stage, after the homologous chromosomes have each divided into two chromatids. (*Strand* and *chromatid* will be employed indifferently as synonyms). Each of the homologues is double at the time of crossing-over and consists of two sister strands. On the partial chiasmatype theory (Belling 1931, 1933; Darlington 1930, 1931), which is probably the correct view, chiasmata appear as soon as the chromosomes divide into halves, and crossing-over must occur practically simultaneously with this division of each chromosome into two chromatids, just after the end of pachytene. Each crossover is associated with a chiasma figure, a breakage of one strand, on Darlington's theory, inducing a break in one of the remaining three strands. Rejoining takes place in a criss-cross fashion, with exchange of partners. The two chromatids of each of the paired chromosomes are involved unequally, one crossing over and the other staying intact. Thus in each chiasma only two and not all four of the strands are involved. It follows from this that if r is the mean number of chiasmata formed on a particular segment, the corresponding amount of genetical crossing-over is $\frac{1}{2}$r, *i.e.*, the map length of the segment is 50r cm.

Implicit in this last deduction is the assumption that sister strand crossing-over does not occur, for if it did, a mean frequency of one chiasma would correspond to less than 50 cm map distance, since a chiasma involving the two chromatids derived from the same chromosome would be genetically undetectable in a normal diploid. As a result

of work on the attached X or ring X varieties of Drosophila, it appears highly probable that sister strands do not cross over ( L. V. Morgan 1933; Weinstein 1936). From the standpoint of formal genetics of ordinary diploids its occurrence or non-occurrence is of no importance. It becomes a question of interest only when it is desired to correlate cytologically observed chiasmata with genetically detectable exchanges of material. It will be assumed in the sequel that crossing-over is always between non sister strands so that every cytological chiasma corresponds to a genetically detectable cross-over.

With four strand crossing-over, interference may be of two kinds. The first is known as *chiasma interference,* and is a reduction of the probability of chiasma formation in the neighborhood of a chiasma already established. *Chromatid interference* is a non-random relationship between those two of the strands which cross-over at a second chiasma with respect to the two crossing over at the first. Chiasma interference may be detected by a study of the distribution of the chiasmata themselves, since it is a property of the chiasma as a whole and not of the chromatids involved. Haldane (1931) noted that in the absence of chiasma interference, the relative frequency of bivalents showing r chiasmata should be the *rth* term of a Poisson series

$$e^{-m} \, m^r / \lfloor r$$

in which $m = \bar{r}$ is the mean number of chiasmata on a bivalent. In this case $V(r)$, the variance of r, would be equal to m or $\bar{r}$. Positive interference, on the other hand, would show itself by a greater clustering of the observed values of r about $\bar{r}$. That is, by an inequality $V(r) < \bar{r}$. In all cases examined this latter type of relation has been verified, the variance of chiasma frequency distribution being often as low as $\frac{1}{4}$ or $\frac{1}{6}$ of the mean. Parallel evidence of genetical interference of the same order of intensity has been obtained by experiments in which the whole of a chromosome has been marked out by a net of mutant genes allowing of a complete study of recombination in the chromosome concerned. (Mather 1933).

Chromatid interference is more difficult to detect cytologically. Whereas chiasma interference is universally found, evidence of strong chromatid interference is much rarer. The cause of chromatid interference might be inequality of the tensile or torsional strength of the strands, or the fact that when a strand has been involved in a chiasma the tension along it is in consequence somewhat reduced, so that a second break is rendered less likely. Possibly both factors operate.

A point of interest is that with four strand crossing-over chiasma

interference is by itself incapable of producing genetical recombination values in excess of 50%. In an intuitive way this might be conjectured to be the case, since without interference any one strand has an even chance of being involved or not in a particular chiasma, and therefore the chances of an odd or even number of exchange points being formed on a long interval of the strand are equal. Arguing more precisely, it is easy to see that in the absence of interference, the probability that, in a set of n chiasmata, a strand will be involved in exactly r cross-overs is the coefficient of $\lambda^r$ in the generating function $(\frac{1}{2} + \frac{1}{2}\lambda)^n$ i.e., the binomial term $\frac{1}{2}_n \binom{n}{r}$. (For on each of the n occasions it has a chance $\frac{1}{2}$ of being involved or not involved). Let now $a_0, a_1, a_2, \ldots$ be the probabilities of formation of $0, 1, 2, \ldots$ chiasmata on any segment of the bivalent. Then the frequency of occurrence of a strand with r cross-overs is the coefficient of $\lambda^r$ in the generating function

$$P(\lambda) = \sum_{n=0}^{\infty} a_n \, (\tfrac{1}{2} + \tfrac{1}{2}\lambda)^r = a_0 + \sum_{n=1}^{\infty} a_n \, (\tfrac{1}{2} + \tfrac{1}{2}\lambda)^n$$

As usual the recombination over the segment is

$$y = \tfrac{1}{2}\{1 - P(-1)\}$$

which equals $\frac{1}{2}(1 - a_0)$, since $P(-1) = a_0$.

Consequently, if on a long arm the probability of no chiasmata tends to a limit $a > 0$, then $y \to \frac{1}{2}(1 - a)$. This proof, though written more compactly, is the same as that given by Mather (1938) and by other writers, but a different treatment is given by Emerson and Rhoades (1933).

It follows that genetical recombination in excess of 50% is evidence that some chromatid interference is operating, with or without chiasma interference. Such values have been reported in mice for the factors dilute and wavy (Fisher and Mather 1936a; 1936b), and for sex with both shaker and wavy (Wright 1947). Other cases are those of Wellensiek (1929) in Pisum and Clausen (1926) in Viola. For a given degree of chiasma interference the recombination may exceed 50% on some long segments if the chromatid interference is above a certain level of intensity, but will not do so if there is chromatid interference which is below this level. As far as a single strand is concerned chromatid and chiasma interference need not be separated for most purposes, but have their effects jointly subsumed in the joint probability distribution of the points of exchange on the strand.

### 3. *Interference Across the Centromere*

Most of the evidence, both genetical and cytological, demonstrates independence of the two arms of a chromosome separated by a centromere (Mather 1936). The centromere may therefore be taken for theoretical purposes as an insulator suppressing all or almost all interference between the arms.

Graubard (1932) and Schweitzer (1935) claimed to have found positive interference across the centromere in chromosome II of Drosophila, but Stevens (1936) showed that their conclusions were incorrect, being based on an incorrect estimate of coincidence (*c.f.* Section II.2.*b*). Genetical evidence showing negative interference in regions close to the centromere in chromosome III of Drosophila has been summarized by Kikkawa (1935). A further result on this chromosome has been obtained by Pätau (1941), analysing data of Gowen (1919) by Steven's method, and shows that interference is negative for regions *se-D* and *ss-e* separated by a region containing the centromere, but is positive between *se-D* and *e-ro* which are separated by a larger region containing the centromere. This seems to be the only example of positive genetical interference operating across the centromere. In any case, positive or negative, it is much weaker than that within the arms.

The only other organism for which there is genetical evidence of interference is Neurospora, where Newcombe's analysis (1941) of data of Lindegren and Lindegren (1937) demonstrated negative interference.

The cytological evidence has agreed with genetical findings. Independence of the arms has been shown in *Fritillaria chitralensis* (Bennett 1938) and in *Uvularia perfoliata* (Barber 1941). In other species which have been examined any significant degree of interference which has been observed is negative (Callan 1940 in Anilocra; Newcombe 1941 in Trillium). The exception has been two species of Diptera where, in *Culex pipiens* and *Dicranomyia trinotata*, Pätau found positive interference across the centromere. This was confirmed for Culex by Callan and Montalenti (1947). However, a different interpretation is possible.

### 4. *Chiasma Formation as a Serial Process*

Mather (1936, 1937), on analysis of both genetical and cytological data, concluded that the formation of chiasmata upon a chromosome arm does not occur in random order, but is a serial process in which the centromere plays the determining role.

The spatial distribution of chiasmata can be explained on the assumption that they are formed in a regular time sequence commencing at the centromere. The first or proximal chiasma on the arm forms at

a mean (cytological) distance from the centromere (the *differential distance*), which is correlated with the length of the chromosome arm. The second chiasma forms at a distance from the first which is probably constant throughout the chromosome complement of the organism. This latter distance (the *interference distance*) is not the same in size as the differential distance. Chiasma formation therefore depends on two parameters, namely the differential and interference distances.

The curves of chiasma frequency plotted against (cytological) chromosome length in organisms whose chromosomes have a large range of size, are in the form of an ascending parabola with a flat lower end, where the chiasma frequency approximates to unity, no matter what the size of the chromosome, and an upper sloping portion where the chiasma frequency increases approximately linearly with chromosome length. The character of this curve can be explained as depending on (a) the formation of a first chiasma irrespective of the length of the chromosome but occupying a distance adjacent to the centromere which is positively correlated with the length of the chromosome arm, and (b) the formation of a second and subsequent chiasmata, each at a mean distance from its direct predecessor dependent on interference and constant throughout the chromosomes.

On Mather's theory therefore the proximal chiasma is the first in point of time to be formed and is characterised by a distribution curve $g(u,T)$. Here u is cytological distance from the centromere and T the cytological length of the arm. The probability of the first chiasma being found in du is $dp = g(u,T)du$. The differential distance is the mean length $\bar{u}_D = \int_0^T ug(u,T)du \div \int_0^T g(u,T)du$. Mather takes the view that the formation of a first chiasma is almost certain, so that $\int_0^T g(u,T)du \doteqdot 1$ and $\bar{u}_D$ is approximately equal to the first moment $\int_0^T ug(u,T)du$.

If $X_1$, $X_2$, $X_3$ . . . are the chiasmata, successively established at increasing distances $u_1$, $u_2$, $u_3$, . . . from the centromere, then $X_r$ has a distribution, conditional on that of $X_{r-1}$, given by

$$dp = f(u_r - u_{r-1})\, du_r,$$

and the interference distance $E(X_{r-1}X_r)$ is

$$\bar{u}_1 = \int_0^T uf(u)du \div \int_0^T f(u)du$$

Mather's theory is confirmed by analysis of data on the cytological distribution of crossing-over in *D. melanogaster* (Mather 1936). Some kind of localization of exchange points has to be assumed to explain the differential effects of environmental factors, such as temperature, x-rays,

etc. on crossing-over in various parts of the chromosomes. (Plough 1917; Graubard 1932; Muller 1925). The region near the centromere is affected more than the distal ones.

A comparison of cytological and genetical maps shows little crossing-over near the centromere and more in the distal regions, whether the chromosome is one or two armed. These facts suggest that the centromere plays a determining role in crossing-over, and are exactly what is to be expected on the hypothesis of serial formation, which would imply minimum crossing-over and maximum sensitivity for the centromeric region.

In a number of cases interference has been found to be lowest at the centromere and to increase steadily becoming largest at the distal end. Anderson and Rhoades (1931) found this for the X chromosome in Drosophila, using the quotient Q to measure the interference (Section II.3). Mather points out that this variation disappears if we transform map distance into cytological distance, so that interference is uniform on the cytological map, as it should be on the serial hypothesis.

Using transformation equations based on the binomial $(\frac{1}{2} + \frac{1}{2}\lambda)^n$ (compare Section III.2) where n is the number of chiasmata, multipoint crossing-over data may be converted into chiasma frequency data (assuming chromatid interference is negligible). (Mather 1936). With the centromere as origin it is then possible to determine the cytological distribution curves of the first two or three chiasmata $X_1$, $X_2$, $X_3$, ... Besides deriving the form of g(u,T), the conditional distribution function f(u) can be obtained. g(u,T), f(u) come out as unimodal curves. It is found that the differential distance is variable between chromosomes, its magnitude being a direct function of chromosome length, while the interference distance appears to be substantially constant within and between chromosomes (Mather, 1936, *cf.* Schweitzer 1935). Thus the serial hypothesis is confirmed.

## IV. A GENERAL MATHEMATICAL THEORY OF RECOMBINATION

### 1. *Long Chromosome Arms*

Mather's theory of the serial formation of the chiasmata affords the basis on which a general theory of recombination of wide applicability may be based. (Fisher *et al.* 1947, Owen 1947).

It is convenient to start by considering long chromosome arms, and to make the following assumptions.

(*a*) The two arms of a bivalent are independent.

(*b*) There exists a metric t (or u) (called the *interference metric*)

in terms of which any strand of the tetrad may be mapped. The centro-mere is taken to be the locus t = 0.

(c) The frequency distribution of points of exchange upon a strand of metrical length T is the same as that upon the proximal segment (O,T) of a strand of infinite length over which t ranges from 0 to ∞. (This assumption is subject to later modification).

(d) Points of exchange are formed in serial order starting at the centromere.

(e) Chiasma and chromatid interference and bivalent competition need not be considered separately but may be regarded as having their effects subsumed in the functions g(u), f(u) to be defined below.

(f) *Differential distance.* The probability that the first exchange point $E_1$ be formed in the interval du situated at distance u from the centromere is dp = g(u)du.

(g) *Interference distance.* If $E_r$ is the $rth$ point of exchange the probability (conditional on the establishment of $E_{r-1}$ in dv) of the establishment of $E_r$ in dv, at distance v from the centromere, is

$$dp = f(v - u)dv = f(w)dw,$$

where w, the distance $E_{r-1}E_r$, is the $rth$ intercept length. (This assumption is equivalent to the statement that all intercept lengths except the first $(OE_1)$ are distributed independently with the same distribution dp = f(w)dw).

These assumptions enable the calculation of all the quantities of genetical interest in terms of the unknown functions g(u) and f(u). It is convenient to introduce a further convention as follows:

(h) The metric is so chosen that the mean intercept length, $\bar{u} = \int_0^\infty uf(u)du$ is equal to unity. That is to say, the interference distance is 1 unit. This can always be done by transforming the metric by means of a multiplicative constant, and has the desirable result of making the map distance approximate asymptotically to the metric when both are large.

(i) Lastly we suppose that local interference is complete, *i.e.* $f_1(0) = 0$.

In the mathematical development various auxiliary quantities have to be introduced.

Define by dp = $g_r(u)$ du the distribution of $E_r$, and by dp = $f_r(u)du$ the distribution of $E_{r+1}$ conditional on the establishment of $E_1$.

Then, by (f) and (g),

$$f_r(u) = \int_0^u f_{r-1}(v)f(u-v)dv, \tag{15}$$

$$g_r(u) = \int_0^u g_{r-1}(v)f(u-v)dv, \tag{16}$$

$$g_1(u) = g(u).$$

Defining the generating functions

$$G(\lambda,u) = \sum_{r=0}^{\infty} \lambda^{r-1} g_r(u); \quad F(\lambda,u) = \sum_{r=0}^{\infty} \lambda^{r-1} f_r(u),$$

we have, by (15) and (16),

$$G(\lambda,u) = g_1(u) + \int_0^u F(\lambda,u-v)g_1(v)dv,$$

$$F(\lambda,u) = f_1(u) + \int_0^u F(\lambda,u-v)f_1(v)dv.$$

The expected density of exchange points is clearly $G(1,u)$ and is therefore

$$E(u) = \frac{dx}{du} = g_1(u) + \int_0^u F(1,u-v)g_1(v)dv.$$

If $x(t_1, t_2)$ denote the map length of the segment $(t_1, t_2)$, then

$$x(t_1, t_2) = \int_{t_1}^{t_2} G(1,u)du.$$

Denote by $p_r(t)$ the probability of exactly $r$ exchange points occurring in the proximal segment $(0,t)$, and let $P(\lambda,t)$ denote the corresponding generating function $\sum_{r=0}^{\infty} \lambda p_r(t)$.

Then it may be shown that

$$\frac{d}{dt} p_r(t) = g_r(t) - g_{r+1}(t); \quad \frac{d}{dt} p_0(t) = -g(t)$$

and

$$P(\lambda,t) = 1 + (\lambda - 1) \int_0^t G(\lambda,u)du$$

If $x(t) = x(0,t)$ is the map length from the centromere and $y(t)$ the recombination with the centromere then

$$x(t) = \int_0^t G(1,u)du$$

$$y(t) = \int_0^t G(-1,u)du$$

To proceed further we need to particularize slightly. In any case realism requires that interference be assumed to be zero over long ranges. Hence on the infinite strand every exchange point has at least one successor, so that $U_1 = \int_0^\infty f(u)du = 1$. Also at large distance $f(u)$ must tend to its non-interference value which is the Poisson term $ke^{-kt}$. Apart from these restrictions $g(u)$ and $f(u)$ have to be chosen in some way or other to fit empirical findings and cannot be determined theoreti-

cally. For qualitative investigation it is adequate to start by taking $g(u) = f(u)$.

Under these conditions it may be proved that as $t \to \infty$,

(a) $y(t) \to \frac{1}{2}$

(b) $x(t) \approx t - \frac{1}{2}I$

(c) $V(r) \approx (1 - I)x(t)$

where $V(r)$ is the sampling variance of the number of exchange points r observed on the segment $(0,t)$, and I is the quantity $1 - V_t$, where $V_t = \int_0^\infty (u - 1)^2 f(u) du$ is the variance of the curve $f(u)$.

It will be seen from (c) that I is a measure of the amount of concentration of the values of r about the mean $\bar{r} = x(t)$ over and above the Poisson value (Compare Section III.2) and so is a measure of the mean intensity of interference. (b) shows that I is a measure of the defect by which the map distance falls short of the metric in long segments. When there is zero interference x and t will be identical. I is therefore a convenient index of the average effect of the interference. This is a natural conclusion in view of the fact that $I = 0$ corresponds to a unit value of $V_t$ when $f(u)$ has its maximum degree of dispersion (subject to the restrictions already imposed on the first two moments) while $I = 1$ corresponds to $V_t = 0$, *i.e.* to maximum concentration of the interference curve and complete local interference, though not to complete interference in the sense of Section II.4.

It is worthwhile considering the logical status of the familiar result (a) $y \to \frac{1}{2}$, which has appeared again in the present context. It may be shown that this holds in virtue only of the fact that $\int_0^\infty f(u) du = 1$. In general if $\int_0^\infty f(u) du = c$, then as $t \to \infty$, $y(t) \to 1/(1 + c)$ (Owen 1949). $c = 1$ is equivalent to the assumption that every exchange point on a long arm has at least one successor, which in turn is the same as saying that interference is vanishing at long distances.

For qualitative investigation the family of curves

$$f_b(u) = \frac{a + 1}{a - 1} \left\{ e^{-u\left(\frac{a+1}{a}\right)} - e^{-u(a+1)} \right\} \quad (1 \leqslant a < \infty)$$

has useful properties.

$$V_t = \frac{a^2 + 1}{(a + 1)^2} = 1 - \frac{1}{b} = 1 - I$$

where

$$b = \frac{1}{I} = \frac{1}{1 - V_t} = \frac{(a + 1)^2}{2a};$$

and $2 \leqslant b < \infty$ for $a \geqslant 1$, while the corresponding range of I is $\frac{1}{2} \geqslant I > 0$.

Rather simple formulae are got for x and y, namely:

$$x(t) = t - \frac{1}{2b} + \frac{1}{2b} e^{-2bt},$$

$$y(t) = \begin{cases} \frac{1}{2} - \frac{1}{2}e^{-bt}\{\cosh \omega_1 t + \frac{b}{w} \sinh \omega_1 t\} & , b \geqslant 4; 0 \leqslant I \leqslant \frac{1}{4}. \\ \frac{1}{2} - (2t + \frac{1}{2})e^{-4t} & , b = 4; I = \frac{1}{4}. \\ \frac{1}{2} - \frac{1}{2}e^{-bt}\{\cos \omega_2 t + \frac{b}{w} \sin \omega_2 t\} & , 2 \leqslant b \leqslant 4; \frac{1}{4} \leqslant I \leqslant \frac{1}{2}. \\ \frac{1}{2} - \frac{1}{2}e^{-2t}(\cos 2t + \sin 2t) & , b = 2; I = \frac{1}{2} \end{cases}$$

where $\omega_1 = \sqrt{b^2 - 4b}$, $\omega_2 = \sqrt{4b - b^2}$.

Table 2 shows $x(t)$, the map distance from the origin for b = 2,4, and $\infty$ (*i.e.*, I = $\frac{1}{2}$, $\frac{1}{4}$, and 0).

It will be seen that for b $\geqslant$ 4, *i. e.* $0 \leqslant I \leqslant \frac{1}{4}$, y (t) behaves 'normally' rising steadily to a limiting value of $\frac{1}{2}$ at t = $\infty$. But for all curves of this family with greater intensities of interference ($\frac{1}{4} \leqslant I \leqslant \frac{1}{2}$), y(t) rises above 50% and then oscillates indefinitely about the 50% level. Hence for sufficiently intense interference on long enough arms the recombination fraction may be expected to show oscillations and have some maxima and minima on either side of the 50% level. In particular there may be pairs of loci which, though linked, show exactly 50% recombination and apparently segregate independently.

The following table shows the positions of the earlier maxima and minima for I = $\frac{1}{2}$, when we have the confluent form f(u) = $4ue^{-2u}$.

## TABLE 1

Maxima and Minima of Recombination Fraction When f(u) = $4ue^{-2u}$ (I = ½)

| t | x(t) | Maxima of y(t) | Minima of y(t) |
|---|---|---|---|
| ½π | 158.16cm | 52.161% | |
| π | 289.21cm | | 49.907% |
| π | 446.24cm | 50.004% | |
| 2π | 603.32cm | | 50 − 1.73 × 10⁻⁴% |

Since chromosome lengths above 200cm are rare, much reality need not be attached to the later entries, but the first maximum, nevertheless, may be observable, though its position and magnitude will be modified by the precise degree of interference operating.

It need hardly be stressed that there is no theoretical basis for the exponential form of f(u) or the particular one $4ue^{-2u}$.

## TABLE 2

Map Distance From Centromere as Function of Metric for
Various Degrees of Interference

| t | x(t) in cm. I = ½ | x(t) in cm. I = ¼ | x(t) in cm. I = 0 |
|---|---|---|---|
| 0.00 | 0.000 | 0.000 | 0 |
| 0.05 | 0.468 | 0.878 | 5 |
| 0.10 | 1.756 | 3.117 | 10 |
| 0.15 | 3.620 | 6.265 | 15 |
| 0.20 | 6.233 | 10.024 | 20 |
| 0.25 | 9.197 | 14.192 | 25 |
| 0.30 | 12.530 | 18.634 | 30 |
| 0.35 | 16.165 | 23.260 | 35 |
| 0.40 | 20.047 | 28.010 | 40 |
| 0.45 | 24.132 | 32.842 | 45 |
| 0.50 | 28.383 | 37.729 | 50 |
| 0.60 | 37.518 | 47.604 | 60 |
| 0.70 | 46.520 | 57.546 | 70 |
| 0.80 | 56.019 | 67.521 | 80 |
| 0.90 | 65.683 | 77.509 | 90 |
| 1.00 | 75.485 | 87.504 | 100 |
| 1.10 | 85.307 | 97.502 | 110 |
| 1.20 | 95.208 | 107.501 | 120 |
| 1.30 | 105.138 | 117.500 | 130 |
| 1.40 | 115.092 | 127.500 | 140 |
| 1.50 | 125.062 | 137.500 | 150 |
| 1.60 | 135.041 | 147.500 | 160 |
| 1.70 | 145.028 | 157.500 | 170 |
| 1.80 | 155.019 | 167.500 | 180 |
| 1.90 | 165.013 | 177.500 | 190 |
| 2.00 | 175.009 | 187.500 | 200 |
| 2.10 | 185.006 | 197.500 | 210 |
| 2.20 | 195.003 | 207.500 | 220 |
| 2.30 | 205.001 | 217.500 | 230 |
| 2.40 | 215.000 | 227.500 | 240 |
| 2.50 | 225.000 | 237.500 | 250 |

## TABLE 3

Percentage Recombination Fraction Over $(t_1, t_2)$

| Interval $(t_2 - t_1)$ | $y_{12}$ $(t_1 = 0)$ | $y_{12}$ $(t_1 = 0.4)$ | $y_{12}$ $(t_1 = 0.8)$ | $y_{12}$ $(t_1 = 1.2)$ |
|---|---|---|---|---|
| 0.00 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.05 | 0.468 | 4.073 | 4.800 | 4.947 |
| 0.10 | 1.747 | 8.237 | 9.548 | 9.813 |
| 0.15 | 3.667 | 12.403 | 14.167 | 14.523 |
| 0.20 | 6.078 | 16.495 | 18.598 | 19.023 |
| 0.25 | 8.847 | 20.451 | 22.793 | 23.266 |
| 0.30 | 11.858 | 24.224 | 26.720 | 27.224 |
| 0.35 | 15.015 | 27.781 | 30.358 | 30.878 |
| 0.40 | 18.231 | 31.094 | 33.691 | 34.215 |
| 0.45 | 21.439 | 34.148 | 36.714 | 37.232 |
| 0.50 | 24.583 | 36.936 | 39.431 | 39.934 |
| 0.60 | 30.507 | 41.709 | 43.971 | 44.427 |
| 0.70 | 35.754 | 45.451 | 47.409 | 47.805 |
| 0.80 | 40.204 | 48.257 | 49.883 | 50.211 |
| 0.90 | 43.829 | 50.252 | 51.549 | 51.811 |
| 1.00 | 46.663 | 51.574 | 52.565 | 52.765 |
| 1.10 | 48.781 | 52.356 | 53.078 | 53.224 |
| 1.20 | 50.282 | 52.726 | 53.220 | 53.320 |
| 1.30 | 51.268 | 52.795 | 53.104 | 53.166 |
| 1.40 | 51.847 | 52.659 | 52.823 | 52.857 |
| 1.50 | 52.113 | 52.393 | 52.450 | 52.462 |
| 1.60 | 52.154 | 52.059 | 52.039 | 52.035 |
| 1.70 | 52.039 | 51.699 | 51.630 | 51.617 |
| 1.80 | 51.830 | 51.347 | 51.250 | 51.230 |
| 1.90 | 51.569 | 51.023 | 50.912 | 50.890 |
| 2.00 | 51.292 | 50.738 | 50.627 | 50.604 |
| 2.10 | 51.021 | 50.499 | 50.394 | 50.373 |
| 2.20 | 50.773 | 50.306 | 50.212 | 50.193 |
| 2.30 | 50.556 | 50.157 | 50.076 | 50.060 |
| 2.40 | 50.374 | 50.047 | 49.981 | 49.967 |
| 2.50 | 50.227 | 49.969 | 49.917 | 49.907 |
| 3.00 | 49.916 | 49.888 | 49.882 | 49.881 |

The choice of the exponential form is to be regarded as a mere computational device leading to formulae in terms of elementary functions. It will be shown later that the confluent form $4ue^{-2u}$, may have a general utility, because it corresponds to an intensity of interference approximately equal to the Kosambi level, and therefore in many organisms may represent a suitable approximate form of the interference function. It also happens to be a close mathematical mimic of the function $\frac{d}{du} \left\{ - \text{sech } \frac{\pi u}{2} \right\}$, which was selected by Fisher on analogy with the Kosambi mapping function, and employed with some success by Fisher *et al.* in their treatment of the sex chromosomes in the house mouse (*loc. cit.*). A mathematical model of the sex chromosome was set up of the kind put forward here, and it was shown that the system of recombinations sex $-$ wv$_2$ = 56.07%, sex $-$ sh$_2$ = 56.73%; wv$_2$ $-$ sh$_2$ = 31.06% (each with a standard error of about 2.3%) could be explained if the factors sh$_2$ and wv$_2$ were situated at respective distances of about 85cm, 120cm from the sex determining portion.

For a general segment $(t_1, t_2)$ a slightly more complicated theory applies. If the simplification $g(u) = f(u)$ is made, the relevant generating functions are

$$P(\lambda,t_1,t_2) = 1 + (\lambda - 1) \int_{t_1}^{t_2} F(\lambda,t_1,u)\,du,$$

$$F(\lambda,t_1,u) = F(\lambda,u) + (1 - \lambda) \int_0^{t_1} F(1,v)\ F(\lambda,u - v)\,dv,$$

and we have $\quad y(t_1 t_2) = \int_{t_1}^{t_2} F(-1,t_1,u)\,du.$

There is no addition theorem for $y$.

With the confluent form $4ue^{-2u}$, we get

$$y(t_1,t_2) = \tfrac{1}{2} - \tfrac{1}{2}e^{-2t_2}\left\{ e^{2t_1} \cos 2(t_2 - t_1) + e^{-2t_1} \sin 2(t_2 - t_1) \right\}$$

which depends not only on the metrical length $t_2 - t_1$ of the segment but on its position. Table 3 shows the percentage recombination fraction over $(t_1, t_2)$ calculated by this formula for a range of lengths $(t_2 - t_1)$, and for four positions of the initial terminus $t_1$ (at the centromere and at $t_1 = 0.4, 0.8,$ and $1.2$).

## 2. *Arms of Short or Medium Length*

The treatment of the previous section implies for an arm of metrical length T, that the expected density of exchange points

$$E(u) = x'(u) = F(1,u) = 1 - e^{-2bu}$$

starts from an initial value of zero at the centromere and rises steadily to a terminal value of $1 - e^{-2bT}$ which, T being assumed large, is nearly unity. The map length of the arm is very nearly $T - \frac{1}{4}I$, which simulates the length-chiasma frequency relationship of Mather, but is too low. This is because we have not arranged for the arm to have an obligatory chiasma. Otherwise our model is in general qualitative agreement with the ideas of Mather. The large and approximately uniform density of exchange points near the terminus, agrees with the hypothesis that the determining role in chiasma formation is played by the centromere, practically to the exclusion of the terminus.

A device by which the calculus as set up may be modified for a finite arm is to apply an argument of selection of cases. With function g(u) and f(u) as before, we calculate the probability of any particular situation on the segment (0,T) of a hypothetical infinite arm *conditional on there being no points of exchange on the segment* (T, ∞). The conditional probability thereby obtained is then taken to be the actual probability of the given situation occurring on the actual arm of metrical length T.

Mathematically this leads to quite simple formulae.
The generating functions $P\ (\lambda,t_1,t_2)$ and $F\ (\lambda,t,u)$ are now replaced by

$$P(\lambda,t_1,t_2,T) \quad \text{and} \quad F(\lambda,t,u,T) \quad \text{where}$$

$$P(\lambda,t_1,t_2,T) = 1 + (\lambda - 1) \int_{t_1}^{t_2} F(\lambda,t_1,u,T)du$$

$$\text{and} \quad F(\lambda,t,u,T) \text{ is defined by}$$

$$F(\lambda,t,u,T) = F(\lambda,t,u) \frac{\Omega(T - u)}{\Omega(T)},$$

where $\quad \Omega(T) = e^{-kt}\{1 + \int_0^t e^{kv}F(1,v)dv\}.$

On inserting special values for f(u) it is found that the oscillations of y(t) with maxima in excess of 50% may still occur within the length of an arm if T be sufficiently large. It is found that with the Kosambi level of interference recombinations between the terminus and an interior locus may be as great as 60%, and between the centromere and some loci as great as 55%.

So far a greater number of detailed results have been worked out for the simplified case when $g(u) = f(u)$. This gives a chiasma-frequency-chromosome length curve of the correct parabolic shape but makes the frequency low at the neighborhood of the centromere. It may therefore need revision when the cytological evidence for an obliga-

tory chiasma on short chromosomes is further strengthened. This adjustment merely entails the insertion of an appropriate form for the differential segment function $g(u)$. This form will probably need to be made explicitly dependent on the parameter T.

The formulae obtained when $g(u) = f(u) = 4ue^{-2u}$ (and $k = 2$) are of some interest. We have

$$x_{12} = \left[ u \tanh 2\,T + \frac{\cosh 2(T - 2u)}{4 \cosh 2\,T} \right]_{t_1}^{t_2}$$

$$1 - 2y_{12} =$$
$$\frac{\cosh 2(T - t_2 + t_1) \cos 2(t_2 - t_1) + \sinh 2(T - t_2 - t_1) \sin 2(t_2 - t_1)}{\cosh 2\,T}$$

For a given value of $t_1$, the functional relationship between the x and y of the segment may be calculated, though not in finite terms. For short segments we have (Section II.3)

$$x = y + \int_0^y 2\,C_0\,v dv$$

where $C_0$ is the marginal coincidence.
If $t_1 = 0$, the initial terms come out as $x = y + \frac{1}{3}\,y^2 + \ldots$
Elsewhere, for $t_1 > 0$ the initial terms are

$$x = y + \frac{4}{3}\,y^3 \frac{\cosh^2 2T}{\{\sinh 2T - \sinh 2(T - 2t)\}^2} + \ldots$$

and the marginal coincidence is

$$\frac{2y \cosh^2 2T}{\{\sinh 2T - \sinh 2(T - 2t)\}^2}$$

It will be seen that as $t_1$ is moved out from the centromere, the marginal coincidence, having the high value of $\frac{1}{3}$ at the centromere, at first falls sharply to a smaller value, and then diminishes steadily towards

a terminal value of $\frac{y}{2 \tanh^2 2T}$. At mid-arm $t_1 = \frac{1}{2}T$, and

$$C_0 = \frac{2y}{\tanh^2 2T}$$

For large values of T, this approximates to the value $2y$, which is precisely the Kosambi level. The initial terms of $x = x(y)$ are then

$x = y + \frac{4y^3}{3} +$ as in the Kosambi mapping function (II.2). Hence in the middle of long arms $f(u) = 4ue^{-2u}$ represents interference at the Kosambi level.

### 3. *Frequencies of Modes of Gamete Formation*

Given any set of n linked loci $t_1, t_2, \ldots, t_n$ it is possible in general theory to specify completely the relative frequencies of the $2^{n-1}$ distinct modes of gamete formation.

If $p_{r_1 r_2 \ldots r_{n-1}}$ is the joint probability of there being exactly $r_1$, $r_2$, $\ldots$, $r_{n-1}$ points of exchange in the segments $(t_1, t_2), \ldots (t_{n-1}, t_n)$, a generating function $P(\lambda_1, \lambda_2, \ldots, \lambda_{n-1}; t_1, t_2, \ldots, t_n)$
is constructed, and is a function of the n metrical values $t_1, t_2, \ldots$, $t_n$ as well as the $(n-1)$ dummies $\lambda_1, \lambda_2, \ldots, \lambda_{n-1}$.

The relative frequencies of the modes of gamete formation are derived from the $2^{n-1}$ special values of P got by taking for the $\lambda$'s all possible combinations of the two values $+1$ and $-1$. For example, given three genes $ABC$ in one chromosome and alleles $abc$ in the homologue, then every result of meiosis is a gamete of one of the four pairs of types

| $ABC$ | $ABc$ | $aBC$ | $AbC$ |
| $abc$ | $abC$ | $Abc$ | $aBc$ |

If $\pi_1$, $\pi_2$, $\pi_3$, $\pi_4$, denote the relative frequencies of these pairs of types, and $P_1$, $P_2$, $P_3$, $P_4$ stand for the quantities

$$P(\ 1,\ 1) \qquad\qquad P(\ 1,-1)$$
$$P(-1,\ 1) \qquad\qquad P(-1,-1).$$

Then

$$\pi_1 = \tfrac{1}{4}(P_1 + P_2 + P_3 + P_4)$$
$$\pi_2 = \tfrac{1}{4}(P_1 - P_2 + P_3 - P_4)$$
$$\pi_3 = \tfrac{1}{4}(P_1 + P_2 - P_3 - P_4)$$
$$\pi_4 = \tfrac{1}{4}(P_1 - P_2 - P_3 + P_4),$$

and this type of linear connection between the gametic frequencies $\pi_1, \ldots, \pi_{2^{n-1}}$ and the special values of P extends generally to n factors.

The generating functions P satisfy a recurrence relation

$$P(\lambda_1, \lambda_2, \ldots, \lambda_{n-1}; t_1, t_2, \ldots, t_n)$$
$$= P(\lambda_1, \lambda_2, \ldots, \lambda_{n-2}; t_1, t_2, \ldots, t_{n-1}) +$$
$$+ \int_{t_{n-1}}^{t_n} F(\lambda_1, \lambda_2, \ldots, \lambda_{n-1}; t_1, t_2, \ldots, t_{n-1}, u)\ du$$

where $F(\lambda_1, \lambda_2, \ldots, \lambda_{n-1}; t_1, t_2, \ldots t_{n-1}, u)$ is a density function which is an appropriate generalization of $F(\lambda, t, u, T)$.

For three linked loci, the frequencies of modes of gamete formation

reduce, of course, to linear functions of the three recombination fractions, coming out as

$$\pi_1 = 1 - \tfrac{1}{2}(y_{12} + y_{23} + y_{13})$$
$$\pi_2 = \quad \tfrac{1}{2}(- y_{12} + y_{23} + y_{13})$$
$$\pi_3 = \quad \tfrac{1}{2}(y_{12} - y_{23} + y_{13})$$
$$\pi_4 = \quad \tfrac{1}{2}(y_{12} + y_{23} - y_{13})$$

For a greater number than these the gametic frequencies cannot be expressed entirely as linear functions of the segmental recombination fractions $y_{ij}$. For instance with four loci the eight frequencies are

$$\pi_1 = 1 - \tfrac{1}{2}(y_{12} + y_{13} + y_{23}) \quad - \tfrac{1}{2}(- y_{13} + y_{14} + y_{34}) + w$$
$$\pi_2 = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \tfrac{1}{2}(- y_{13} + y_{14} + y_{34}) - w$$
$$\pi_3 = \quad \tfrac{1}{2}(- y_{12} + y_{13} + y_{23}) - \tfrac{1}{2}(y_{23} - y_{24} + y_{34}) + w$$
$$\pi_4 = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \tfrac{1}{2}(y_{23} - y_{24} + y_{34}) - w$$
$$\pi_5 = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \tfrac{1}{2}(y_{12} - y_{14} + y_{24}) - w$$
$$\pi_6 = \quad \tfrac{1}{2}(y_{12} - y_{14} + y_{24}) - \tfrac{1}{2}(y_{12} - y_{13} + y_{23}) + w$$
$$\pi_7 = \quad \tfrac{1}{2}(y_{12} - y_{13} + y_{23}) \quad\quad\quad\quad\quad\quad\quad - w$$
$$\pi_8 = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad w$$

where w is a function of the four metrical parameters $t_1, t_2, t_3, t_4$.

With five the number of additional functions is four. A general enumeration of the additional functions has not yet been made for n linked loci.

## V. Concluding Remarks

The occurrence of genetical recombination affords the observational material enabling the determination of the serial order of the genes in chromosomes. Linkage and recombination require that Mendel's Laws of factorial segregation be restated in modified form. However, as Fisher (1947) has remarked, the essential character of the original laws, which is a combinatorial one, is preserved. With any prescribed set of loci, linked or unlinked, the frequencies of the modes of gamete formation are, under given conditions of the intra- and extra-nuclear environment, independent of the particular allelic factors occupying the loci.

An important part of genetical work, since the discovery of linkage, has consisted in the translation of experimental values for the frequencies of gamete formation into information about the serial order of the genes involved. This, in general, has to be done by a process of successive approximation whereby data referring to various sets of genes (having one or more members in common) are numerically combined. No doubt, the final work in mapping any particular chromosome will always have to be of this nature.

Attempts at a theoretical formulation of any of the questions involved have been rather few. The most useful mathematical instrument contributed to the subject appears to be the Kosambi mapping function, which is largely an intelligent empiricism, but a very good approximate representation of the facts as they are found in several organisms. Its logical status is that of an ingenious and appropriate computational device, by which the first steps in successive reduction of the data to the map form may be short circuited.

In recent years however, the combination of cytological and genetical study has, by illuminating the actual mechanism of crossing-over, opened the way to a theory of a different character, sketched in Sections III and IV. It need hardly be stressed that such a theory can never purport to represent reality exactly or universally, and contains elements which cannot be determined by theoretical considerations alone but which must be inserted on a empirical basis. For instance, it will never be possible to develop tables for the conversion of recombination into map length which shall be applicable universally to all chromosomes or even to all chromosomes of the same organism. Also, such a theory is inevitably associated with some degree of idealization of reality. A theory of this kind plays, however, a useful role in science and is of the same status as, say, the statistical mechanics of imperfect fluids, which though a general theory, and formally accurate to a high degree of approximation, involves constants for which only empirical values may be supplied. Such a theory has two main roles. The first is a descriptive one: it is capable of subsuming a number of externally unrelated phenomena within the same logical structure, and exhibiting them as products of the same nexus of cause and effect. Secondly, even though perhaps it can only be brought into very approximate numerical agreement with observation, it may provide mathematical tools which can lighten and speed the practical work of analysis and interpretation of data. It is too early to judge how effective in either of these ways the modern theory of recombination can be made, but the question is worthy of further pursuit.

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# Corn Breeding *

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As the white man discovered and explored America, he found Indian corn (*Zea mays* L.) growing throughout the length and breadth of both continents. Disregarding South America and attempting no complete catalog, he found long-season flints growing in Cuba and Florida, early flints growing in New England and Canada, early flints and flour corns at the head waters of the Missouri River, late flour corns with special adaptation to the semi-deserts of our Southwest, and various types

throughout Mexico. Some of the corn grown by the Indians in Virginia in 1607 was dent corn as was some of that the remains of which have been taken from the mounds of the Mississippi basin. Here then was our heritage from the Indians: a plant incapable of survival in the wild, but one that had been selected and adapted to the completely diverse environments indicated by its wide distribution. Improvement has not stopped.

## I. CORN BREEDING BEFORE HYBRID CORN

Corn breeding has progressed from the most simple mass selection through more intensive methods up to those now being used in the production of hybrid seed corn. The older methods were reviewed in detail by Richey (1922, 1927b) and are well summarized by Hayes and Immer (1942). They require only brief attention here as background.

### 1. *Mass Selection*

It is inconceivable that a mixture of shelled corn from unselected ears should be used for seed. Consequently, a certain amount of mass selection has been practiced since the earliest times. At first this may have been no more than selection for soundness of grain and suitable maturity. Soon, however, selection for ear type had begun, and by the early 1800's recommendations were made to select from the standing stalks in the field so as to obtain better plant types. This selection established the varieties extant at the end of the century with their definite adaptations to the varied conditions required by an expanding American agriculture. That further mass selection within these improved and adapted varieties in the early 1900's could not be shown to increase yield appreciably, is absolutely no evidence that the previous selection among the more primitive kinds also had accomplished nothing as is sometimes stated.

With the advent of the corn shows, ear-type selection, which previously had been a means to an end, became an end in itself. Later, the influence of the various ear-characters on yield were studied in many experiments. A survey of these experiments by Richey (1922) showed that long, heavy ears with fewer kernel rows, lower shelling percentages and less deeply indented kernels tended in general to yield more than ears with the contrasting characters. This was largely contrary to the teachings of the corn shows. In a specific study of continuous selection for ear type, Garrison and Richey (1925) found that close selection for any of the types resulted in lower yields, with extreme departures yielding less than types nearer the varietal mode. Crossing between types tended to restore yield, with the larger increases coming from crosses

between the more divergent parents. They concluded that close selection to type was akin to mechanical inbreeding.

### 2. *Varietal Hybridization*

In addition to its use for obtaining new combinations from which to select, varietal hybridization has been tried from time to time as a means of directly augmenting the yield of corn. The first definite experiments appear to have been those of Beal (1880) in Michigan. These were followed by those of workers in several of the Experiment Stations and the U. S. Department of Agriculture. Richey (1922) has tabulated the results of 244 such comparisons. Of these, 82% yielded more and 18% yielded less than the mean of the parents, and 56% yielded more than the better parent. The proportion of hybrids superior to the best varieties in the area of the experiments could not be determined exactly, but would have been very small. At any rate, the prospects of gain from the method have been so slight that it has never come into practical usage.

### 3. *Ear-to-Row Breeding*

The ear-to-row method of corn breeding was introduced by the Illinois Agricultural Experiment Station about 1896. It consists in determining the relative productiveness of a number of open-pollinated ears in individual row plantings. Seed from the higher yielding ears then is increased under any one of several different systems. Although ear-to-row breeding was practiced extensively during the early 1900's, it failed to live up to its expectations. Under the most favorable conditions an increase of perhaps 5 bushels per acre was the maximum that could be had from the immediate progeny of the tested ears and the gain from later generations was even less (Richey 1922). Advances in our knowledge of plot technic and of genetics point clearly to two major reasons for the failure of ear-to-row breeding: (*a*) The methods used were inadequate to determine the more productive ears; (*b*) The more productive ears usually were so primarily because they were superior chance hybrids which, of course, did not breed true.

## II. Corn Breeding After Hybrid Corn

The breeding of hybrid corn involves the application of three basic principles: (*a*) The establishment by inbreeding and selection of lines that breed true, more or less, for certain characteristics; (*b*) The determination of which lines are most satisfactory for the particular purpose; and (*c*) The practical utilization of the lines in one or another kind of hybrid. To these should be added the more recent development (*d*) The

improvement of existing lines. Aside from (d), this departs from Shull's (1909) original suggestion for hybrid corn only in recognizing that the hybrid used can be other than a single cross, thereby covering the important suggestion of double crossing by Jones (1918a). Shull was not the first to inbreed corn or to make hybrids between inbred lines. Darwin (1876) noted the lesser vigor of inbred corn than of crossbred corn. Shamel (1905) reported yields for four-time selfed corn and for a cross of the three-time selfed parent. The inbred parent yielded 6.2 lbs. from 100 stalks compared to 50.5 lbs. for the cross. Hybrid corn developed, however, as a result of progress in fundamental genetics. The basic research of East, of Emerson, and of Hayes in the genetics of corn contributed much. So did the important research in the genetics of the fruit fly and other organisms. Shull (1909) deserves full credit for the initial suggestion of crossing selfed lines as a practical means of obtaining better corn yields. That suggestion was of little moment, however, until genetic progress showed a reasonable basis for so doing.

## 1. *Producing Hybrid Seed Corn*

Inbred strains may be combined into several different kinds of hybrids. The simplest of these is the single cross, or hybrid between two strains. The seed of the cross usually will not appear noticeably different from self-pollinated seed of the pistillate parent. The vigor of hybridity becomes evident, however, shortly after germination of the crossed seed begins. A three-way cross is the hybrid of a single cross between two inbred strains and a third inbred strain. It is customary to use the single cross as the female parent and the third inbred strain as the male parent in producing a three-way cross. The crossed seed produced on the hybrid plants is superior in quality and quantity to that produced on inbred plants as in single crosses. Double crosses are hybrids between two single crosses, involving four different inbred strains. Here, both the male and female parent plants are vigorous hybrids, the seed quality and production are high, and there is every possible assurance of abundant pollen from the male parent, which is not true when this parent is an inbred strain. The cross of an inbred with a variety has been variously designated as a line-variety cross, an inbred-variety cross and a top cross. Jones (1922) first reported data on such crosses, and Lindstrom (1931) gave results with both sweet and dent corn. Davis (1929) suggested using inbred-variety crosses for testing the combining value of inbred lines. Its use in this connection (its only use at present) will be discussed later. As of now, almost all commercial hybrid field corns are double crosses. Double crossing, suggested by Jones in 1918, provided a more practical means of utilizing inbreds for this purpose than

did Shull's (1909) suggestion. Single crossing is used extensively, however, with sweet corn.

In the production of hybrid seed it is customary to reproduce the inbreds and to produce the parent single crosses each year. It is usual to maintain small stocks of the inbreds by hand pollinating. These then are sometimes increased once in blocks isolated from other corn by 40 to 60 rods and the product used as seed parent in single-cross production. Seed for the male parent should always be hand pollinated if at all possible. Regardless of what kind of hybrid is involved, the seed is produced for commercial use by growing rows of the two parents in an isolated field and detasseling the plants of the female parent before they shed pollen. In general, a field for this purpose should be not less than 40 rods from other corn. From two to four rows of the female parent can be planted to every row of male parent. The customary ratios are 2 to 1 for single crossing, and 3 or 4 to 1 (6 to 2 is very common in the Corn Belt) for double crossing.

First-generation seed of single crosses ordinarily is used to produce double-crossed commercial seed. Sometimes, however, it has been necessary to use second, or, as it is usually called, "advanced" generation seed. Kiesselbach (1930) reported yields for many double crosses made between the $F_1$, $F_2$ and $F_3$ of single crosses. As an average there was no significant difference among them. In experiments by Hayes et al. (1931), double crosses between the $F_1$, $F_2$ and $F_3$ of the same singles had relative yields of 101, 103 and 112. The differences are explained most simply by some selective gene modification. When one single-cross parent must be used in an advanced generation it is used as the pollen parent if possible. In this way the seed production is not decreased by the one-third loss customary in $F_2$ below $F_1$, and pollen shedding is prolonged by the greater irregularity of the $F_2$ male.

## 2. *Establishing Inbred Lines*

A final hybrid is the product of many years' selection and experimentation. During that breeding period all pollinations are made by hand. Ear shoots are protected from stray pollen by being covered with paper bags from before until after the silks emerge. Pollen from the selected tassels that also have been protected then is applied to make the desired mating, and the pollinated ear shoot is again protected. A number of variations in this technic are practiced some of which are described and illustrated by Richey (1927b) and Jenkins (1936).

Self-fertilization is the usual method of inbreeding used to initiate new lines of corn. Good plants of one or more varieties are self-pollinated, that is, pollen is placed on the silks of the same plant from

which it came. The best of the resulting ears are planted, an ear to a row, and good plants within these rows again are self-pollinated, and so on for several generations. Each year, however, only the ears from the best plants from the best rows are selected for continuing the various strains.

With a continuation of this inbreeding there is a marked increase in the uniformity of the plants within any progeny row, although the differences from row to row are extreme. Some strains are discarded almost at once because of grossly unfavorable characters. Others are better and are continued. After some five to six generations of self-pollination the strains breed practically true for whatever characters they possess. Every plant of any strain then is practically like every other plant. After that it is unnecessary to self-pollinate in propagating a strain. Pollination between plants of a strain is essentially like self-pollination. Jones (1939) reported that plant height decreased during some five generations of selfing whereas a decrease in yield might continue for longer, perhaps twenty generations. He noted differences between sister lines segregating after various numbers of selfings, but considered these to be mutations rather than due to continuing segregation. In later papers, Jones (1945) and Singleton (1943b) have reported on certain such cases in more detail. In general, however, most corn breeders have found their established lines to breed reasonably true after perhaps six or seven generations of selfing.

### 3. *Selection Criteria*

The relation of various characters of inbreds to their yield and that of their single crosses has been studied extensively to determine effective selection criteria. Kiesselbach (1922) found a general relation between the yields of inbreds and their crosses. Richey (1924) and Richey and Mayer (1925) found that some lines consistently produced high-yielding crosses, both in different combinations and in successive generations. Jones and Mangelsdorf (1925) found little relation between characters of lines in the first and fifth generations, but Hayes (1926) reported significant positive correlations for certain characters in several successive generations of inbreeding. Nilson-Leissner (1927), Jorgenson and Brewbaker (1927) and Jenkins (1929) determined correlations among certain characters, including yield, in inbred lines. They also determined the correlations for many of these characters in the parent inbreds and their hybrid progeny. In general these were positive and many of them were significant statistically, showing objectively that selection in inbred lines for characters desired in hybrids would be effective.

Special attention is directed to the correlations of yield in the inbreds with the yield of their hybrids. In general, these were positive but not very large. As pointed out by Richey (1945a), however, most of the correlations were computed for the mean yields of the two parents with the yields of their individual hybrids. Such correlations involve not only the average value of the germ plasm, but also the way that different genetic complements may or may not nick. But Jenkins (1929, Table 13) reported correlations also for the yields of individual inbreds with the mean yields of their several crossbred progeny. Such mean yields tend to average out nicking effects and measure the average combining value. These correlations, naturally, tended to be much higher. In fact they are appreciably of the same order (and meaning) as correlations for the yield of inbred-variety crosses with average single-cross performance. Clearly, then, to the extent that visual selection has chosen high-yielding inbreds it will also have selected for high combining value (Richey 1945a).

Hayes and Johnson (1939) report a multiple correlation of 0.6660 for twelve characters of inbreds with the yields of the corresponding inbred-variety crosses. Holding date of silking constant to eliminate the important influence of maturity, left a value for R of 0.5310. Hayes (1946) stresses the fact that the inbreds had been selected for those characters indicating their vigor and productiveness, and that this selection had been reflected in higher-yielding hybrids. Sprague (1946b) comments that this 0.6660 indicates a closer relation than that found by Jenkins (1929). Jenkins' multiple correlations, however, were based on his Table 12. The correlations for inbred yield with average crossbred yield in his Table 13 are more critical. These were 0.25, 0.41, 0.45, 0.64, and 0.67. The corresponding correlation reported by Hayes and Johnson (1939) was 0.2474. On this basis, the relations in Jenkins' experiments were at least as close as the others.

## 4. *Testing Inbred Lines*

The ultimate use of inbred lines is in hybrids and their final selection probably can best be based on hybrid performance. In the earlier years, the inbreds were crossed *inter se* in groups and tested. The labor involved in such a program was tremendous. A more efficient method was available in the use of inbred-variety crosses. This had been suggested by Davis (1929), but the suggestion had been overlooked. Jenkins and Brunson (1932) reported extensive data comparing the ranking of inbreds by their performance in inbred-variety crosses and by their average performance in a number of single crosses. The experiments involved several varieties and different locations. The seven correlations

between inbred-variety and average single-cross production ranged from 0.53 to 0.90, the higher correlations being for inbreds that had previously been selfed for six generations.

Johnson and Hayes (1936) studied the combining value of inbred lines of Golden Bantam through their performance in single crosses and in inbred-variety crosses. They found that the lines poor in inbred-variety crosses were also poor in single crosses, and *vice versa*. They obtained some good single crosses, however, when one parent was a high combiner. Testing inbreds in inbred-varietal crosses has also been tried at a number of other experiment stations and has proved satisfactory for the preliminary testing of inbreds. It can of course measure only the average combining value of the inbreds, just as does average single-cross performance. Single-cross tests, however, provide information also on the way that certain lines will nick. Theoretically, it would seem that the average combining value of new inbred lines would be indicated most perfectly by their crosses with the variety from which they were derived, unless a rather large number of single crosses could be used. It must be recognized, however, that the test yield of an inbred in an inbred-variety cross will have a much larger error component as it will have had only four, five or six replications as against the forty, fifty or sixty replications represented by the mean of ten single crosses. The use of inbred-variety crosses should be restricted to a preliminary purging, to be followed by single cross testing.

The use of inbred-variety crosses for testing combining value has certain disadvantages. If lines from several varieties are involved, no one variety is likely to provide an unbiased test for all. Again, Sprague (1939) has shown that some ten plants must be used adequately to represent the variety. This involves considerable labor, particularly when a variety is used as the seed parent for the test crosses. These difficulties and other considerations have led to trials of other testers. Double crosses or synthetics offer much the same problems as varieties. They may be so constituted, however, as to emphasize selection for particular attributes of the inbreds. Thus, a synthetic susceptible to lodging, disease, or what not would promote more critical testing for resistance to these characteristics (Sprague, 1946a). Beard (1940) has compared the use of single crosses and of a variety for testing. He concluded that the single crosses were at least equal to the variety. It seems obvious, however, that a single cross must select primarily for combining value with the particular single cross used, and Federer and Sprague (1947) have concluded from a statistical study that increasing the number of testers in such cases will improve the estimate of combining value more than to increase the number of replications.

The problem of how best to test inbred lines depends somewhat on the stage of inbreeding when the testing is to be done. It accordingly will be considered further at the end of the next section.

## 5. *Time of Testing Inbred Lines*

In the earlier days of hybrid corn breeding, no definite tests for combining value were contemplated until the lines had been selfed for three or four generations. More recently it has been proposed that a comparison of original selfs in, test crosses could be used to eliminate lines with low combining value at the very beginning. This had its inception primarily in an interpretation of a comprehensive experiment by Jenkins (1935) and in the results of an experiment by Sprague (1939). The latter showed very marked differences in combining ability among the plants of an open-pollinated variety. Jenkins interpreted his data as showing that inbred lines acquired their individuality in combining value very early in the inbreeding process, and that selection then was not effective in improving this characteristic.

Sprague (1939) showed variation in combining value between plants of an open-pollinated variety sufficient to permit practical selection among them. Jenkins (1940) found very little variation in combining value among individual plants within $S_1$ progenies. This situation and its corollaries have been advanced as in favor of early testing. The argument seems confused. Actually, if all of an initial set of $S_1$ plant families are carried to homozygosis the variation among them then will be greater than at the beginning. It follows that the early elimination of entire families, with more extensive selection among the remaining ones may decrease the effectiveness of the program.

Jenkins' (1935) data were for inbred-variety crosses of a number of inbred lines of the Lancaster and Iodent varieties made after one to eight successive generations of selfing (except $S_7$). Disregarding the crosses made of the $S_1$ lines, the average trend in both varieties was essentially constant. Moreover, the variance due to differences between lines was significantly larger than that due to interaction between line and generation. These facts led Jenkins to the conclusion already noted. Richey (1945a) analyzed these data in another way and reached different conclusions. He grouped the lines into those that were in the better or poorer fractions of all lines at different stages of inbreeding. Discarding the five poorer lines (out of a total of twelve) in $S_2$-$S_3$, would have eliminated three having high yields at fixation in $S_6$-$S_8$. Moreover, there would have been no larger a proportion of good lines at that time. Further analysis showed that the seven better and five poorer lines in $S_6$-$S_8$ ranked that way also (as averages) in $S_4$-$S_5$, but did not differ in

$S_1$ or in $S_2$-$S_3$. Finally, the lines that were better in $S_4$-$S_5$ and $S_6$-$S_8$ were those in which selection in $S_4$-$S_5$ had been largely successful in choosing the better of two sibs. In the lines that were poorer at fixation, on the other hand, selection in $S_4$-$S_5$ had more frequently chosen the poorer sib. As Richey (1945a) pointed out, the situation indicated by his analysis is in better agreement with genetic expectation than is the situation indicated by Jenkins' (1935) analysis. Certainly there is segregation of genes influencing the growth of the inbreds themselves during the early generations of selfing. The correlations between inbred and hybrid performance already noted make it seem likely that these genes also influence combining value.

Preliminary data on an experiment with early testing have been presented by Sprague (1946a). These include a comparison of the yields of test crosses of six $S_0$ plants and of twenty $S_1$ progeny plants of each. He noted that most of the correlation of 0.85 arose from the differences between the means of the higher four and lower two families. Richey (1947) pointed out that the opportunity of obtaining high-yielding $F_2$ progeny from Sprague's family 130, with a parent yield of only 82.5 bushels, was about as good as to obtain one from the three parent lines with the higher yields of 93, 93 and 100 bushels. He concluded that the data indicated the expenditure of considerable effort for a very small gain from early testing.

Richey (1947) showed graphically the partial results of research on this problem by Dr. A. M. Brunson. In these, the mean yield in test crosses of the progeny of thirty higher-yielding $S_0$ plants was about twenty bushels above the corresponding value for five lower-yielding $S_0$ plants. Among the thirty higher yielding plants, however, the regression of mean progeny yield on parent yield was negative, though not significantly so. Here too, then $S_0$ testing adequately indicated the few very poor combiners: It did not, however, distinguish among the better 85%. Singleton and Nelson (1945) working with sweet corn, found no correlation for the yields of test crosses of $S_0$ plants with those of their progeny in $S_1$, $S_2$ and $S_3$ and concluded that it was not possible to detect combining ability earlier than $S_3$.

The correlations between inbred and crossbred performance noted above have been for the third and later generations of selfing. Unpublished data kindly supplied by Dr. E. Broadus Browne suggest that inbred performance in $S_1$ may sometimes be as good or better a criterion than test crosses made with the $S_0$ plants. The data are the correlations for average single cross performance (a) with test-cross yields, and (b) with inbred yields, the inbred yields being determined from sib-crossed seed produced for the purpose. In all, fourteen $S_1$ lines were crossed in

the 91 combinations. Seven of the lines were chosen because their parent plants were high yielding in test-cross combination, and seven because the parents were low yielding. Three of the inbreds yielded very little and would have been discarded from any breeding program. The results for the experiment excluding these and using only eleven lines accordingly also were computed. The correlations obtained when the fourteen, and when only eleven entries were included are shown in the following table.

| Yields correlated | Coefficients of correlation for the number of entries stated | |
| | 14 | 11 |
| --- | --- | --- |
| Test crosses and single-cross means | 0.87 | 0.82 |
| Sibs and single-cross means | 0.70 | 0.93 |

All correlations are significant at the 1% point. For the fourteen entries, test-cross yields gave somewhat better predictions. Among the lines that might have continued in a breeding program, however, selfed performance was the better criterion of average single-cross performance and also, of course, of the potential value of the inbreds for seed production. The importance of this last consideration can be attested by all who have selected inbreds from an open-pollinated variety.

How to test is partly dependent on when the testing is done. Richey (1945a) presented a theoretical consideration of different test criteria, with major emphasis on selecting for dominant favorable genes. Test crosses were shown to mask inherent variation so greatly as to make them unsuitable for distinguishing between closely related lines. It was suggested that this influence might account in part for the small differences Jenkins (1940) found between $S_2$ siblings. Richey (1945a) also pointed out that test crosses could not distinguish whether two dominants of equal importance were at one or at two loci, the latter being amenable to improvement whereas the former is fixed. Selfed progeny performance, on the other hand, tends to overemphasize the influence of major deleterious recessive genes that will be eliminated in the long run. This is most important in the early stages of inbreeding, and probably would be negligible among lines being derived from crosses between previously established inbreds. His major conclusions follow:

Test crosses are a good criterion of combining value at any stage in the program, as of that time. They are not good indicators of prospective combining value, however, until fixation has reasonably been achieved.

Selfed performance on a progeny basis is not a good criterion of ultimate combining value until selection has eliminated recessives of larger individual effects and lower frequencies.

With effective selection against recessives of major effect and progress toward fixation, progeny performance of selfs and of crosses will tend to tell more nearly the same story. Selfed performance then will be satisfactory for selecting within families, while crossed performance will be more suitable for selecting among families.

## 6. *Predicting Hybrid Performance*

Richey and Sprague (1932) reported the yields of eleven double crosses and of the component single crosses. They then computed the average yields of the two parental combinations and of the four non-parental combinations. There were marked differences among double crosses involving the same four inbreds in different combinations, but yields were erratic and there was no consistent relation between the double-cross yields and either of the above single-cross averages.

Jenkins (1934) reported correlations for the yields of double crosses, (a) with the mean-yield of the six possible single crosses, (b) with the mean yield of the four non-parental combinations, (c) with the mean of all single cross data involving the lines, and (d) with the mean of the inbred-variety crosses of the four parent inbreds. Method (b) would be expected to give the higher correlation on the basis of genetic theory. The correlations for methods (a), (b) and (c), however, were 0.75, 0.76, and 0.73 respectively, showing little difference for the three methods. The correlation for the inbred-variety crosses was 0.61, still significant.

The effectiveness of predicting the yields of double crosses from the mean yields of the four non-parental single crosses has now been tested at many experiment stations. Doxtator and Johnson (1936) and Anderson (1938) found very high correlations at Minnesota. Hayes *et al.* (1943) report a correlation of 0.48 for predicted and actual yields of 114 double crosses. This accounts for only 23% of the variance in yield, but is highly significant. In later experiments, Hayes *et al.* (1946b) found correlations of 0.72 and 0.72 for the yield predicted from 1940 and from 1941 data with the actual average yield in 1943-1944. It should be noted, however, that a single-cross test under extreme conditions may not be too valuable for predicting results to be expected under average conditions. That, however, is only a detail to be kept in mind. The method has been found satisfactory and has been accepted for general use.

Doxtator and Johnson (1936) particularly emphasized the importance of the way of combining any four lines. In detail, the best way can be determined only by experiment. In general, however, crossing the two lowest yielding single crosses will produce the highest yielding double cross. Intensive studies by Eckhardt and Bryan (1940a, 1940b) show that the highest yielding combinations where two parental varieties were involved were those in which the two lines from one variety came

into the double from one side and those from the other variety came in from the other side. They also found that combinations of this kind tended to be more uniform in such characters as plant and ear height and length and weight of ear. Where early $(E)$ and late $(L)$ lines were used (1940b), combinations of $(E \times E)$ $(L \times L)$ were more uniform in several characters associated with maturity than were combinations of $(E \times L)$ $(E \times L)$. Pinnell (1943) obtained conflicting results. One $(E \times L)$ $(E \times L)$ cross was more uniform and another was less uniform than $(E \times E)$ $(L \times L)$. He concluded that even though two $E$ (or $L$) lines were alike phenotypically, they might be unlike genotypically, providing opportunity for segregation in the double cross. Such differences probably need little consideration, particularly as the importance of uniformity has been much overemphasized.

As noted earlier, some 82% of 244 varietal hybrids had higher yields than the average of their parents. This would lead to an expectation that crosses between inbreds from different varieties would tend to be more productive than crosses between inbreds from the same variety. This expectation has been justified by the general experience of corn breeders. It carries forward, moreover, into double crosses and is doubtless the basis for the results of Eckhardt and Bryan (1940a) already referred to. The importance of this diversity influence has been noted by several investigators. Thus Wu (1939), Hayes and Johnson (1939), Eckhardt and Bryan (1940a), Johnson and Hayes (1940) and Cowan (1943) have stressed the importance of this element of performance in hybrids. It is almost certainly only one more manifestation of hybrid vigor.

## 7. Special Methods

Suggestions have been made from time to time for using what may be called special methods in isolating or improving corn inbreds. Dobzhansky and Rhoades (1938) suggested using inversions in identifying strains carrying superior genes on specific chromosomes. A strain homozygous for a suitably marked inversion is crossed with an inbred line. The $F_1$ then is backcrossed to the inversion strain. Differences between the progeny plants homozygous and heterozygous for the inversion will be due to the total differential influence of the genes carried on the inverted and on the normal segments of that chromosome. By testing several inbred lines, their relative value for the specific chromosome segment could be estimated. Since corn has ten chromosomes, the labor of a complete analysis would be tremendous, as the authors noted. Singleton (1941) questioned whether the differences would be large enough to be practically measurable. Sprague (1941), however, found

significant differences in plot yield and in kernel weight in a comparison
of normal and inverted segments of the fifth chromosome. He notes that
the segment from dent corn was superior to that from sweet corn.
Singleton (1941) suggested a modification of the Dobzhansky and
Rhoades (1938) method. He also proposed what he calls a "multiple
recessive" method for locating favorable genes. Both might have value
for some specific research, but, as of the present, seem to involve too
much time and labor to be practical.

Burnham (1946), from a theoretical basis, outlined a method for
obtaining homozygotes directly, by doubling gametes with the aid of
translocations. He assumes a multiple-translocation stock such that its
$F_1$ crosses with normal corn will have a ring containing the twenty
chromosomes at meiosis. Such a plant should produce two kinds of
functional gametes largely corresponding to those of the two parents;
other gametic combinations abort in corn. The selfed $F_2$ progeny from
such an $F_1$ plant should comprise heterozygotes with the chromosome
ring, and two kinds of normals each homozygous for one of the two
parental gametic combinations. These could be recognized by suitable
tests. In commenting on this suggestion, Richey (1947) questioned
whether immediate achievement of homozygosis might not be disadvan-
tageous by fixing deleterious recessives before they could be eliminated
by selection. He noted that Macaulay (1928) had suggested that even
selfing might proceed toward homozygosis too rapidly for best results.

Stadler (1944) has proposed gamete selection as a way of obtaining
improved inbred lines. This involves (a) crossing an established, "elite,"
inbred with an open-pollinated variety; (b) testing the individual $F_1$
plants of this cross in tester crosses; and (c) testing the $S_1$ progeny of
the exceptional $F_1$ plants. As outlined, then, the method involves two
concepts, (a) obtaining a random sample of gametes from an open-
pollinated variety to use in improving an elite inbred, and (b) selecting
intensively among those gametes by objective test in the $F_1$ of their
crosses with the gametes of the elite inbred. It has been pointed out by
several (Burnham, 1946; Hayes et al., 1946; Richey, 1947) that the test-
ing and selection among the $S_1$ progeny of the $F_1$ plants involves nothing
more than is involved in testing within any $F_2$ for selection. It is merely
another application of the principle of early testing.

Richey (1947) on the basis of an analysis of early testing, as already
reviewed, questioned the validity of such testing as a sound breeding
method. He, and Hayes et al. (1946a), also questioned the need for the
initial test of the $F_1$ crosses between the elite inbred and the open-pol-
linated variety. Data reported by Hayes and Johnson (1939) showed
that some high-combining lines could be selected from crosses between

established inbreds even when one parent was low-combining. Assuming that the elite gamete was $A, B, C, r, s$, with only dominants as of value, Richey (1947) noted that testing with a recessive might indicate a varietal gamete carrying $A, B, C, R, s$ as superior to a gamete carrying $a, b, c, R, S$. The latter, however, could be potentially more valuable as a base for improvement, since $R$ and $S$ are both lacking in the elite gamete. This false indication from the test cross arises from its failure to distinguish between two dominants of equal weight when they are at one (allelic) and at two loci, as pointed out by Richey (1945a).

Though disagreeing with Stadler (1944) as regards the early testing, Richey (1947) completely agreed with him as to the important need for sampling further the germ plasm of open-pollinated varieties and as to the greater efficiency of sampling for gametes rather than zygotes. He emphasized the value of using gametes from an adapted variety for improving superior but unadapted inbreds from another area, and noted that preliminary observations through three generations in Tennessee indicated this method of adaptive improvement as of much promise.

A system of recurrent selection was suggested by Hull (1945), designed particularly for obtaining inbreds to use in specific combinations. The plan involves (a) selfing a number of plants and crossing them on a specific tester, (b) testing the crosses, (c) intercrossing the $S_1$ lines from the plants best in crosses, and (d) repeating this cycle, using the intercrossed seed from the previous cycle as foundation material. Hull suggested that with about 100 plants tested and using the ten best for continuation, four cycles should have an efficiency equivalent to testing more than 10,000 plants from the original stock. No test of the method has been completed, but the restoration of heterozygosity between selections seems to offer definite advantages. *A priori*, one can question the advisability of the basic concept of selecting for a particular combination rather than for the best average germ plasm as is done when a more general test is used.

## 8. *Progressive Improvement of Inbred Lines*

In the earlier stages of the hybrid corn development all new inbreds had to derive from open-pollinated corn. After the first inbreds were established, however, attempts were made to improve them in various ways. Whether the products of such attempts constitute "new lines" or "improved old lines" is partly a matter of definition. Certainly, some of the preceding discussion has concerned the improvement of existing lines and some of that in this section will concern establishment of essentially new lines.

Among the first methods to be tried was that of "second cycle" or

"pedigree" selection as it has variously been called. It follows essentially the pedigree method of breeding small grains. Two existing inbreds are crossed and new inbred lines are selected from the cross. The methods used may be any of those previously discussed. As a rule, the parent lines are chosen because they complement each other. Thus, an inbred which has high yield in hybrids but which lodges or is susceptible to some disease, may be crossed with an inbred resistant to lodging or to the disease in question. Selection then of course is for the desired combination.

The best evidence as to the possibilities in this method is from extensive and intensive experiments in Minnesota. Wu (1939), Hayes and Johnson (1939) and Johnson and Hayes (1940) have reported on various phases and stages of those investigations. Some of their findings bearing on the theories of corn breeding already have been noted. Practically, inbreds were obtained which were superior as such, and which yielded more in hybrids or yielded equally well within a shorter growing season, which had more resistance to corn smut, and which lodged less. There is no reason to suppose that additional improvement can not be achieved in another cycle, and so on. It should be emphasized, however, that such a program will result in an ever-narrowing network of germ plasm unless "new blood" is brought in from time to time. The use of gametes from open-pollinated corn (or from outside inbreds) will avoid this narrowing tendency.

In a modified form of second-cycle improvement, the original cross between two inbreds is backcrossed to one of them (the recurrent parent) one or more times before beginning to select in self-fertilized lines. It was pointed out by Richey (1927a) that such a procedure might well recover the more important part of the recurrent parent genotype, but improve it by retaining desired characters from the non-recurrent parent. Experimental results will be discussed later in connection with convergent improvement. Here, it need only be noted that one or two generations of backcrossing frequently have preceded selfing in an improvement program, and that good lines have been developed both with and without backcrossing as clearly shown by Hayes et al. (1946a). Backcrossing is used primarily, of course, when one of the parent inbreds is generally superior to the other but needs some definite kind of improvement.

Experiments on a special application of backcrossing, designated "Multiple Convergence," were reported by Richey (1946b). It was shown that appreciable hybrid vigor could be obtained in crosses between related lines derived by backcrossing in parallel a few generations to a common parent inbred. The related lines were much alike and like the

recurrent parent. Their crosses accordingly could be used in place of that parent and had enough more vigor to reduce the hazards of seed production. Crosses of unrelated crosses between such converged lines also afford a possible basis for obtaining much of the uniformity of single crosses in what are mechanically double crosses.

The progressive improvement of inbreds by crossing and reselecting occurred as a natural development of hybrid corn breeding. Much of the secondary improvement sought was in inbreds being used for hybrid production or in others that would have been in use except for some major defect. Richey (1945a) has suggested, however, that progressive improvement could be planned in advance as a means of obtaining better inbreds from a variety. To emphasize the planned continuity of effort, it was designated "Cumulative Improvement." This would differ from former practice only in that the initial selections would be made specifically for further crossing and selection. Accordingly, lines that otherwise might be discarded because of some fault could be retained for improvement. Also, the second phase of selection would begin relatively sooner.

### 9. Synthetic Varieties

A synthetic variety is a complex hybrid among several inbred lines, propagated as a variety. It is produced by growing a mixture of the inbreds, or more usually a mixture of the component single crosses, in isolation with open pollination. Their possible use was suggested by Hayes and Garber (1919), but, so far, has not seemed practically worthwhile. Hayes (1926) reported the yields of five synthetics, each derived from one variety, in comparison with the parental yields. The synthetics ranged from 12% less to 17% more productive than their parents. It was not possible at that time, however, to have only high-combining inbreds so that the evidence is not too critical. The same is true for the synthetic varieties reported on by Sprague and Jenkins (1943). The inbreds involved had been chosen originally because they possessed various plant and ear characters. That the yields of the advanced generations of these synthetics no more than about equalled the yield of adapted open-pollinated varieties, therefore, is not surprising.

Hayes et al. (1944) have provided more critical evidence of the possibilities of synthetics. Twenty inbred lines were tested for yield in all possible combinations, and the eight with the highest average yields were brought together into a synthetic. This was maintained for a time by mass selection from good plants, but without selection for ear type. Its yield was definitely superior to that of a comparable adapted variety, and nearly equal to that of Minhybrid 403, a good double cross for the locality of the test.

The yield of the $F_2$ or other advanced generation under random mating can be predicted through a formula given by Wright (1922).

$$F_2 = F_1 - \frac{(F_1 - P)}{n}$$

in which n is the number of parent lines, P is their mean yield, $F_1$ is the mean yield of their single crosses, and $F_2$ the mean yield of these crosses in $F_2$, which is equivalent to the synthetic mixture. This formula is based on an assumption of simple, additive genic action. That this is justified with corn is indicated by the agreement of predicted and observed yields in various experiments. Thus, Neal (1935) found close agreement for single, three-way, and double crosses, and Kinman and Sprague (1945) found agreement for 45 single crosses. The latter concluded that the most efficient number of lines to use in a synthetic would differ with the range in combining ability among the available inbreds. For the ten inbreds studied, they concluded that four to six would be most efficient. Examination of their data, however, reveals that four of the ten lines they used had definitely lower yields in crosses than did the other six. More extensive trials might locate a larger number of inbreds with more nearly equal combining value. Given the average yields of parents and single crosses, the most efficient number in any specific case can be predicted from Wright's formula.

For the present, synthetic varieties have little place for practical corn production in the United States. If and when high-combining inbreds become available in larger numbers, and if and when these are materially higher-yielding as inbreds than are those now available, the picture may change. Until then, however, possible use of synthetics would seem to be restricted to areas where for some reason the use of double-crossed seed is not practical.

### III. Hybrid Vigor and Corn Breeding

Hybrid vigor is not peculiar to corn, but a discussion of modern corn breeding without consideration of hybrid vigor is unthinkable. Shull (1914) suggested the convenient term "heterosis" for the stimulus of heterozygosis, and it has come into general use as synonymous with hybrid vigor. More recently, Mather has concluded as follows: "Heterosis is due to a poor relational balance brought about by artificial selection. The concept of heterosis is now extended to include all types of such unbalance, natural and artificial" (1943, p 63). It is difficult to see why a stimulus resulting in greater vigor should be assigned to an unbalance of any kind. Furthermore, the proposed extension in concept would totally deprive the term of any specific meaning. That would be unfor-

tunate. In considering here the possible causes of heterosis, therefore, the older term hybrid vigor generally will be used, and when heterosis is used, it will be synonymous with hybrid vigor.

No conclusive evidence has been accumulated on the extent to which hybrid vigor is a cause of the high yields of corn hybrids. Whaley (1944) has called attention to the fact that the idea of a hybrid as "super-normal" pervades the literature. He further notes that a concept of the hybrid as normal and the inbred parents as inferior better fits the facts of corn breeding. This is incontrovertible. It is well supported by the fact that the individual hybrid corn plants are not superior to the best plants of the open-pollinated varieties. It is the modal performance of the hybrid that is superior, resulting in the larger yields. It is the uniformity in hybrid vigor interacting with a corresponding uniform superiority in quantitative and qualitative attributes that produces the productive whole. No one element, however, need be greatly above average since they will be cumulative in their effect. Under this hypothesis, the best hybrids will be those having a minimum superiority above high-yielding parents; it is supported by the fact that the higher-yielding inbreds have tended consistently to produce the higher-yielding hybrids. On the other hand, Hull (1945) assumes a superiority for the heterozygote over both homozygotes. Under that hypothesis, hybrid vigor itself is a direct cause of superiority, and the best hybrids will have a maximum superiority above low-yielding parents. The weight of the evidence appears to be strongly against Hull's hypothesis.

The classical examples of hybrid vigor in plants were reviewed and described by East and Hayes (1912). Studies by Kiesselbach (1922) showed that corn hybrids matured many more, slightly larger cells in an equal time than did their inbred parents. Ashby (1930, 1932) attempted to show that corn hybrids made their excess size by an equal growth rate acting on a larger embryo. Sprague (1936) and others have investigated this problem and come to different conclusions. Whaley (1944) has reviewed the earlier contributions in detail. Wang (1947) found that "the larger size of hybrid maize kernels is the result of differences in growth rate." The problem is one of physiology rather than genetics, however, and needs little attention here. As East (1936) pointed out, the larger size of seed or seed parts may be a manifestation, but not a cause of hybrid vigor.

In describing how hybrid vigor is expressed, East (1936) emphasized that it is manifest in the organism as an entity by increased general metabolic efficiency, thus eliminating an excess of development in a single character as an example of hybrid vigor. Such a requirement would not eliminate instances of simple hybrid vigor that are provided

by crosses among the several known recessive dwarfs in corn. The dwarfs have all parts of the plants smaller, and hybrids between them exceed the average of the parents in height and in many other respects. In fact, their excess growth would be very well covered by East's detailed description.

Justification for including such simple examples as instances of hybrid vigor is offered by the situation in the sorghums. Hybrids between different sorghums have long been cited as manifesting extreme vigor. Genetic analysis showed later that most of the excess size is the result of interaction among a few genes (Martin 1936). Quinby and Karper (1945) recently have reported on this situation in milo. They found two genes for internode length, and three which determine internode number through their control of photoperiod response. The demonstration of this relatively simple situation certainly does not convert the phenomenon from an example of hybrid vigor into something else. And the inclusion of such simple cases may help to an understanding of the more complex.

## 1. *Explanations of Hybrid Vigor*

The most typical example of hybrid vigor of the kind needing explanation is provided by the excess vigor of hybrids between selfed lines of corn and the decrease in vigor accompanying their inbreeding. Various explanations for this have been advanced. The oldest of these, physiologic stimulation, has been described by Shull (1914), as follows: "In other words, hybridity itself—the union of unlike elements, the state of being heterozygous—has, according to my view, a stimulating effect upon the physiological activities of the organism." This explanation is strictly non-Mendelian. It describes a condition which may be illustrated by an electric cell. An increase in the quantities of the diverse elements would result in a greater current. Aside from the fact that vigor increases more or less proportionally with changes in heterozygosity, this explanation appears to have no supporting evidence.

The interaction of dominant favorable genes is a second and the most generally accepted theory at the present time. It was first suggested by Keeble and Pellew (1910) as a result of their researches with pea hybrids. They specifically predicated the interaction of more than one pair of genes for the different characters. Nevertheless, the early refusal to accept their hypothesis was based on conditions which would obtain only if relatively few genes were involved. Bruce (1910) offered a similar Mendelian explanation of hybrid vigor from a purely mathematical basis. The interaction of dominant favorable genes as proposed by Bruce (1910) and by Keeble and Pellew (1910) was rejected as an

explanation by various early workers on the grounds that it would result in a skew distribution in $F_2$ and that it should be possible to recover individuals homozygous for the dominant favorable genes concerned. Collins (1921) showed clearly, however, that these objections were not valid if the number of dominant favorable genes concerned was of the order of twenty or more. In the meantime, Jones (1917) had suggested that linkage between favorable and unfavorable genes would prevent skewness and recovery of multiple dominants. As East (1936) has emphasized, Jones' (1917) suggestion was linkage, and not the interaction of dominant genes. But linkage, by itself, can not cause hybrid vigor. As Richey (1945b) recently emphasized, then, the dominance theory may be attributed most appropriately to Bruce and to Keeble and Pellew, and the date of placing hybrid vigor on a Mendelian basis should be 1910, that of their papers, rather than the date of Jones' (1917) paper as has been stated by Jones (1942).

Another possible basis for hybrid vigor has been discussed from time to time during the past thirty years. This is the complementary action of alleles at a single locus. Singleton (1943a, 1943b) has reported a diminutive mutant, C30, whose hybrids with the parent P39 strain show hybrid vigor, and which produces more vigorous hybrids with some unrelated strains than does P39. More recently, Jones (1944, 1945) has reported other mutants whose hybrids with the parent strains show vigor. Jones (1945) has interpreted these as evidence for intra-allelic interaction. So far, these seem to be the only instances that have been noted in corn. Quinby and Karper (1946), however, reported a pair of alleles, *Ma ma*, the heterozygote of which differs from either homozygote, contributing to a superiority for the hybrid. Gustafson (1946), too, reports instances of superiority for allelic heterozygotes and interprets data of some earlier workers as indicating a similar situation. Hull (1945) also postulates a superiority for the heterozygote, or overdominance, implying intra-allelic interaction. He bases his conclusion on the yields of the $F_1$ and $F_2$ hybrids between selfed lines of corn in relation to the parent yields. He finds regressions in such cases that he explains as influenced by overdominance. To the extent that such intra-allelic interaction may occur, it will operate indistinguishably from the complementary action of completely linked genes. It will be interesting to learn whether its observed frequency may indicate it later as a probable cause of any important part of hybrid vigor.

The mechanism usually assumed as a basis for possible intra-allelic stimulation is that one gene is dominantly favorable for one essential process, its allele being dominantly favorable for another. This is no more than dominant favorable interaction at the allelic level. Gustafson

(1946) suggests a means by which genes that are deleterious when homozygous could be beneficial in the heterozygote. Such a situation could occur if a non-dominant gene conditioned the production of a substance deleterious in excess but beneficial in a lesser quantity, as in the intermediate heterozygote. The allelic homozygote having none of the beneficial substance also would be inferior.

East (1936) proposed a type of intra-allelic action with dominance lacking. His hypothesis involves a series of alleles at locus $A$, for example, such as $A^1$, $A^2$ . . . $A^4$. Each allele of higher number is assumed to have a positive active function, diverging farther and farther from that of $A^1$. Thus, $A^1 A^4$ would have greater physiologic efficiency than $A^1 A^2$. With the lack of dominance assumed by East, $A^1 A^4$ would function less effectively for the process controlled by the individual alleles than would $A^1 A^1$ or $A^4 A^4$, although the final functioning of the heterozygote might well be superior. Negatively, East's (1936) proposal seems to have stemmed from his unwillingness to accept the dominance and recessiveness of what he termed normal and defective genes as a basis for hybrid vigor.

## 2. *The Role of Recessives*

Garber and Rowley (1927) and Mangelsdorf (1928) found that corn plants heterozygous for "recessive" defective seeds were very slightly inferior to their dominant homozygotes. Wentz and Goodsell (1929) found no significant relation between yield and frequency of recessive defects in nineteen varieties of corn. Woodworth (1930) selfed a large number of plants of the Station Reid variety. The seed from ears segregating for no obvious seed, seedling, or mature plant recessive defects was mixed and tested against the parent variety. The results of several such tests were conflicting. The "recessive-free" seed yielded slightly more in general, but not significantly so. Kemp (1929) has postulated a situation in which recessives are held in a population by linkage with favorable dominants. He then shows statistically the results of such a situation. One of these is that maximum yields can not be had from stocks in which such recessives have been eliminated.

A lack of preciseness in the use of the term "recessives" probably has led to some differences in theory and interpretation that are more apparent than real. Thus, Sprague (1946b) states in one paragraph of his summary, "Recessive characters probably have little or no influence on heterosis." In his second following paragraph he notes, however, that, ". . . dominant favorable genes are more important in heterosis than physiologic stimulation. . . ." But dominant favorable genes can be effective in hybrid vigor only as they operate in opposition to their

less favorable recessives. The real contrast should be between defectives of major and of minor individual effects, but both of which are recessive. It seems clear that the suppression of grossly defective recessives has no part in hybrid vigor of the kind obtained in crosses between corn inbreds. That, however, need not exclude the influence of the suppression of minor deleterious recessive genes, which may occur in large numbers with imperceptible individual effects, as recently has been reemphasized by Jones (1942) and Richey (1945a, 1946a). Genes for unfavorable functioning may be recessive only because their alleles can function adequately for both in the heterozygote. This is the situation East (1936) illustrated by his "two-cylindered engines running on one cylinder." It certainly is what happens with regard to some minute chromosomal deficiencies in corn (McClintock, 1944). The adequate functioning of certain heterokaryons between *Neurospora* strains is significant in this connection. Two strains in which different genes necessary to the formation of different essential substances have been inactivated can function as heterokaryons. (Reviewed by Horowitz *et al.*, 1945). The results of Dodge *et al.* (1945) have added significance. These workers found that genes in different haploid nuclei giving vigor in heterokaryons could be combined into a single haploid nucleus.

### 3. *Convergent Improvement*

Convergent improvement was suggested by Richey (1927a) as a means for distinguishing between two theories as to the cause of hybrid vigor, and also as a basis for improving inbred lines without changing their combining value. It involves (*a*) crossing two inbreds, as *A* and *B*, (*b*) backcrossing to one of them (the recurrent parent) in successive generations while selecting for desired characters from the other (nonrecurrent) parent, and (*c*) selfing with selection, to homozygosis. Operations (*b*) and (*c*) are to be carried on in parallel, using each inbred as a recurrent parent. Its bearing on the explanation of hybrid vigor inheres in the following facts: The "recovered" inbreds, *A'* and *B'* can differ from their recurrent parents only in what each may have acquired from its non-recurrent parent. To the extent, then, that *A'* and *B'* do differ from *A* and *B*, they are more nearly alike than are *A* and *B*. It follows that the cross *A'* × *B'* will be less heterozygous than *A* × *B* and, if equal in yield, the evidence will be for the interaction of dominant favorable genes as against physiologic stimulation.

Richey and Sprague (1931) reported yields for lines being "recovered" from six single crosses backcrossed, with selection, for from one to four generations each. They also reported the yields for two crosses between recovered lines and for four crosses of recovered lines with their

non-recurrent parents. They found the yields both of the recovered lines and of the crosses to be very slightly but consistently above expectation on the basis of randomness. They concluded that the data supported the interaction of dominant favorable genes as a more probable explanation for hybrid vigor than physiologic stimulation. They also pointed out that the suggestion they obtained of hybrid yields even in excess of the theoretical might indicate that genes of partial dominance were involved in addition to those with complete dominance.

Gowan *et al.* (1946) have reported a study with Drosophila from which they concluded that the general trend pointed distinctly toward a genic basis rather than toward physiologic stimulation as a cause of hybrid vigor. These authors have questioned the validity of Richey and Sprague's (1931) analysis of their convergent improvement data. A check, however, indicates the analysis to have been correct for the method used and that the method used is justifiable. Gowen *et al.* (1946) also appear not to have known that additional data had been reported by Murphy (1942) confirming the conclusions of Richey and Sprague (1931). More recently, Hayes *et al.* (1946a) have reported on extensive tests of convergent improvement. Their data, too, are in agreement with Richey and Sprague's (1931) conclusion that the more probable cause of hybrid vigor is the interaction of dominant and partially dominant favorable growth genes. The data from all three sources also indicate the value of crossing and backcrossing as a means of improving inbreds under certain conditions.

## IV. Quantitative and Qualitative Inheritance

The controlled modification of quantitative and qualitative characters is, of course, of the essence of corn breeding. However, among the more than 300 genetic factors detailed in the summary of linkage in maize (Emerson *et al.*, 1935), and which are essentially qualitative, only a very few have any importance in corn breeding. These are genes affecting the color, texture and composition of the kernels, resistance to disease, and others of similar practical implication. The important relation of quantitative characters to corn breeding recently has been stressed by Hayes (1946). The importance of this relation already has been noted here in connection with the discussion of selecting inbreds. It could not well be otherwise. Quantitative characters either are the direct physical components of yield or are closely associated with such components. Moreover, the inheritance of most characteristics determining adaptation, resistance to insects, diseases and the like is primarily quantitative. Emerson and East (1913) showed that quantitative inheritance in corn was controlled by multiple genes with relatively small,

more or less equal effects, and with dominance lacking. Later research on this subject has been reviewed in detail by Smith (1944).

## 1. *Adaptation*

Adaptation to the area in which hybrids are to be grown is essential to yield and quality. Thus corn farmers in the North have suffered severe losses from trying to grow hybrids that could not mature within the short season available. On the other hand, sale of hybrids from the Corn Belt to southern farmers also has been seriously detrimental. These hybrids mature too quickly to utilize the full available season and, frequently, at a period when conditions are conducive to heavy damage to quality. Furthermore, they tend to be susceptible to injury by diseases and insects which either do not occur or are unimportant where the hybrids were developed.

Lindstrom (1943) found that the number of days from planting to silking or tasseling increased from the $F_1$ to the $F_2$ to the $F_3$ in several crosses between earlier and later maturing inbreds. He interpreted this as evidence for the dominance of genes for earliness. A more reasonable explanation would seem to be a decrease in heterozygosis and consequent vigor associated with the inbreeding. It certainly has been the general experience in corn breeding that crosses between *heterozygous* parents are strictly intermediate in maturity, clearly indicating a lack of dominance in either direction. On the other hand, crosses between *inbreds* of like season are earlier maturing than the parents: just another manifestation of the more efficient functioning of hybrids. An exception to the intermediacy of maturity may be expected in some crosses. Thus, many tropical varieties of corn require a short day for flowering, a condition that seems to be more or less recessive (but not monogenic) in crosses with the longer-day varieties of the temperate zone. Crosses between such varieties, then, will be influenced by both of these types of interaction.

Tolerance to heat and drought is of particular importance in the more arid areas, but has an important relation to yield everywhere. Heyne and Brunson (1940) showed that genes for such tolerance were located on at least two chromosomes, 5 and 9. They further concluded that the sugary gene, *su*, predisposed to susceptibility, and that glossy seedlings due to $gl_1$ and $gl_2$, but not those due to $gl_3$, were resistant to heat in the seedling stage.

In the Corn Belt a tight husk extending much beyond the end of the ear serves no important purpose and increases the cost of harvest. To the South, however, adequate husk protection from insect attack becomes increasingly important. In fact, some of the southern inbreds must have the husks cut back to insure adequate fertilization. Singleton (1946)

has reported the occurrence of such a condition in sweet corn at the Connecticut station. Obviously, the extension of the husk beyond the ear tip will depend upon both the length of the ear and the length of the husk. In a study of this problem, Freeman (1945) investigated the genetics of husk length, ear length, and days-to-silking, using translocation stocks as testers. He found genes for long husks to be located in chromosomes 1, 2 and 8 in inbred Florida 1, and in chromosomes 1, 2 and 3 in inbred Florida 2. This would indicate at least four different genes (or gene groups) as affecting husk length in the inbreds studied. Similarly, genes for ear length appeared to be carried by chromosomes 1, 3, 4, 5 and 8 in Florida 1, and in chromosomes 4, 5 and 9 in Florida 2. Genes for long season were found in chromosomes 3, 5 and 8 in Florida 1, and in these and also in chromosomes 1 and 2 in Florida 2.

## 2. *Disease Resistance*

The inheritance of resistance to diseases in corn may be monogenic or, more usually, complex. Thus, Mains (1926, 1931) reported a gene for resistance to race 3 of rust (*Puccinia sorghi*). Later, Rhoades (1935) located this gene on the long arm of chromosome 10, using x-ray induced deficiencies and cytologic examination in addition to trisomic tests. Ullstrup (1944) found two physiologic races of *Helminthosporium carbonum*. Resistance to one of these, race I, is due to a single dominant gene. Studies by Ullstrup and Brunson (1946) indicated the locus for the resistance vs. susceptibility genes (*Hm hm*) to be on chromosome 1, and about twenty units to the left of *Br br*. Observation suggests that there also are modifying genes that affect the time and severity of the attack in plants homozygous for the susceptible recessive allele. Resistance to bacterial wilt caused by *Bacterium stewartii* was studied by Wellhausen (1937). He reported three dominant genes for resistance, $Sw_1$, $Sw_2$, and $Sw_3$. Of these, $Sw_1$ was most potent and $Sw_3$ had little effect alone, but supplemented the action of $Sw_1$ and $Sw_2$ when in combination with them. Bacterial wilt is more serious in sweet corn than field corn, and an outstanding example of breeding for disease resistance is the development of the hybrid sweet corn, Golden Cross Bantam (Smith, 1933).

Resistance to the various root, stalk, and ear rot diseases has been definitely demonstrated to be heritable and strains more or less resistant have been isolated. Little or nothing is known of the genetics of this resistance, however, beyond the fact that it appears to be of the mutiple-gene type. The same is largely true for *Helminthosporium turcicum* (See, for example, Elliott and Jenkins, 1946) and *H. maydis*, and for corn smut due to *Ustilago maydis* (Jones, 1918b; Immer, 1927; Hoover,

1932). For corn smut, however, additional evidence as to detailed complexity is available. Thus, Burnham and Cartledge (1939) found associations between smut reaction and region of interchange in 13 translocation stocks. Saboe and Hayes (1941) used a similar technic, with interchanges marking 15 arms of the 10 chromosomes. They reported genes for smut resistance in the short arm of chromosome 6 and in the long arms of chromosomes 7 and 8. In other crosses, a gene for resistance was indicated as in the long arm of chromosome 3 or 5 and another as in the long arm of chromosome 5 or 8.

### 3. *Resistance to Insects*

Some of the complexities of breeding for resistance to insects are well illustrated in a report on breeding corn resistant to the European corn borer, *Pyrausta nubilalis*, (Meyers *et al.*, 1937). Thus, fewer borers per plot occurred in later maturing hybrids, but more borers in hybrids that were taller during the period of oviposition. To select for low final infestation, then, might well lead only to strains lacking early vigor, and that were too late maturing for the locality. Deviations from the infestations predicted from the regression on maturity and early plant height, however, provided a satisfactory selection criterion. It was concluded that there were inherent differences in resistance, and that use of the more resistant inbreds would permit hybrids with 20% fewer borers than the average open-pollinated varieties of those days. It also was shown (Meyers *et. al.*, 1937) that there were differences among inbred lines in the percentage establishment from a given degree of egg infestation. These results were later confirmed and extended (Patch, 1943; Patch and Everly, 1945). Nothing suggestive of immunity or of genetically simple resistance has been found, however, the mode of inheritance apparently being typical of multiple factor interaction.

Reference already has been made to the importance of husk protection in the South. Kyle (1918) showed that long, tight husks largely prevented damage by the black or rice weevil, *Sitophilus oryza*, except as access was had to the grain through holes made by other insects. He also showed that long, tight husks materially reduced damage from the corn ear worm, *Heliothis armigera*, as had been reported previously (Collins and Kempton, 1917) for sweet corn breeding experiments. More recently (Painter and Brunson, 1940; Blanchard *et al.*, 1941; Dicke and Jenkins, 1945), it has been demonstrated clearly that certain inbred lines regularly transmit resistance to ear-worm damage to their hybrids. Moreover, Painter and Brunson (1940) concluded that other, more subtle causes than husk protection were indicated as being concerned with resistance. They also noted, as did Richey (1944), that marked differ-

ences occurred in susceptibility to ear-worm damage to the tender young leaves or "bud." This, of course, must be apart from husk protection. Again, the mode of inheritance seems typically quantitative, but Richey (1947) found indications of linkage for a gene concerned with resistance with the $Y$ $y$ locus.

Heritable differences among inbred lines in their resistance to the corn leaf aphid, *Aphis maidis,* were reported by Walter and Brunson (1940) and by Snelling *et al.* (1940). Huber and Stringfield (1942) found a significant correlation between resistance to aphids and to European corn borer damage. They suggested that aphid resistance might be used as a criterion in selecting for corn borer resistance. More recently, Walter and Brunson (1946) report an experiment on selecting for divergent aphid reaction in three long-time self-fertilized lines. They concluded that there appeared to be a possibility for effective selection for insect resistance in some lines, but not in all. They also concluded that aphid reaction is probably inherited quantitatively on a complex factorial basis.

Differential resistance to the following insects also has been reported and presumably is heritable. Southern corn rootworm, *Diabrotica 12-punctata,* (Bigger *et al.,* 1938); chinch bugs, *Blissus leucopterus,* (Holbert *et al.,* 1935 and Snelling and Dahms, 1937); grasshoppers, *Melanoplus spp.* (Brunson and Painter, 1938); and white grubs, *Phyllophage spp.* (Hoegemeyer, 1941). In none of these cases was there any indication of simple inheritance.

## 4. *Composition of the Grain*

The inheritance of yellow *vs* non-yellow, and of sugary *vs* non-sugary endosperm are, of course, the earliest reported cases of Mendelian inheritance in corn (Correns, 1901). Both involve chemical composition. Hauge and Trost (1928) showed the association between the yellow pigment and vitamin A. Mangelsdorf and Fraps (1931) showed a direct relation between vitamin A content of the endosperm and the number of $Y$ genes present. Similar results were obtained later by Johnson and Miller (1938, 1939), and Randolph and Hand (1940) have shown that the provitamin A fraction of the carotenoids could be increased about 40% by doubling the chromosomes.

The importance of the sugary gene, *su,* needs no emphasis to those who are familiar with the immense sweet-corn production for which it is the base. Mangelsdorf (1947) recently has reported duplicate genes that determine what he has called amylaceous-sugary endosperm. One of these is an additional allele at the *Su su* locus and has been designated *su* $^{am}$. The other, *du,* is located on chromosome 10. In conjunction with

the ordinary *su* gene, *du* determines a "supersugary" endosperm. Mangelsdorf (1947) discusses the possible use of these genes in sweet corn breeding, and Cameron (1947) discusses the chemico-genetic implications in a companion paper. Andrew *et al.* (1944) note that in canning tests, sweet corn recessive for the waxy gene (*su wx*) was considered superior to sweet corn dominant at the waxy locus.

The gene for waxy corn, *wx*, was so named by Collins (1909) because of the waxy appearance it gives to otherwise starchy endosperms. According to Hixon and Sprague (1942) the waxy starch is entirely amylopectin, whereas the ordinary corn starch is about 78% amylopectin and 22% amylose. This results in a difference in properties and permits waxy starch to be used in many products in which tapioca starch had been considered indispensable. Corn varieties and hybrids with waxy starch have been developed and are being grown commercially. They were of special value during the later war years in supplying a substitute for the tapioca that was not available. An additional allele, *wx_a*, has been reported by Brimhall *et al.* (1945) as having been obtained from Argentina. So far, this allele appears to offer nothing of economic interest.

That the chemical composition of dent corn can be strikingly modified is abundantly shown by the classical experiments at the Illinois Experiment Station. After twenty-eight years of continuous selection (Winter, 1929), the protein content had been modified from an original 10.9% to 16.6% and 8.4% in the high- and low-protein selections, or a spread of 8.2%. Similarly, the oil content had been shifted from an original 4.7% to 9.9% and 1.5% in the high- and low-oil selections, or a spread of 8.4% in oil content. At that stage, there were no indications that the high-protein and high-oil strains had reached a limit; little change had occurred in the low-protein strain in twenty years, and a large percentage of germless kernels in the low-oil strain indicated that it was near a physiological limit to further reduction. These results are in accord with quantitative inheritance in general, as were those of East and Jones (1920) and Hayes (1922) working with protein content of corn under self fertilization. Student (1934) analyzed Winter's (1929) data and estimated that not less than 20-40 genes were concerned in determining oil content, possibly 200-400, and that the number was not at all likely to be of the order of 5-10.

The importance of adequate niacin in avoiding certain malnutritional disturbances is well known, and appeared to warrant a survey of the possibilities of breeding for higher niacin content. The following statements are based on the report of such a survey by Richey and Dawson (1948). Varieties of corn differ widely in niacin concentration,

the southern varieties tending to be higher. Inbred lines also differ, the range for twenty-four established inbreds being from 13.9 to 53.3 micrograms per gram. Hybrids tended to be intermediate between their parents, with the seed parent generally exercising more influence than the pollen parent. Three generations of plus and minus selection in self-fertilized lines from a variety with an average of 22.0 micrograms per gram (and an indicated maximum for individual ears of about 37 micrograms) resulted in strains averaging from 14.3 to 42.7, and with one ear having 50.6 micrograms per gram.

Evidence was completely for ordinary quantitative inheritance in this material, dent corn. The results of Mather and Barton-Wright (1946), however, indicated the *Su* gene for starchy seeds as dominant for lower niacin concentration than its *su* allele for sugary. This appears to be a pleiotropic effect of the *Su su* alleles.

## V. CONCLUDING REMARKS

The foregoing discussion has been centered primarily around methods of breeding and the characters to be controlled. In concluding, it is desirable to summarize briefly from the standpoint of the different kinds of genes that are involved. The four categories used are those of convenience, as is the order of their consideration. In the first category may be placed qualitative genes, genes with major individual influence on characters of immediate practical importance. Examples are grain color, texture, or composition, disease resistance, and the like. Action may be dominant or intermediate, and monogenic or more complex. Such genes are amenable to the simplest breeding technics and provide no serious problem. A second category, also offering no difficulty, comprises grossly deleterious recessives. These are eliminated rapidly and almost automatically under modern breeding methods.

The genes in the remaining two categories have small individual effects. In one group are the genes controlling multiple-factor, quantitative, or blending inheritance. They generally lack dominance and are cumulative in effect, arithmetically, or geometrically. The cumulative influence may be immediately apparent, or not expressed until some threshold is reached. Several different genes may have equal effects and therefore be indistinguishable individually. Their existence can be proven, however, through linkage tests. The genes with small individual effects comprising the fourth category are those involved in hybrid vigor, if the interaction of dominant favorable genes is the correct explanation of that phenomenon. Complete or partial dominance of the more favorable alleles must, of course, be the rule for these genes. It is the genes in these two categories that are all important in corn breeding. To-

gether they determine the quantitative potential of the plant and the final expression of that potential as limited by the efficiency of functioning. They are the "yield genes" producing the results obtained in the corn breeding experiments that have been reviewed in the preceding pages. Because of their small individual effects they are recognized only in their mass actions, and the interaction of the two groups can not always be differentiated. Thus, a plant may produce a small ear either because it carries genes for small ears, or because it functions too inefficiently to permit production of the potentially larger ear. The breeder accordingly must work with both groups more or less as a whole.

In its first stages, hybrid corn breeding isolated inbred lines with better than average genes in both of these categories. Crossing such lines tended to suppress the recessive genes for defective functioning and, in the better hybrids, to bring the resulting more efficient functioning into combination with superior quantitative and qualitative potentials. It has now been demonstrated that superior inbreds can be obtained from the higher genetic levels of hybrids among the initially isolated lines. The difficulty of further improvement of course is greater, but the reason for that difficulty remains the same: that of selecting for mass actions by large numbers of genes with small individual effects. How much farther empirical progressive improvement can go is uncertain, but until more efficient methods are devised it appears the only recourse.

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# Parthenogenesis in Animals*

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## Contents

## I. Modes of Reproduction in Animals

One of the basic phenomena in living organisms is fertilization, *i.e.,* the fusion of two sexually differentiated cells and nuclei, which sooner or later is followed by a chromosome reduction. Fertilization together with the alternation of nuclear phases is an essential condition for the continuation of life, *i.e.,* reproduction in most organisms, namely in all those with sexual differentiation. A number of animals and plants are, however, capable of reproduction without fertilization. For more detailed information see the reviews of Hartmann (1929) and Hämmerling (1940). Both one- and many-celled animals may reproduce *asexually.* Many Protozoa are able to reproduce asexually in that a one-celled individual divides into two cells, which phenomenon belongs to *agamogony.*

The asexual reproduction of Metazoa again is *vegetative,* the reproduction taking place by means of cell groups consisting of a number of somatic cells. The most common mode of animal reproduction is, however, *sexual reproduction or gamogony or zygogenesis.* In this case the reproduction is carried out by sexually differentiated male and female gametes which possess the reduced or azygoid ("haploid") chromosome number. In fertilization they fuse forming a zygote which thus receives an unreduced or zygoid ("diploid") number of chromosomes. In many animals fertilization fails, however, secondarily. These animals develop *parthenogenetically by which is meant the development of the egg cell into a new individual without fertilization.* The first to use the term parthenogenesis in this sense was v. Siebold in 1856. (If a male gamete develops without fertilization the phenomenon is called *androgenesis.* This is accomplished in a way in the merogony experiments carried out in certain animals. In them a nucleus-free egg is fertilized and the nucleus of the sperm develops further in the plasm of the egg.) Parthenogenesis is the prevailing mode of reproduction in the animals in which the fertilization fails secondarily.

In plants we speak of mictic reproduction, its opposite being named apomixis, which means reproduction without mixis, *i.e.,* without both the fusion of two reproductive cells and adjoining alternation of nuclear phases. To this apomictic reproduction belong, in addition to parthenogenesis, other modes of reproduction; for details see the reviews by Rosenberg (1930), Fagerlind (1940) and Gustafsson (1935, 1944, 1946, 1947a and 1947b). This depends on the fact that plants have a distinct alternation of generations in that a zygoid sporophyte and an azygoid gametophyte alternate, which increases the possibilities of different apomictic types of reproduction. It is, however, to be noticed that parthenogenesis is not a part of apomixis, as is sometimes supposed, but a quite different group of reproductive phenomena. Thus parthenogenesis comprises such types of reproduction as cannot be included in apomixis since they are of mictic nature, *e.g.,* automictic parthenogenesis; see p. 207ff., below. The result of this is that systems made of the modes of reproduction in plants (*cf.* Fagerlind and Gustafsson, *op. cit.*) do not as such apply to animals.

## II. Occurrence of Parthenogenesis in Animals

Parthenogenesis is a very common phenomenon in the animal kingdom, forms with parthenogenetic reproduction being found in most animal groups. It is consequently natural that parthenogenesis and cytological questions connected with it have been much studied, the respective literature being very extensive. Concerning this subject we have

also a number of comprehensive reviews in regard to animal partheno-
genesis. We may refer especially to the following, Winkler (1920), Ankel
(1927, 1929), Vandel (1931a) and White (1945, p. 267-302).

It is not possible here to mention all the animal groups in which
parthenogenesis has been found; for details see Vandel's review (1931a,
p. 18ff.). From this paper we notice that in few of the greater animal
groups are parthenogenetically reproducing forms absent. In vertebrata
normal parthenogenesis is unknown (with the possible exception of cer-
tain fish hybrids) though some vertebrata have been artificially induced
to reproduce parthenogenetically. Neither has parthenogenesis so far
been found in such big insect orders as the dragon-flies, Odonata, or the
true bugs, Hemiptera (Heteroptera). [In the present paper the system
and nomenclature of Storer (1943) have been used.]

The various animal groups differ greatly in regard to the frequency
of parthenogenetic forms. Some big animal groups are wholly (or almost
wholly) characterized by parthenogenetic reproduction. Such are the
flukes (Trematoda: order Digenea), rotifers, water fleas (Cladocera) and
aphids (these all have a heterogonous alternation of generations; *cf.*
p. 235ff.). In moths parthenogenesis is found in several Psychids, though
parthenogenetic forms are exceptions among other Lepidoptera. A sim-
ilar situation prevails for instance in beetles in which parthenogenesis
is common only in a few subfamilies of weevils. In a large number of
animals parthenogenetic forms occur only sporadically among bisexual
forms.

### III. Systems of Parthenogenesis

Parthenogenesis is a very wide collective concept. It can be sub-
divided into several types which in turn may be organized into different
systems. Thomsen (1927, p. 79) has already pointed out that in sys-
tematizing the cases of parthenogenesis three different principles may be
followed. The different cases of parthenogenesis may thus be consid-
ered from the following points of view (a) mode of reproduction, (b) sex
determination, and (c) cytology.

If we consider parthenogenesis with reference to *reproduction* it may
be classified as follows:

A. Occasional or accidental parthenogenesis or tychoparthenogenesis.
   Unfertilized eggs develop occasionally through parthenogenesis.
B. Normal parthenogenesis.
   1. Obligatory parthenogenesis. The egg develops always parthe-
      nogenetically.
      a. Constant or complete parthenogenesis. All generations are
         parthenogenetic.

    b. Cyclical parthenogenesis. One or more parthenogenetic generations alternate with a bisexual generation. (Heterogony)

    c. Paedogenesis. The eggs of individuals at larval stage develop parthenogenetically. Is closely connected with cyclical parthenogenesis.

  2. Facultative parthenogenesis. An egg may either be fertilized or develop parthenogenetically.

Although most of these terms were already used in the above sense at the end of the nineteenth century, they were built up into a system in the beginning of this century.

In regard to *sex determination* the following three types of parthenogenesis may be distinguished:

  A. Arrhenotoky. Unfertilized eggs develop parthenogenetically into males. In all these cases fertilized eggs develop into females.

  B. Thelytoky. Unfertilized eggs develop into females.

  C. Deuterotoky or amphitoky. Unfertilized eggs develop into both sexes.

This terminology too was used at the end of the last century; *cf.* Winkler (1920, p. 15).

On the basis of cytological data the cases of parthenogenesis have been classified in several ways. A more detailed recapitulation of these systems is found in Suomalainen (1940b, p. 11-18). In the oldest systems, *viz.*, the ones of Winkler (1908, 1920) and Hartmann (1909), only two main groups are differentiated. Winkler distinguishes somatic and generative parthenogenesis. The first group comprises the cases in which chromosome reduction is absent in the developing eggs, the second those cases in which reduction takes place. Hence generative parthenogenesis according to him includes also the cases in which chromosome reduction at first occurs but in which the zygoid chromosome number is later restored. Hartmann again uses the terms diploid and haploid parthenogenesis as these, according to him, better describe the facts. This terminology does not, however include the cases of polyploid parthenogenesis.

In the systems of parthenogenesis by Thomsen (1927, p. 86-89) and Ankel (1929, p. 331-342) based on cytological conditions, three main groups are distinguished. It is true that Thomsen also regards generative parthenogenesis as the opposite of somatic parthenogenesis but his definition of these terms differs from the one of Winkler. Somatic parthenogenesis is thus divided by Thomsen into automictic and apomictic

parthenogenesis. Ankel again regards the three types as equivalent. Both in Thomsen's and Ankel's systems emphasis is laid on the following points: whether the chromosome number of the individual concerned is zygoid or azygoid, and how the zygoid number is maintained. In this way we obtain the following system (the definitions are somewhat changed):

A. Generative or haploid parthenogenesis. Partheno-produced individuals develop from eggs in which a chromosome reduction takes place, receiving consequently an azygoid (haploid) number of chromosomes.

B. Somatic parthenogenesis. Partheno-produced individuals have a zygoid (diploid or polyploid) chromosome number.
   1. Automictic parthenogenesis or parthenogamy. Regular chromosome conjugation and reduction occur in the eggs developing parthenogenetically. The zygoid chromosome number is restored through the fusion of two azygoid nuclei, the formation of a restitution nucleus or endomitosis. This corresponds to White's (1945) meiotic parthenogenesis.
   2. Apomictic parthenogenesis. Neither chromosome reduction nor fusion of nuclei nor any corresponding phenomenon (see above) takes place in the eggs developing parthenogenetically. This type corresponds to the somatic parthenogenesis of Winkler and Ankel and the ameiotic parthenogenesis of White (1945).

In the opinion of Whiting (1945) the division just described may give rise to confusion and useless discussion, since the chromosome number as a result of somatic polyploidy often fluctuates in different tissues, which makes the criteria for haploidy frequently uncertain. Therefore he extends the term haploid parthenogenesis to comprise all cases of origin from a single reduced maternal nucleus irrespective of whether the animals are later haploid or not. He thus uses the term in the same sense as Winkler. In my opinion the system of Thomsen, described above, does not cause confusion, if we only hold to the conception that diploidy and haploidy in this connection refer to the chromosomal conditions in the germ line nuclei.

It may be mentioned that the unfertilized eggs of many normally bisexual species begin to develop, although the development as a rule sooner or later comes to a standstill. We have in this case a rudimentary or incomplete parthenogenesis: *cf.* Vandel (1931a, p. 275-278). In many animal groups unfertilized eggs may be induced to develop parthenogenetically with the aid of different methods (certain chemicals,

mechanical irritation, temperature changes, etc.). This artificial parthenogenesis is not dealt with in the present paper; for details see the reviews by Tyler (1941) and Peacock (1944).

## IV. RACES WITH DIFFERENT MODES OF REPRODUCTION

In many parthenogenetic animals—in all those with obligatory parthenogenesis—reproduction is exclusively parthenogenetic. In such forms males are usually completely unknown.

In other parthenogenetic animals both parthenogenetic and zygogenetic reproduction are present. Such is the case in cyclical parthenogenesis in which parthenogenetic generations alternate with a bisexual generation. Other species again occur as two races, the one bisexual and the other parthenogenetic. These will be considered later (p. 223ff.).

A single species may also include several different parthenogenetic races. So, for instance, two such races are known in the white-fly, *Trialeurodes vaporariorum*. In the so-called "American" race parthenogenesis is facultative and arrhenotokous (Schrader, 1920). The eggs undergo a reduction and may either be fertilized or develop through parthenogenesis. In the former case a diploid female is produced, in the latter a haploid male. The parthenogenesis of the so-called "English" race of the same species is obligatory and thelytokous (Thomsen, 1927). The diploid chromosome number is here restored through automixis (for details *cf.* p. 209). Corresponding races have been found in the saw-fly, *Diprion polytomum* (Smith, 1941). Its two races even have divergent chromosome numbers, the arrhenotokous race possessing twelve chromosomes (2n), the thelytokous race 14. Also in certain other saw-flies corresponding races have been observed (Comrie, 1938) though they have not been studied in detail.

In the coccid genus Lecanium (Thomsen, 1927)—at least in *L. hesperidum* and *L. hemisphaericum*—there are two different parthenogenetic races. In one of them, the so-called bisexual-parthenogenetic race, parthenogenesis is facultative, in the other obligatory. In both cases parthenogenesis is thelytokous. Both races are diploid. The eggs of the former race undergo two meiotic divisions and may either be fertilized or develop parthenogenetically. The fertilized eggs give rise both to males and females, the parthenogenetic eggs exclusively to females. In the latter case the diploid chromosome number is restored through automixis (*cf.* p. 209). Males are unknown in the race with obligatory parthenogenesis, which is of apomictic character.

Also animals with cyclical parthenogenesis may occur as biologically divergent races, because the bisexual phase of the cycle may fail secondarily.

## V. Chromosomal Conditions in Parthenogenetic Animals

### 1. *Generative or Haploid Parthenogenesis*

In generative or haploid parthenogenesis (*cf*. p. 197) a regular chromosome conjugation and reduction take place in the eggs. In spite of this the eggs may develop either through fertilization or without it. In the former case the egg gives rise to a zygoid (diploid) female, in the latter again to an azygoid (haploid) male. The eggs of a single female may thus develop into both females and males, the latter being of parthenogenetic origin. Generative parthenogenesis is therefore always facultative and arrhenotokous. [Mittler (1946) has stated that virgin females of the white-fly *Trialeurodes vaporariorum*, when subjected to high temperature (about 38°C.), may produce parthenogenetically in addition to the males occasional females too, which are diploid. These exceptional females arise probably by means of high temperature through an altered maturation process, the diploid number of chromosomes being thus retained in the egg.] The haploidy of the male and the diploidy of the female are to be understood in such a way that the germ-line nuclei in the male contain half the number of chromosomes found in the germ-line of the female.

This type of parthenogenesis has been observed in the following animal groups: Rotifera, Homoptera (certain coccids and aleurodids), Thysanoptera, Coleoptera (Micromalthus), Hymenoptera and Acarina (Tetranychidae and Tarsonemidae).

Of the reviews concerning generative parthenogenesis we may refer to the ones by Schrader and Hughes-Schrader (1931), White (1945, p. 267-279) and Whiting (1945).

*Oogenesis.* In all animals belonging to this type, oogenesis is regular. The chromosomes pair in the oocytes in the usual way, and the bivalents result from conjugation and chiasma-formation. Crossing over occurs accordingly in the oocytes. The eggs undergo two meiotic divisions which reduce the chromosome number.

*Spermatogenesis.* Since all the males of this type are haploid, their spermatogenesis occurs without chromosome reduction. Spermatogenesis is, in consequence, exceptional, as the chromosomes do not pair nor cross over. As a result usually only one equational, meiotic division is present. This division may, however, reveal meiotic features, for instance in certain animals the chromosomes are more contracted than in mitosis. Reduction may also fail in other ways. The different possibilities are described in the following.

(a) *The first meiotic division is abortive.* This type of spermatogenesis is found in most Hymenoptera studied; the only exceptions are

*Telenomus fariai* and possibly a few Polistes species (*cf.* below). The Hymenoptera have both cytologically and genetically been more extensively studied than other animals with generative parthenogenesis. This animal group has therefore been of great value for our knowledge of male haploidy. Of the numerous papers dealing with the cytology of the Hymenoptera the following may be mentioned (for papers on the thelytokous Hymenoptera *cf.* pp. 208 and 214; Sanderson's (1932) paper contains a detailed survey of the work done on the cytology of Hymenoptera published up to 1932):

Suborder 1. Chalastrogastra.

Horn-tails, Siricidae: *Sirex cyaneus* (Peacock and Gresson, 1931).

Saw-flies, Tenthredinidae: *Pteronidea ribesii* (Sanderson, 1932), Diprion (5 species) and *Neodiprion sertifer* (Smith, 1941).

Suborder 2. Clistogastra.

Braconid wasps, Braconidae: *Habrobracon juglandis* (Torvik-Greb, 1935; Speicher, 1936).

Scelionid wasps, Scelionidae (belongs to Proctotrupoidea subfamily): *Telenomus fariai* (Dreyfus and Breuer, 1944) (*cf.* p. 201).

Chalcid wasps, Chalcididae: *Paracopidosomopsis floridanus* (Patterson and Porter, 1917; Patterson, 1917), *Pteromalus puparum* (Dozorceva, 1936), *Melittobia chalybii* (Schmieder, 1938).

Gall wasps, Cynipidae: *Neuroterus baccarum* (Doncaster, 1910, 1911, 1916; Dodds, 1938, 1939), 7 other species (Dodds, 1938).

Ants, Formicidae: *Camponotus herculeanus* (Lams, 1908).

Colonial wasps, Vespidae: *Vespa crabro* (Meves and Duesberg, 1908), Polistes (5 species) (Machida, 1934; Pardi, 1947) (*cf.* p. 203).

Bees, Apidae: *Apis mellifica* (Nachtsheim, 1913), *Osmia cornuta* (Armbuster, 1913).

In most Hymenoptera the first division in the male is abortive. A first division spindle is formed (in some species a unipolar spindle, in others a bipolar one), but the chromosomes do not divide. In the first division of a number of Hymenoptera (*e.g.,* Habrobracon, Paracopidosomopsis, Vespa, Apis) the division of the cytoplasm is carried out so that a non-nucleated cytoplasmic bud is pinched off and soon degenerates. In others (*e.g.,* Pteronidea, Diprion, Melittobia) not even the plasm divides in the first division. In the second meiotic division of the male the chromosomes divide mitotically (except in Telenomus). In this division the cytoplasm divides equally, resulting in the formation of two functional spermatids from each primary spermatocyte. Only in the apids does the cytoplasm divide unequally in the second division, since in them also this division results in the formation of a small, but this time nucleated bud. In the apids every primary spermatocyte gives rise to only one functional spermatid.

It is to be noticed that in Habrobracon spermatogenesis is similar in the haploid and diploid males (Torvik-Greb, 1935). Although the

spermatocytes of the latter possess a diploid number of chromosomes, the chromosomes do not pair and the first meiotic division is abortive. The resulting spermatids will thus be diploid. A corresponding case has been found by Smith (1941, p. 276) in the exceptional diploid spermatocytes of *Diprion polytomum*. This proves that the special type of meiosis found in the hymenopteran males is genetically determined.

*Micromalthus debilis*, the only beetle with generative parthenogenesis found so far, resembles the Hymenoptera as to its spermatogenesis (Scott, 1936, 1938). This species, which is the only representant of its genus and family, combines parthenogenesis with paedogenesis (*cf.* p. 237). Its four different kinds of reproductive females (three of them are larvae) are diploid (2n = 20). The male develops through parthenogenesis and is haploid somatically too at both early and later stages of development. The first spermatocyte division is completely abortive as regards the division of the chromosomes and the cell. A unipolar spindle is, however, formed as a rule. The chromosomes in the first anaphase—resembling mitotic chromosomes—move away from the single pole with their centromeres hindermost. The second spermatocyte division is in all respects a normal mitosis, and results ultimately in the formation of two normal sperms. It is not known whether the males actually fertilize the adult females, *i.e.*, if Micromalthus has any sexual reproduction (*cf.* p. 237).

(b) *The second meiotic division is abortive.* *Telenomus fariai* which belongs to the family Scelionidae deviates considerably from other Hymenoptera in its spermatogenesis (Dreyfus and Breuer, 1944). Its first meiotic division is an ordinary mitosis and gives rise to two secondary spermatocytes. The second division in turn is abortive, one of the resulting cells being a small non-nucleated bud. The last spermatogonial division too, seems to be unequal in regard to the cytoplasm. Only the larger daughter cell forms a primary spermatocyte whereas the smaller degenerates.

(c) *Only one equational meiotic division takes place.* In certain animals belonging to this group, in spite of the occurrence of only one equational division, some relics of meiotic character are found, *viz.* a long prophase and the intensive contraction of the metaphase chromosomes (iceryine coccids) or a distinct growth stage in the spermatocytes (*Aleurodes proletella*, Polistes). Other forms again (*Trialeurodes vaporariorum*, acarids) differ in this respect, the spermatocyte division being similar to the spermatogonial divisions and other mitoses.

With the exception of certain thelytokous forms, the rotifers have a heterogonous alternation of generations. In them the obligatorily thelytokous generations alternate with generations in which partheno-

genesis is facultative and arrhenotokous, being thus representatives of amphigony (*cf.* pp. 235-236). The eggs of the thelytokous females thus undergo only one equational meiotic division (*cf.* p. 213). In the eggs of the arrhenotokous and gamic female the chromosomes pair regularly, and the eggs undergo two meiotic divisions. Such a haploid egg may develop either into a diploid female or a haploid male, depending on whether the egg is fertilized or not. Studies concerning the cytology of the rotifers (for a detailed account see the review by Ankel, 1927) have given contradictory results, and many details need reinvestigation. Some authors regard even the haploidy of the rotifer males as doubtful. According to Whitney (1929) the males of *Asplanchna amphora* are, however, haploid. They produce two types of sperms. The bigger ones which develop directly from the secondary spermatocytes arising as a result of one meiotic division, have a haploid number of chromosomes. A part of the secondary spermatocytes divides again, however, with an irregular distribution of chromosomes. Through this division small spermatids are formed, which develop into small non-functional spermatozoa.

In the order Homoptera the males of iceryine coccids and certain white-flies are haploid and develop parthenogenetically. Of the former group the following animals have been studied as to their mode of reproduction and cytology: *Icerya purchasi* (Schrader and Hughes-Schrader, 1926; Hughes-Schrader, 1927), *I. littoralis, I. montserratensis, Echinicerya anomola, Crypticerya rosae* (Hughes-Schrader, 1930a and 1930b) and *Steatococcus tuberculatus* (Hughes-Schrader and Ris, 1941). In all these species the male possesses only two chromosomes and the female four. Spermatogenesis in the haploid males involves a single meiotic division of equational character (*cf.* however, p. 201). *I. purchasi* differs from the other species just mentioned in that the females have become hermaphrodites, and the males are very rare. The hermaphrodite individuals are not able to copulate with each other, but only with the males. Usually they are, however, self-fertilizing. In their gonads both eggs and sperms are formed. A part of the cells in the center of the gonads is already at an early stage converted from diploid into haploid through a process unknown in detail. These cells give rise to the testis tissue of the hermaphrodite gonads, the ovarial part being formed by the more peripheral diploid cells. Hermaphrodite individuals are thus diplohaploid mosaics. Their spermatogenesis is similar to that of the males, that is to say only one meiotic division of equational nature takes place. It is, however, to be noticed that in exceptional cases sperms may arise from diploid cells also in relatively restricted regions of the gonad. These cells undergo two meiotic divisions which result in the reduction of the chromosome number, which becomes haploid though no synapsis is

present. The first division is equational for all four chromosomes, and the second division effects a reduction in that two chromosomes go to each pole. This suggests that "the single equational division of the male Iceryini is not a genotypically determined character, but is merely a mechanical consequence of haploidy" (White, 1945, p. 276).

In most aleurodids studied, *viz.*, in the arrhenotokous ("American") race of *Trialeurodes vaporariorum* (Schrader, 1920), in *Aleurodes proletella* and in *Dialeurodes citri* (Thomsen, 1927) the males are haploid and develop parthenogenetically. Also in their spermatocytes only one equational meiotic division is present (*cf.* p. 201). The parthenogenesis of the "English" race of *Trialeurodes vaporariorum* is obligatory and thelytokous (*cf.* p. 209).

In certain Polistes wasps, contrary to other Hymenoptera, the first spermatocyte division is, according to Machida (1934), "entirely wanting"; it is not present even as abortive. After the growth period only one equational division takes place and is followed by the metamorphosis into spermatozoa. This needs, however, reinvestigation especially as Pardi (1947) has in two other Polistes species observed that spermatogenesis contains an abortive first division in accordance with other Hymenoptera.

The haploidy of the male and the generative parthenogenesis are very pronounced in the mites belonging to the families Tetranychidae and Tarsonemidae, of which the following species have been investigated: *Tetranychus bimaculatus* (Schrader, 1923), *Pediculoides ventricosus* (Pätau, 1936) and *Pediculopsis graminum* (Cooper, 1939). In all these species the male has three and the female six chromosomes. The spermatogenesis of the two latter species is very imperfectly known, but probably all these species have a single equational maturation division. According to Schrader no meiotic features can be discerned in it, for which reason it may be said that the last spermatogonia develop into spermatozoa. In certain other mites arrhenotoky has also been observed, whereas the males of still other species are diploid. Thelytoky has also been found in some mites (*e.g.*, Amblyomma). A more detailed account of these data is found in the papers of André (1935) and Whiting (1945, p. 252-253).

Arrhenotoky is found in a number of thrips (*e.g. Anthothrips verbasci*, Shull, 1917). Unfertilized eggs develop only into males, while the offspring of mated females contain both sexes. The parthenogenesis of thrips (both arrhenotoky and thelytoky) is cytologically wholly unanalyzed, but it is probable that the males of the arrhenotokous species are haploid. Many thrips are thelytokous.

*The chromosome number in the somatic cells.* Although the males

of the animals described above develop through parthenogenesis from haploid eggs, many tissues of the male (as well as of the female) are highly polyploid, as the chromosome number has been multiplied endomitotically. This somatic polyploidy is especially conspicuous in some Hymenoptera. It is, however, probable that at least most hymenopteran males even in their polyploid tissues contain half the number of chromosomes found in the corresponding tissues of the female. This is suggested by Sanderson's (1932, p. 387) observations on the saw-flies. In regard to the honeybee Whiting (1945, p. 237) presents the possibility "that, due to regulation [for details see Nachtsheim, 1913], the drone bee has, in general, the same chromosome numbers as the female, although it originates from a haploid egg. Nuclei of the male approximate in size those of the female." This is, however, uncertain. As to the size of the cells in the both sexes we must notice that although in Habrobracon the diploid male cell size is considerably larger than the diploid female cell size, and the haploid male cell size approaches the diploid female cell size, this does not justify the assumption that the haploid male would have in its somatic cells the same number of genomes and the diploid male double the number, as compared with the (diploid) female. The relative size of the cells depends rather on the effect of the genes situated in the chromosome segment acting as a sex allele (see below), which effect is stronger in the male (Whiting, 1945, p. 244; Grosch, 1945). Maleness as such is an effective factor in regard to cell size.

In some animals (Micromalthus, Acarida) many tissues of the male even at a late stage are still haploid.

*Sex determination.* In generative parthenogenesis, parthenogenesis itself has become a means of sex determination. It is clear that in these animals the sex cannot be determined by the same principle as in the animals in which sex determination is founded on the sex chromosome mechanism and on the balance between male- and female-determining genes. In these organisms the quantitative relation of the sex chromosomes and the autosome genomes, and hence the relation of the sex determining genes, differs in the two sexes, forming the basis of sex determination. In animals with generative parthenogenesis the females are diploid, but the males haploid; if they possessed sex chromosomes, the relation of the sex chromosomes and the autosome genomes would thus be the same in the two sexes. Sex determination must take place in them according to some other principle. The numerical relation of the two sexes shows considerable variation in these animals, deviating remarkably often from 1:1.

The studies of P. W. and A. R. Whiting (1925) and A. R. Whiting (1927) on Habrobracon have thrown new light on these questions.

It was shown by them that in this animal fertilized eggs may on close inbreeding also develop into diploid males. These are, however, of low viability and almost sterile. It was thus proved that Habrobracon males need not inevitably be haploid. That the diploid males, in fact, develop from fertilized eggs may be demonstrated genetically, as they receive paternal in addition to maternal genes. P. W. Whiting has later shown (1940, 1943, 1945) that the sex in Habrobracon is determined by a series of multiple alleles, of which at least nine different ones are known $(x_a, x_b—x_i)$. An individual which has any two different alleles is female, and an individual which has only one (haploid individual) or two similar alleles (homozygous individual) is male. The homozygosity of the diploid males is explained by the fact that they arise by close inbreeding. It is to be noticed that according to Whiting each member of these allelic series is to be regarded, not as a single gene, but as a chromosome segment, perhaps relatively long and containing many different genes, which do not cross over. Sex determination in Habrobracon is, in a sense, polygenic, but as crossing over does not take place between the segments, the various groups of genes act as single allelic factors. It is clear that the genes for secondary as well as for the primary sex characters must be located within these segments.

The question thus arises whether sex determination in other animals with generative parthenogenesis is similar to that found in Habrobracon. Whiting (1945; 1947, p. 16) is of the opinion that "the complementary scheme of sex determination may, at least tentatively, be extended to other hymenopterons and to the remaining groups of invertebrate animals with haploid males." The same type of sex determining mechanism as is found in Habrobracon may, to begin with, be supposed to prevail in other hymenopterans, as the generative parthenogenesis of the Hymenoptera probably has a common origin. According to Dozorkeva (1936) the sex of Pteromalus is determined in the same manner as in Habrobracon, and recently the same condition has been shown in the honeybee (cf. Whiting, 1947). It is true that diploid males are not known in other animals of this type except in Habrobracon, and even in it only in laboratory breeds. This fact may, however, depend on a complete inviability of the diploid males. In the honeybee there is definite evidence of a series of lethal alleles which act as do the sex alleles of Habrobracon. The Habrobracon system of sex determination may thus possibly characterize the whole order Hymenoptera.

This system cannot, however, be prevalent in forms which always have a close inbreeding. Such are e.g., the egg-parasite Telenomus (Dreyfus and Breuer, 1944) in which the daughters and sons of a single mother copulate before leaving the egg shell of the host, and the chalcid

wasp Melittobia (Schmieder, 1938; Schmieder and Whiting, 1947) in which the first eggs of a virgin female develop parthenogenetically into males, the first of which may even mate its own mother. In this case one of the sex alleles of the female must be the same as in the male. If the diploid homozygous males were inviable, one half of the fertilized eggs ought to remain undeveloped. This does not happen, at least in Melittobia, in which 90% of the eggs in highly inbred females give rise to females. This suggests that the close-crossed species of Hymenoptera may possibly have some method of sex determination fundamentally different from that in Habrobracon. Schmieder and Whiting (1947) suppose that multiple sex allelism might be the more primitive and general method of reproductive economy in animals with generative parthenogenesis, and that the close-crossed species have adopted some other system.

*Icerya purchasi*, which as a rule is self-fertilizing, probably behaves in the same manner as the close-crossed Hymenoptera. If its sex determination is similar to the one found in Habrobracon, a considerable part of its eggs ought to remain undeveloped. Correspondingly in *Trialeurodes vaporariorum* which has both an arrhenotokous and an obligatorily thelytokous race, the heterozygosity of the sex alleles would be lost in the thelytokous race, when the diploid chromosome number is reestablished by means of a doubling of the chromosome set in the first cleavage nucleus (*cf.* p. 209). The conditions partly correspond in the thelytokous race of the saw-fly, *Diprion polytomum,* which also has both an arrhenotokous and a thelytokous race. In this case the diploid chromosome number is restored, when the second polar nucleus fuses with the egg nucleus. Heterozygosity would be maintained only if the chiasmata in the bivalent containing the sex alleles were formed in such a manner that these alleles would divide post-reductionally (for details *cf.* Smith, 1941). This suggests that, in addition to certain Hymenoptera, in other animals with generative parthenogenesis sex might also be determined on some other principle. It does not seem probable that such a complicated sex determining mechanism as that of Habrobracon would have arisen independently in seven widely differing animal groups.

Some authors (Dozorkeva; Dreyfus and Breuer) suggest that they have found in the Hymenoptera, studied by them, a heteromorphic chromosome pair, and regard these chromosomes as a morphologically differentiated pair of sex chromosomes. The matter needs reinvestigation, however, as it is difficult to understand how Hymenoptera could possess such sex chromosomes. Bauer (1937, p. 4) thinks that the different size of the chromosomes might depend on genetically inactive

heterochromatin, the heteromorphic chromosomes representing a pair of autosomes.

## 2. *Automictic Parthenogenesis*

In automictic parthenogenesis (p. 197) the early stages of meiosis in the egg are quite normal, *i.e.*, similar to the ones in animals with fertilization. The chromosomes pair at zygotene, crossing over occurs between them, and they form bivalents. As a result of chromosome reduction during the meiotic divisions the zygoid nuclear phase in the eggs becomes azygoid. The zygoid phase is, however, soon restored in that two azygoid nuclei (the egg nucleus and the second polar nucleus, or two cleavage-nuclei) fuse. The fusion of the nuclei of different animals in fertilization, amphimixis, is replaced in automixis by the fusion of two nuclei of a single individual. In a number of automictic forms the same result is attained in that the halves of the divided chromosomes, which are usually included in separate nuclei in the second division, remain in the same nucleus owing to the formation of a restitution nucleus or some kind of endomitosis. Since in automictic parthenogenesis (at least in typical cases) the nuclear phases alternate and the nuclei fuse (mixis), this kind of parthenogenesis cannot be included in apomictic reproduction (*cf.* p. 194). The animals developing as a result of this type of parthenogenesis always have a zygoid soma.

This mode of reproduction, which is very rare in higher plants (*cf.* Gustafsson, 1946, p. 27), is relatively common in animals. So far it has been found in the following species:

Nematoda: *Rhabditis monohystera.* Female-producing eggs, certain thelytokous Rhabditis species (Bělař, 1923, 1924). The parthenogenesis of the Rhabditis species is pseudogamy, *i.e.* the eggs develop only if the sperm penetrates them, though the sperm then degenerates without taking part in the later development of the egg.

Crustacea: Anostraca. The parthenogenetic races of *Artemia salina* (Artom, 1911, 1931; Gross, 1932; Barigozzi, 1936, 1946; Haas and Goldschmidt, 1946).

Insecta. Orthoptera: Phasmidae. Parthenogenetic phasmids (Cappe de Baillon *et al.*, 1934a and b, 1935, 1937, 1938).

Orthoptera: Acrididae. Tychoparthenogenetic eggs of the tettigids (Nabours, 1925, 1929, 1937; Robertson, 1930).

Homoptera: Aleurodidae. *Trialeurodes vaporariorum*: theiytokous race (Thomsen, 1927).

Homoptera: Coccidae. The parthenogenetic eggs of the bisexual-parthenogenetic races of *Lecanium hesperidum* (Thomsen, 1927, 1929) and *L. hemisphaericum* (Suomalainen, 1940c).

Lepidoptera: Psychidae. The thelytokous races of *Solenobia triquetrella* and *S. lichenella* (Seiler, 1923, 1927, 1939; Seiler and Schaeffer, 1941), *Apterona helix* (Narbel, 1946).

Hymenoptera: Tenthredinidae. *Pristiphora pallipes* (Comrie, 1938), the thelytokous race of *Diprion polytomum* (Smith, 1941).
Hymenoptera: Ichneumonidae. *Nemeritis canescens* (Speicher, 1937).
Hymenoptera: Cynipidae. The female-producing eggs of the spring generation in *Neuroterus baccarum* (Doncaster, 1910, 1911, 1916; Dodds, 1939).

*Pairing of chromosomes in the oocytes.* The maturation of the automictic eggs is clearly of meiotic nature. The chromosomes pair at zygotene, and crossing over and chiasma formation take place in the usual manner. As a result of this, bivalents are present in the later phases of oogenesis. The presence of chiasmata has been verified in the parthenogenetic eggs of at least the following animals: the tettigids, the bisexual-parthenogenetic race of *Lecanium hesperidum*, the thelytokous race of *Diprion polytomum* and the spring generation of *Neuroterus baccarum* (only indirect evidence). The possible formation of chiasmata in the eggs of the parthenogenetic psychids is difficult to observe owing to the small size and strong contraction of the chromosomes. The question of chiasmata in these psychids is open, especially as certain Lepidoptera do not seem to have any chiasmata in the oocytes (*cf.* Bauer, 1939, p. 587). Though some forms with automictic parthenogenesis (Artemia, certain phasmids, Solenobia) are polyploid, no multivalents have been observed in them.

*Meiotic divisions.* The eggs undergo two meiotic divisions (one of them may be abortive). In the eggs of all parthenogenetic animals with automixis the metaphase plates in the first meiotic division reveal the azygoid number of bivalents. In most of them the first division is morphologically reductional, the second equational. In coccids (and possibly in aleurodids) we find the opposite situation. These animals have post-reduction, *i.e.* the first meiotic division is equational and the second reductional (Ris, 1942). The meiotic divisions result in the reduction of the chromosome number in the eggs. In some animals of this type the second meiotic division is, however, ineffective (see below). In the female-producing eggs of the spring generation in the gall wasp, *Neuroterus baccarum,* and in the eggs of the psychid *Apterona helix* no polar nuclei are formed (*cf.* pp. 211-212).

*Restoration of the zygoid chromosome number.* Since chromosome reduction occurs in the automictically parthenogenetic eggs, the zygoid chromosome number must later be restored, as animals of this type have a zygoid soma. This may happen in different ways.

(*a*) *The cleavage nuclei fuse.* This mode is represented by the thelytokous races of *Solenobia triquetrella.* The tetraploid race (Seiler, 1923, 1927) has 124 chromosomes in its somatic nuclei. The chromosomes pair regularly in the eggs, the first metaphase revealing 62 bi-

valents. The paired chromosomes separate in the first division and divide equationally in the second division. The egg thus contains 62 univalent chromosomes which number may be counted also in the nuclei which arise through the first cleavage division. The second cleavage division gives rise to four nuclei in all, which still possess 62 chromosomes. These nuclei then fuse in twos, the zygoid chromosome number (124) being thus restored. The diploid parthenogenetic race (Seiler and Schaeffer, 1941) resembles the former, its chromosome number being, however, only one half of the above number.

The chromosome cycle of the thelytokous ("English") race of *Trialeurodes vaporariorum* corresponds to the case just described, with the difference that its zygoid chromosome number is restored somewhat earlier, *viz.*, in the first cleavage division. The halves of the divided chromosomes remain in the same nucleus owing to endomitosis or the formation of a restitution nucleus (details unknown).

(b) *The second polar nucleus fuses with the egg nucleus.* A good example of this type of automictic parthenogenesis is found in the bisexual-parthenogenetic race of *Lecanium hesperidum* (2n = 14). The egg cell undergoes two meiotic divisions which result in the formation of four azygoid nuclei, *viz.*, the egg nucleus and three polar nuclei, each of them having seven chromosomes. The eggs of this race may either be fertilized or developed through parthenogenesis (*cf.* p. 198). In the former case the diploid chromosome number is naturally restored by fertilization. In the latter case the egg nucleus and after a while also the second polar nucleus sink deeper into the egg. When side by side the chromosomes of the two nuclei begin to spiralize and chromatinize, and seven chromosomes appear in each. The nuclear membranes disappear and both the chromosome groups associate and form the first cleavage metaphase plate, which thus gains the diploid number, *i.e.* fourteen chromosomes.

A corresponding fusion of azygoid nuclei is observed in the eggs of the bisexual-parthenogenetic race in the coccid *Lecanium hemisphaericum*, and in the eggs of the saw-flies *Pristiphora pallipes* and of the thelytokous race of *Diprion polytomum*.

Also in the parthenogenetic races of *Artemia salina* the second polar nucleus may fuse with the egg nucleus (see below).

(c) *The halves of the divided chromosomes remain at the second meiotic division in the same nucleus* owing to the formation of a restitution nucleus or endomitosis. This mode of re-establishment of the zygoid chromosome number is typical in *Solenobia lichenella* (Seiler, 1923, 1939). Its thelytokous race is tetraploid, having the same chromosome number as the corresponding race of *S. triquetrella*. The beginning of oogenesis

is fairly similar in both these species. The paired chromosomes also separate in the first division in *S. lichenella*. The anaphase spindle sinks thereafter deeper into the egg, as is usual before the second division, but no second polar nucleus is formed. The second division is entirely abortive; the chromosomes divide and fall apart already in the first division anaphase plates, the halves remaining in the same nucleus in both the plates. Only two nuclei result accordingly from the meiosis, the egg nucleus and the polar nucleus both of which are zygoid.

Also in certain parthenogenetic nematodes (*Rhabditis monohystera,* some thelytokous Rhabditis species, *e.g., Rh. "XIX"*) the zygoid chromosome number is restored in the manner described above.

In the parthenogenetic races of *Artemia salina* the chromosome behavior at the second meiotic division is highly variable. Of this crustacean, in addition to the diploid bisexual race, several different polyploid parthenogenetic races are known, *viz.,* diploid, tetraploid, octoploid and decaploid (n = 21). In the oocytes of the parthenogenetic races the chromosomes pair and form bivalents. At the first division each bivalent is a small quadripartite body in which the chromosomes are apparently held together by a single chiasma (White, 1945, p. 285). The first division is similar in all eggs. It is, however, to be noticed that the eggs show great variation in regard to the second division. In a number of eggs the second division results in the formation of a second polar nucleus which remains in the egg, and later fuses with the egg nucleus as in Lecanium. The second division may, however, stop at anaphase, the chromosomes of both the egg nucleus and the second polar nucleus remaining in the same zygoid nucleus owing to the formation of a restitution nucleus. In a part of the eggs the second division does not proceed further than metaphase. It is not known in detail how the zygoid chromosome number is restored, but we may suppose it to happen endomitotically as in *Solenobia lichenella*. According to Barigozzi (1946), the eggs of the tetraploid parthenogenetic Artemia females may undergo only one maturation division. In such eggs no chromosome pairing and reduction take place.

The tychoparthenogenesis of the tettigids may also be regarded as belonging to this type of parthenogenesis, though it is somewhat different from the animals described above. The females of certain Paratettix, Apotettix, Telmatettix and Tettigidea species reproduce parthenogenetically as well as bisexually. The unmated females have fewer offspring (only $\frac{1}{4}$) than the mated ones, which shows that only a part of the unfertilized eggs develop through parthenogenesis. The partheno-produced individuals are nearly all females, males being rare exceptions. So in *Apotettix eurycephalus* only thirteen (0.2%) males

were found among 5326 parthenogenetically developed individuals. As is usual in the males, the chromosome number of these individuals was thirteen, the number in the females being fourteen. In all eggs a regular conjugation takes place, and the paired chromosomes separate in the first meiotic division. In those eggs which develop through parthenogenesis no second division occurs, as the division of the centromeres is delayed and the sister chromatids are in this manner held together. The same condition is found in the cleavage metaphase plates. In them, as in the mitoses of the young larvae, the chromosome number varies from seven to fourteen. If seven chromosomes are present, all of them are twice as broad as the usual mitotic chromosomes; if we have eight chromosomes, two are one half of the others, and so on. The broad metaphase chromosomes probably contain four chromatids instead of two, these four chromatids being held together by the centromere which is delayed in its division. Later the division of the centromeres in all the chromosomes catches up with the cell division and becomes synchronous with it; now the halves of even the last broad chromosomes fall apart. Though the cells now contain fourteen separate chromosomes, the homologous chromosomes still have a tendency to lie side by side. The mitoses of the partheno-produced tettigids thus display a clear somatic pairing, though the homologous chromosomes in the fertilized eggs lie at the opposite sides of the cleavage metaphase plates. It is further to be noticed that all the cells of an individual tend to have a similar plan of location of the chromosomes. From this, we understand that the partheno-produced tettigid females are homozygous in the vicinity of the centromeres as has been found experimentally.

(d) *Both the azygoid second division metaphase plates form one zygoid metaphase plate.* The zygoid chromosome number is in this way restored in the thelytokous psychid *Apterona helix.* This species is diploid with 2n = 62 chromosomes. In the first metaphase plate there are thirty-one bivalents, and the same number of univalents is present in both the first division anaphase plates. As usually in Lepidoptera two second division metaphase plates, an inner and an outer, are formed, both containing thirty-one chromosomes and having separate spindles. Before the second division the inner metaphase spindle with its chromosome plate moves to the side of the outer spindle. Finally the two spindles and metaphase plates lie side by side and fuse. The second division metaphase plate thus formed will contain sixty-two univalent chromosomes, which number will be included in the nuclei which arise as a result of the second division. In Apterona no polar nucleus is formed, but both the nuclei resulting from the second meiotic division remain in the egg and divide further.

(*e*) In the thelytokous parasitic wasp *Nemeritis canescens* (2n = 22) both the meiotic divisions deviate from the usual type. The chromosomes pair regularly, though no split in the threads can be seen at diplotene. The first division metaphase plate contains 11 bivalents. The small size of the chromosomes unfortunately prevents a detailed analysis of the bivalents. In both the first division anaphase plates eleven univalents are to be seen. Immediately after the first division these groups, due to the formation of a restitution nucleus, fuse into one metaphase plate of twenty-two univalents. In the second division one haploid set of univalents separates from the other set. In both second anaphase plates eleven univalents are found. The zygoid chromosome number is restored probably through endomitosis in that the chromosomes in the second division anaphase plates divide, the halves remaining in the same nucleus. This is indicated by the fact that the cleavage divisions already reveal twenty-two chromosomes.

(*f*) Parthenogenesis is evidently automictic also during a part of the cycle of the gall wasp *Neuroterus baccarum*. Its spring generation consists exclusively of parthenogenetic females (*cf.* pp. 236-237), which belong to two types: male-producing and female-producing. In both types the chromosomes pair regularly. The eggs of the male-producing females undergo according to Doncaster (1910, 1911) two, and according to Dodds (1939) only one meiotic division. The fact is that their chromosome number is reduced and they develop through arrhenotoky into haploid males. Doncaster and Dodds agree that no polar nucleus is formed, and in this sense no maturation division takes place in the eggs laid by the female-producing females. The metaphase spindle is situated not perpendicular to, but parallel to the margin of the egg. "More than 10 chromosomes (the haploid number of the species is 10) were proceeding to each pole. Two nuclei, both of which later undergo segmentation divisions are reconstituted at the poles" (Dodds, p. 184). In the first cleavage divisions the diploid number of chromosomes, twenty, is seen. Accordingly, the diploid number seems to have been restored so that during the division which may be regarded as the maturation division as well as the first cleavage division, the paired chromosomes first depart and thereafter divide endomitotically at anaphase in the same manner as in *Solenobia lichenella* (*cf.* pp. 209-210).

The parthenogenesis of at least certain phasmids is automictic, since the chromosomes pair and form bivalents, and this in turn is followed by a chromosome reduction. So in *Bacillus rossii*, which has the diploid chromosome number thirty-six (Cappe de Baillon *et al.*, 1937), the parthenogenetic eggs undergo two meiotic divisions. In the first metaphase seventeen-eighteen bivalents are visible (v. Baehr, 1907).

During which phase the zygoid chromosome number of these phasmids is restored, is unknown.

In some papers (Pchani, 1925) it has been suggested that in the eggs of certain parthenogenetic phasmids (*e.g., Carausius morosus*) two equational maturation divisions would take place, and consequently their parthenogenesis would be apomictic. The data underlying this statement need, however, reinvestigation.

### 3. *Apomictic Parthenogenesis*

Apomictic parthenogenesis is characterized by the fact that the oogenesis differs considerably from the oogenesis in those forms which have fertilization or in which parthenogenesis is automictic. In the eggs of animals representing these two types meiosis as a rule comprises chromosome conjugation and chiasma formation followed by chromosome reduction, which results in the change of the nuclear phase from zygoid into azygoid. The zygoid phase is then restored through fertilization or through an automictic fusion of nuclei. In apomictic parthenogenesis these meiotic features are lacking, wholly or in part. No chromosome reduction takes place during the maturation divisions in the egg, and the zygoid chromosome number is thus maintained. The maturation division of most animals belonging to this type is at least partly of meiotic character as the chromosomes during it contract more than in mitosis. The animals which represent this kind of parthenogenesis have naturally always a zygoid soma.

Apomictic parthenogenesis is the most common type of parthenogenesis and it is found in several animal groups. The following cases have been cytologically studied more in detail:

Turbellaria. *Bothrioplana semperi* (Reisinger, 1940).

Trematoda. Eggs that develop parthenogenetically. *E.g. Diplodiscus temporatus* (Cary, 1909).

Nematoda. *Rhabditis aberrans* (Krüger, 1913), *Rhabditis pellio*: "a mutant" (Hertwig, 1920), *Rh. sp.* ("*XX*") (Bĕlař, 1923, 1924).

The parthenogenesis of all these Rhabditis species is pseudogamy.

Rotifera. The eggs of the thelytokous females. *E.g. Hydatina senta* (Whitney, 1909; Shull, 1921), *Asplachna priodonta* (Storch, 1924), *A. amphora* (Whitney, 1929).

Mollusca. *Campeloma rufum* (Mattox, 1937), *Potamopyrgus jenkinsi* (Rhein, 1935; Sanderson, 1940).

Crustacea. Cladocera: Eggs developing parthenogenetically. *E.g. Daphnia pulex* (Kühn, 1908; Mortimer, 1936), the thelytokous race of *D. pulex* (Schrader, 1925), *D. magna* (Mortimer, 1936), *Polyphemus pediculus* (Kühn, 1908).

Ostracoda: *Cypris reptans* (Woltereck, 1898), *C. fuscata* (Schleip, 1909), *Heterocypris incongruens*: thelytokous race (Woltereck, 1898; Bauer, 1940).

Isopoda: *Trichoniscus elisabethae*: thelytokous race (Vandel, 1928).

Insecta. Orthoptera: Blattidae. *Pycnoscelus surinamensis* (Matthey, 1945).
   Orthoptera: Tettigoniidae. *Saga pedo* (Matthey, 1941).
   Homoptera: Aphididae. The eggs which develop parthenogenetically. (Stevens, 1905; v. Baehr, 1909, 1920; Morgan, 1909, 1915; Frolowa, 1924; Schwartz, 1932; Suomalainen, 1933).
   Homoptera: Coccidae. *Lecanium hesperidum* and *L. hemisphaericum*: thelytokous races (Thomsen, 1927), *Aspidiotus hederae* (Schrader, 1929).
   Diptera: Cecidomyidae. Parthenogenetically developing eggs of *Miastor metraloas* (Kahle, 1908; White, 1946a) and of *Oligarces paradoxus* (Reitberger, 1940).
   Coleoptera: Curculionidae. Thelytokous species and races (Suomalainen, 1940 a and b, 1947; Seiler, 1947).
   Hymenoptera: Tenthredinidae. *Thrinax macula* (Peacock and Sanderson, 1939).

*Pairing of chromosomes in the oocyte.* Our knowledge of the earlier stages of oogenesis of many animals with apomictic parthenogenesis is very incomplete. In most animals of this type no trace of chromosome pairing has been observed. This is true of the following:

Rotifera. *Asplachna priodonta.*
Mollusca. *Campeloma rufum.*
Crustacea. Cladocera: The thelytokous race of *Daphnia pulex.*
   Isopoda: *Trichoniscus elisabethae.*
Insecta. Orthoptera: *Saga pedo.*
   Homoptera: Aphidae. *Phylaphis coweni, Phylloxera fallax* and *Ph. caryaecaulis* (Morgan, 1915), *Tetraneura ulmi* (Schwartz, 1932).
   Homoptera: Coccidae. *Lecanium hesperidum.*
   Hymenoptera: *Thrinax macula.*

In a few animals with apomictic parthenogenesis, *viz., Rhabditis aberrans, Cypris fuscata* and *Thrinax macula* young oocytes show a distinct synizesis stage, but no synaptic pairing nor tetrad formation can be observed. Also in Miastor the egg nucleus goes through a series of prophase stages, resembling the pachytene of a true meiosis (White, 1946a, p. 331). The chromosome strands are loosely associated at this stage. Probably, however, we do not here have a real conjugation, but a case of somatic pairing, similar to the one found in many Diptera.

Although chromosome pairing seems to be totally lacking in the parthenogenetic eggs of some aphids (*cf.* above), other aphids behave differently in this respect. So de Baehr (1920) has observed a transient pairing in the parthenogenetic eggs of *Aphis palmae.* The chromosomes pair at zygotene, the young oocyte nucleus revealing in consequence the haploid number of bivalents. Later these conjugated chromosomes fall apart, and the diakinesis nucleus contains again the diploid number of univalents. A similar behavior has been stated by Paspaleff (1929) to occur in the parthenogenetic eggs of the autumn generations in *Macrosiphum rosae* and *Siphonophora rosarum.* The chromosomes pair in the

young oocytes forming bivalents which contract considerably. In the beginning of the growth period of the oocyte, the conjugated chromosomes fall apart however, and the later stages show the diploid number of univalents. In the single meiotic division they divide equationally. Evidently the homologous chromosomes do not cross over, and no chiasmata are formed, which later would keep the bivalents together. The transient chromosome pairing in the parthenogenetic eggs of the aphids deserves a closer examination.

*Maturation division.* The eggs of animals with apomictic parthenogenesis undergo only one maturation division. (The only exceptions are *Pycnoscelus surinamensis* and *Bothrioplana semperi; cf.* below.) The chromosomes divide equationally as in mitosis. In many cases, however, the chromosomes at "diakinesis" and metaphase resemble closely the meiotic chromosomes. They are strongly contracted, being thus short and thick, and deviate accordingly clearly from the mitotic chromosomes. This condition we find, *i.e.,* in *Diplodiscus temporatus, Trichoniscus elisabethae,* parthenogenetic curculionids and *Thrinax macula,* which animals have long and slender mitotic chromosomes. The maturation division of most animals with apomictic parthenogenesis is thus pseudohomeotypic (*cf.* Gustafsson, 1935, 1946). In some species (*e.g.,* Miastor, Oligarces) the chromosomes exhibit a mitotic shape in the metaphase of the maturation division. This seems accordingly to correspond with the so-called somatic division (*cf.* Gustafsson, *op. cit.*) The formation of restitution nuclei, which is relatively common in apomictic plants, has not been observed in animals with apomictic parthenogenesis.

As mentioned above, the chromosomes in the parthenogenetic eggs of the aphids divide equationally. In the eggs which develop into males the X chromosomes, however, pair, the maturation division being reductional in regard to them (Stevens, 1910; Morgan, 1912, 1915; Frolowa, 1924; Schwartz, 1932; Suomalainen, 1933). The pairing of the X chromosomes is not known in its details, because the earlier stages of the "male-eggs" have not been studied. At metaphase the X chromosomes form a bivalent, while the autosomes remain as univalents (some species have several X chromosomes, which form a corresponding number of bivalents). In the maturation division of a "male-egg" one half (usually one chromosome) of the X chromosomes goes to the polar body, the other half (the other chromosome) remaining in the egg. In this way the male obtains one half of the X chromosomes of the female. The aphid male is thus of the XO type, the female representing the XX type.

Seiler (1947) has found that the eggs of the parthenogenetic *Otiorrhynchus sulcatus* and probably also of other parthenogenetic weevils

undergo, in addition to an equational division, "a rudimentary reduction division." The univalent chromosomes arrange themselves in a single plate at the meiotic prophase before the spindle is formed. When the spindle is formed, the chromosomes leave the equatorial plate and are distributed in the spindle. Gradually the chromosomes — now maximally contracted — arrange themselves in a new plate, which is at right angles to the former equatorial plane. After this the chromosomes divide equationally.

Those animals with apomictic parthenogenesis which have two equational meiotic divisions deviate cytologically from all the former. To this type belongs, according to Matthey (1945) the parthenogenetic cockroach *Pycnoscelus surinamensis*. In its oocytes the chromosomes pair, and zygotene and pachytene are quite normal. After this the conjugated chromosomes fall apart, however, since no chiasmata are formed, for which reason a diploid number (38) of univalents are later seen in the eggs. These "pseudotetrads" closely resemble bivalents which condition according to Matthey depends on the centromere repulsion and the distal attraction of the halves of the divided chromosomes. This again Matthey explains as resulting from a division of the chromosomes (also centromeres) before the first metaphase, and the redivision of the divided chromosomes at first metaphase. These chromosomes have accordingly a tetrapartite structure (they have now four centromeres), which brings about their great resemblance to bivalents. Two equational maturation divisions are present, which take place very rapidly. In the first division the halves of the chromosomes separate, in the second the halves of these. The egg thus receives the diploid number of chromosomes (38).

The question of whether certain parthenogenetic phasmids have two equational maturation divisions, as has been suggested (Pehani, 1925), or if parthenogenesis in all phasmids is automictic (*cf.* pp. 212-213) needs reinvestigation. Similarly the older observations on the two equational maturation divisions of the gall wasp *Rhodites rosae* necessarily need revision, since the studies on the cytology of this gall wasp have given results which are contradictory in many respects.

The eggs of the Turbellaria *Bothrioplana semperi* too undergo two equational maturation divisions, although they differ greatly from the usual pattern. In about 90% of the individuals belonging to this flatworm species the male organs do not develop—as a rule the Turbellaria are hermaphrodites—and in the remaining 10% spermatogenesis is abnormal resulting in the sterility of the sperm. The eggs develop thus exclusively through parthenogenesis. The parthenogenesis of Bothrioplana is dioogony; each egg-capsule always contains two eggs which

together give rise to a single embryo. In its maturation divisions the chromosome number is doubled twice followed each time by a reduction. The chromosomes do not pair. In the eggs the diploid number (20) of univalent chromosomes which display a slender mitotic shape is thus to be seen. They divide equationally and the halves go to the two poles, contracting strongly at the same time. Through the formation of a restitution nucleus the chromosome halves—a new longitudinal split is seen in them—come together again forming an irregular plate which will contain the tetraploid number (40) of strongly contracted chromosomes. Thereafter the chromosomes go to opposite poles, one diploid set to each. The chromosomes divide equationally once again already in the first division anaphase plates. The cells arising as a result of the first division thus receive the tetraploid number of chromosomes. After a short interphase they arrange themselves in the second metaphase plate, the homologous chromosomes (chromatids) being situated side by side. At the second division a new reduction (from 40 to 20) takes place, the chromatids of each pair going to different poles. No polar nuclei are formed and consequently the maturation divisions are at the same time the first cleavage divisions. From this it is evident that the parthenogenesis of Bothrioplana is of apomictic nature in spite of the abnormal course of the maturation divisions.

## VI. Genetical Aspects of Parthenogenesis

Genetical conditions in parthenogenetic animals differ in many respects from those in the bisexual, zygogenetic forms. As is known, the most important genetical rules concerning bisexual animals, the Mendelian laws, are based on the free combination of the chromosomes (and the genes situated in them) in meiosis, which as a rule results in the formation of different sorts of gametes in distinct ratios. The recombination of genes in fertilization then gives rise to several partly new gene combinations. In all parthenogenetic animals (except generative parthenogenesis) fertilization is wanting, and in many of them (animals with apomictic parthenogenesis) chromosome pairing is also absent. In consequence, many parthenogenetic animals differ fundamentally from bisexual forms in regard to their genetical conditions.

Parthenogenesis has been considered from the genetical point of view by Smith (1941, p. 297-298) and White (1945, p. 282-283). Parthenogenesis may on cytological grounds be differentiated into several types (*cf.* above); consequently it cannot be a uniform concept from the genetical point of view. In regard to genetical conditions parthenogenesis may be classified as follows.

(a) The progeny is genotypically identical with the mother. This
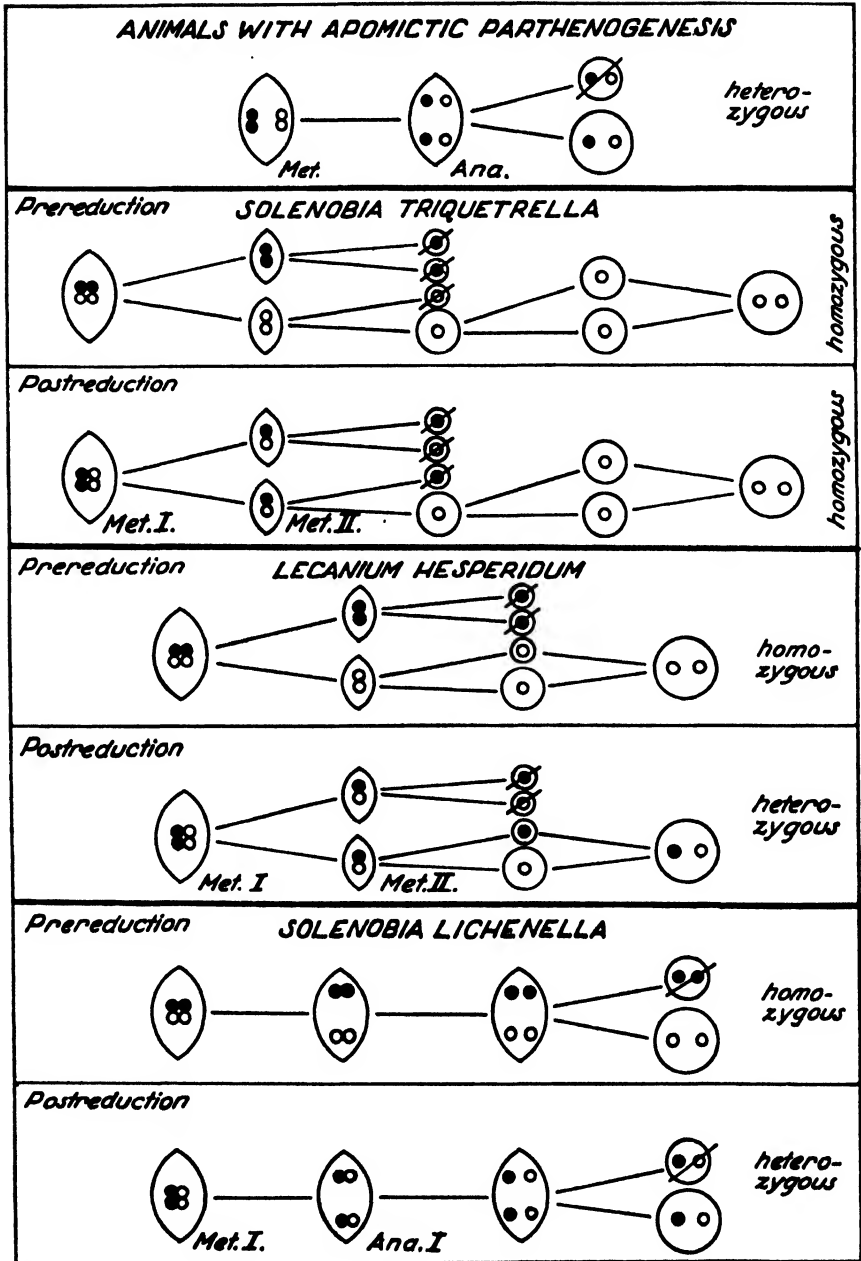
FIG. 1

Diagram showing the genetical consequences of different types of partheno-

genesis in the case of heterozygosity. Details in the text. Of the two possible homozygotes only one has been represented (drawn in outline).
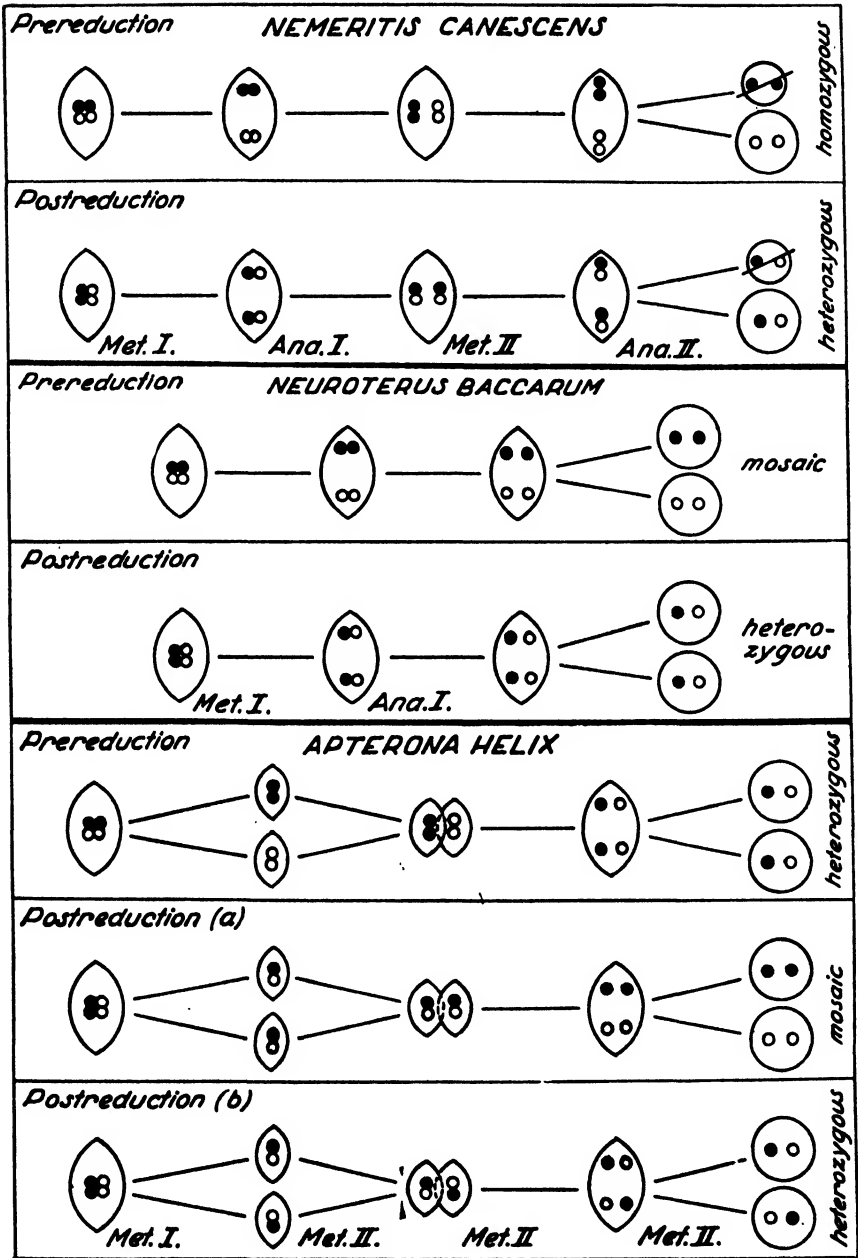
is the case in apomictic parthenogenesis, which from a genetical point of view is the most simple type of parthenogenesis. Meiosis is totally absent and consequently no new gene combinations are possible, the progeny being genotypically similar to the mother. Apomictic parthenogenesis is accordingly combined with great genetical stability. In this case genotypically new forms may arise only through mutations or structural rearrangements. If a gene mutation takes place in an animal with apomictic parthenogenesis, the heterozygosity thus established is maintained in the following generations, because the changed gene in these animals cannot become homozygous. Such animals become gradually heterozygous in regard to more and more gene pairs, as the elimination of recessive mutations by natural selection is impossible. White (1945, p. 283) has pointed out that in animals which for a long time have been apomictically parthenogenetic, the differences in the two chromosome sets have accumulated, so that they, in fact, can no longer be considered as diploids either in a cytological or a genetical sense. It is evident that in animals with apomictic parthenogenesis only dominant mutations become apparent. The higher polyploids among these animals (*Saga pedo,* tetraploid and pentaploid curculionids) occupy a special position in that in them no newly arisen mutations can express themselves, as they are counterbalanced by several doses of the original allele (*cf.* White, 1945, p. 293). In these higher polyploids with apomictic parthenogenesis gene mutations have lost their significance in regard to evolution (*cf.* p. 232).

Automictic parthenogenesis differs genetically from apomictic parthenogenesis in that meiosis and, in consequence, chromosome conjugation and segregation are present. In the eggs of some, at least, of the animals with automictic parthenogenesis crossing over occurs (*cf.* p. 208). (In the following theoretical survey the different types are considered assuming that the bivalents contain chiasmata, though their existence has not been ascertained in all cases.) Since the mode of the restoration of the zygoid chromosome number varies in automictic animals, automictic parthenogenesis also genetically falls into several types.

(b) Heterozygosity is obligatorily replaced by homozygosity in the progeny. This happens in the automictically parthenogenetic animals in which the zygoid chromosome number is restored by fusion of two azygoid cleavage nuclei (*e.g.,* the parthenogenetic races of *Solenobia triquetrella,* the thelytokous race of *Trialeurodes vaporariorum*). These animals are consequently homozygous. Heterozygosity can originate in them only through a mutation and even then the homozygous condition is regained already in the following generation. This type is in this respect the direct opposite of the previous one (apomictic parthenogenesis).

As mentioned earlier, the parthenogenetic races of *Solenobia trique-trella* are representatives of this type. Since the females are heterogametic in Lepidoptera, it is difficult to understand, as Seiler (1942, p. 526-527) has already pointed out, how the diploid Solenobia individuals, developing parthenogenetically, continually succeed in being females and consequently heterogametic. This type of parthenogenesis ought to obligatorily result in homozygosity.

(*c*) Heterozygosity is replaced by homozygosity, if the alleles in question (the heterozygous gene pair) separate at the first meiotic division (prereductional), but it is maintained, if they separate at the second division (postreductional). This condition is prevalent in those animals with automictic parthenogenesis in which the zygoid chromosome number is restored by the fusion of the second polar nucleus with the egg nucleus (*e.g.*, the bisexual parthenogenetic races of *Lecanium hesperidum* and *L. hemisphaericum*; for the genetical aspects of their parthenogenesis see Suomalainen, 1940c, p. 25 *ff.*). This type comprises also those cases of automixis in which the zygoid chromosome number is reestablished through an endomitotical division of the chromosomes in both first anaphase plates (*e.g.*, the parthenogenetic race of *Solenobia lichenella, Rhabditis monohystera*). Also the parasitic wasp *Nemeritis canescens* is a representative of this group (*cf.* p. 212).

The prereductional or postreductional division of the heterozygous allele pair depends on its situation in the bivalent. Let us consider only the most common bivalent type, a bivalent with prereduction in respect to centromere, and provided with one chiasma. (In contradistinction to this, the Lecanium species have postreduction.) If the allele pair concerned is situated proximal to the single chiasma, its separation is prereductional; in this case heterozygosity is replaced by homozygosity. If the allele pair lies distal to the single chiasma, its separation is postreductional, and the heterozygosity is maintained. If more than one chiasma is present, the result depends on the relative positions of the allele pair and the chiasmata. Since the number and situation of chiasmata, as a rule, varies even in the eggs of a single female, certain parts of a bivalent and consequently the gene pairs included in them may separate either prereductionally or postreductionally depending on the number and position of chiasmata. As a result of this a heterozygous female with this type of automictic parthenogenesis may in regard to at least a number of genes give rise both to homozygous and heterozygous offspring.

(*d*) Heterozygosity is maintained if the alleles concerned (the heterozygous gene pair) separate in the second division (postreductionally). If these alleles separate at the first division (prereductionally) a mosaic

of the two homozygous cell sorts originates. This is the case in the gall
wasp *Neuroterus baccarum.* .Even here the postreductional and prere-
ductional division depend on the position of the heterozygous allele pair
in the bivalent. The fact that no pure homozygotes but only mosaics
are produced in the latter case is accounted for by the exceptional
oogenesis of this gall wasp (*cf.* p. 212).

(*e*) Heterozygosity is preserved if the alleles (the heterozygous
gene pair) separate in the first division (prereductionally). If they again
separate in the second division (postreductionally) a heterozygous in-
dividual develops in 50% of the cases, and a mosaic formed by the two
homozygous cell types arises in 50%. These genetical conditions are
represented by the psychid *Apterona helix* (details in Narbel, 1946).
Also in this case the relative situation of the allele pair and the chiasmata
in the bivalent determines the mode of separation of the alleles. Con-
trary to the two previous types, the maintenance of the heterozygosity
is effected by the fusion of both the second metaphase plates, in the case
when the gene pair divides prereductionally. In this manner the genes
(both recessive and dominant) separated at the reduction come to the
same metaphase plate and subsequently divide. If the gene pair in question
tion divides reductionally at the second division, the dominant genes
may go to one pole and the recessives to the other, giving rise to a mosaic,
or one dominant and one recessive gene go to both poles, resulting in
the formation of a heterozygous individual. This depends on the situa-
tion of the different chromosomes in the metaphase plate which has been
formed through the fusion.

It must be noticed that in the most common automictic types of
parthenogenesis (types *b* and *c*) heterozygosity either obligatorily (type
*b*) or at least often (type *c*) is replaced by homozygosity. This implies
that if a gene mutation occurs in such an animal the changed gene
usually soon becomes homozygous. In this way species with automictic
parthenogenesis sooner or later will become split into a number of dis-
tinct races. Automictic parthenogenesis deviates thus considerably
genetically and especially in regard to its evolutionary aspects from
apomictic parthenogenesis.

(*f*) It is not possible here to consider in detail the genetical side of
generative or haploid parthenogenesis. The parthenogenesis of animals
belonging to this type is facultative and arrhenotokous, implying that
fertilized eggs develop into females, similarly to bisexual, zygogenetic
animals, and only the males develop through parthenogenesis. In ani-
mals with generative parthenogenesis a recombination of genes as a
rule takes place in the beginning of the development of the females.
These animals therefore resemble in many genetical respects bisexual

zygogenetic animals. It must, however, be pointed out (*cf*. Smith, 1941, p. 290) that in animals with generative parthenogenesis recessive characters, if controlled by a single gene, will show up immediately in approximately half the male progeny because of the absence of dominant alleles in haploid individuals. Further, the frequency of the recessives increases in forms with generative parthenogenesis relative to that in zygogenetic bisexual forms because all mutated genes in the former act as sex-linked factors. "Thus assuming that an 'autosomal' recessive mutation originated in females of both a facultative and non-parthenogenetic species and that these females are then fertilized by their respective wild type (dominant) males, and further that the $F_1$ progenies, consisting of equal numbers of males and females, intrabreed: from the former there would result a ratio of 9 dominants : 4 heterozygotes : 3 recessives, while from the latter the ratio would be 9 dominants : 6 heterozygotes : 1 recessive" (Smith, 1941, p. 290).

It is to be noticed (*cf*. White, 1945, p. 278-279) that evolutionary genetics of animals with generative parthenogenesis and male haploidy must be very different from the one found in animals in which both sexes are diploid. In the latter the recessive genes are able, and in fact do exist hidden in the heterozygous state in wild populations. Accordingly in such animals most wild populations contain numerous recessive genes. Those species in which the males are haploid cannot possess any such supply of hidden recessive genes in this sense, since all the recessive genes will be subject to the effects of selection in the males (*cf*. above). The consequence is that in animals with haploid males the females must be more homozygous than in animals with diploid males. The greater uniformity thus produced makes them, however, evolutionarily less plastic. Similarly a favorable mutated gene will have a greater chance to spread through a population and to establish itself in a species with haploid males as compared with one with diploid males.

The genetical aspects of sex determination in animals with generative parthenogenesis have already been discussed earlier (pp. 204-207).

## VII. PARTHENOGENESIS AND POLYPLOIDY

### 1. *General Features*

Polyploidy as is known is a common phenomenon in the plant kingdom. It has been established that at least 50% and probably more of the angiosperm species are polyploid (*cf*. Müntzing, 1936, p. 263, Huxley, 1942, p. 140). In the animal kingdom, however, polyploids are comparatively rare. A list of the polyploid animals so far encountered is given

in the reviews of Vandel (1937, 1946), Suomalainen (1940b, p. 94-102) and White (1946b).

In many cases polyploidy occurs in animals in connection with parthenogenesis. In the following parthenogenetic animals polyploidy has been found so far (only cytologically verified cases):

Mollusca. Gastropoda. .*Potamopyrgus jenkinsi*. A tetraploid parthenogenetic race (Rhein, 1935; Sanderson, 1940).

Crustacea. Anostraca. *Artemia salina*. Tetraploid, octoploid and decaploid parthenogenetic races (Artom, 1911, 1931; Gross, 1932; Stella, 1933; Barigozzi, 1936, 1946; Haas and Goldschmidt, 1946).

Isopoda. *Trichoniscus elisabethae*. A triploid parthenogenetic race (Vandel, 1928, 1931b, 1933, 1934, 1940).

Insecta. Orthoptera: Phasmidae. Triploid and tetraploid parthenogenetic races and species (Cappe de Baillon *et al.*, 1934a and b, 1935, 1937, 1938; Cappe de Baillon and de Vichet, 1940).

Orthoptera: Tettigoniidae. *Saga pedo*. A tetraploid parthenogenetic species (Matthey, 1941, 1946; Goldschmidt, 1946).

Lepidoptera: Psychidae. *Solenobia triquetrella* and *S. lichenella*. From both a tetraploid parthenogenetic race (Seiler, 1923, 1927, 1938, 1939, 1942, 1943, 1946).

Coleoptera: Curculionidae. Triploid, tetraploid and pentaploid races and species (Suomalainen, 1940a and b, 1947; Seiler, 1947).

## 2. *Parthenogenesis Combined with Polyploidy in Animals*

In the following the polyploidy in different parthenogenetic animals is described in more detail.

The parthenogenetic snail *Potamopyrgus jenkinsi* occurs possibly as a diploid as well as a tetraploid race. In the waters of the European Continent twenty to twenty-two chromosomes have been observed in this species (Rhein, 1935), whereas in England it seems to have thirty-six to forty-four (Sanderson, 1940). The latter race would thus be tetraploid. Since these chromosome numbers are difficult to determine, they need reinvestigation.

The crustacean *Artemia salina* is found both in the Old and the New World. Its distribution area is, however, very dispersed owing to the fact that it lives as a rule exclusively in water of very high salinity, occurring consequently in salt lakes and salt pans scattered over an enormous area. For its distribution in the Old World see the map by Stella (1933, p. 435) and the review of Barigozzi (1946). The separate strains of *Artemia salina* may even deviate considerably morphologically; a part of the differences may, however, be accounted for by modifications. Several races differing in their cytology have been established. The eggs of the diploid bisexual race (2n = 42) do not develop without fertilization. In addition several parthenogenetic races of Artemia are

known, *viz.*, a diploid (2n = 42), a tetraploid (84 chromosomes), an octoploid (168 chromosomes) and a decaploid (210 chromosomes) race. This last race has been found only in Palestine (Haas and Goldschmidt, 1946). Parthenogenesis in *Artemia salina* is automictic (*cf.* p. 210). The parthenogenetic races consist almost exclusively of females. Males are found as rare exceptions in the diploid and tetraploid races. It is not known what condition determines the origin of these exceptional males. The males of the bisexual races do not copulate with the parthenogenetic females. Polyploid Artemia individuals are obviously bigger than diploid, and the development of the individuals belonging to the parthenogenetic races is more rapid as compared with the representatives of the bisexual races.

Vandel (*op. cit.; cf.* p. 224) has established the same chromosome number (2n = 16) in the isopod genus *Trichoniscus* for four bisexual species (*elisabethae, provisorius, biformatus* and *darwini*). Their eggs do not develop without fertilization. *Tr. elisabethae* exists in addition as a triploid parthenogenetic race ("forme" coelebs) with twenty-four chromosomes, which is considerably bigger than the diploid race. The eggs of the bisexual race undergo two meiotic divisions, giving rise to almost equal numbers of males and females. The triploid race consists almost exclusively of females. Their parthenogenesis is apomictic in that only one maturation division is observed in the eggs (*cf.* p. 213). In the triploid race males are also produced sporadically (Vandel, 1934), the ratio of the sexes being 100 females : 1.6 males. No conjugation is found in the spermatogenesis of these exceptional triploid males. Meiotic divisions number two according to Vandel, but both are equational. Triploid males copulate with triploid females, but no fertilization takes place, and the eggs develop even in this case through parthenogenesis. The diploid males of the bisexual race also copulate with the triploid parthenogenetic females. What condition gives rise to the triploid males is obscure in Trichoniscus also, as the sex determination of isopods as well as that in Artemia is not cleared up.

The question as to the possible polyploidy of the parthenogenetic phasmids may still be regarded as partly open. Certain parthenogenetic phasmids (*e.g., Bacillus rossii*) have the same chromosome number as related bisexual species; for the chromosome numbers of phasmids see the reviews of White (1945, p. 295). These parthenogenetic forms are accordingly diploid. In other parthenogenetic species the chromosome numbers are clearly higher than in the related bisexual species, being, however, rarely exact multiples of one basic number. It is therefore difficult to decide if the species are polyploid and what their polyploidy degree would be, as even related bisexual species may have differing

chromosome numbers. So for instance in the genus Carausius the chromosome numbers (2n) of two bisexual species studied are twenty-two and forty-two (the former of these species belongs according to others to the genus Greenia), while the parthenogenetic species *C. theiseni* has forty to forty-two and *C. furcillus* sixty-four to seventy-five or eighty-five to one hundred chromosomes. Cappe de Baillon *et al.* have on this basis concluded that *C. furcillus* comprises both triploid and tetraploid individuals. In some cases the multiplicity is more obvious. *Phobaeticus sinetyi* seems to be tetraploid, as its chromosome number (52) is exactly twice the number of a related bisexual species (26). *Leptynia hispanica* might be triploid, as it has fifty-two to fifty-six chromosomes, while the bisexual *L. attenuata* has but thirty-six chromosomes. Phasmids may possibly be irregular polyploids (hypoploids or hyperploids), certain chromosomes being represented in their complement more times than others. In many parthenogenetic phasmids sporadical male individuals have been found, though they are very rare. Possibly they are intersexual females; their spermatogenesis is highly irregular and abnormal and consequently no functional sperms are formed (*cf.* Cappe de Baillon and de Vichet, 1940; White, 1945, p. 293).

The large wingless parthenogenetic tettigoniid *Saga pedo* (= serrata) has a wide distribution area in southern Europe, ranging from Spain to the Ural Mountains; see the map by Matthey (1941, p. 130). We must, however, remember that its distribution is very discontinuous and the populations everywhere small. *Saga pedo* has sixty-eight chromosomes, of which six pairs are metacentric including two pairs of X chromosomes. Two bisexual species, *Saga ephippigera* and *S. gracilipes* from Palestine, have thirty-one chromosomes in their spermatogonia (Goldschmidt, 1946; Matthey, 1946). According to Matthey, the chromosome number of the male *S. ephippigera* is thirty-three, which number has also been found by Goldschmidt in one male individual. In these species only the X chromosome is metacentric. Goldschmidt is of the opinion that it seems plausible that the establishment of a supernumerary pair of chromosomes may have occurred in *S. pedo* previous to the doubling of the chromosome number. The four pairs of metacentric autosomes in *S. pedo* would appear to have evolved by intrachromosomal rearrangements in originally acrocentric chromosomes. Parthenogenesis in *Saga pedo* is apomictic (*cf.* p. 214). Of this species only two or three males have been found which possibly are intersexual like the exceptional phasmid males. It is to be noticed that *Saga pedo* is of smaller size than the diploid bisexual Saga species mentioned above.

Both *Solenobia triquetrella* and *S. lichenella* are represented not only by a diploid bisexual race but also by a tetraploid parthenogenetic race.

In the former species, in which parthenogenesis has been thoroughly studied by Seiler (*op. cit.; cf.* p. 224), a diploid parthenogenetic race has been found as well. Tetraploid Solenobia individuals have 124 and the diploid specimens 62 chromosomes respectively. Parthenogenesis in Solenobia is automictic, although the zygoid chromosome number is restored in a different manner in each species (*cf.* pp. 208-210). The parthenogenetic females resemble each other closely, the tetraploid individuals being, however, considerably bigger than the diploid ones (also bisexual). When the parthenogenetic female has emerged from the pupa it begins to lay eggs almost immediately. The gamic female in its turn waits for the male, laying eggs only after copulation. The male may even copulate with a parthenogenetic female, if this has just emerged from the pupa, and has not yet begun to lay eggs. If the tetraploid parthenogenetic and the diploid bisexual race are crossed, the progeny is triploid and intersexual, as may be expected. They have as a rule three autosome genomes, but only two X chromosomes owing to the female heterogamety of the Lepidoptera. The $F_1$ individuals may be partly male and partly female, being sexual mosaics. In the fertilized eggs of the parthenogenetic female the nuclei of the egg and sperm cells do not fuse immediately but the egg nucleus divides a few times, and the resulting nuclei may fuse with each other or with one or more sperm nuclei which have penetrated the eggs. The cells of the $F_1$ animals may accordingly develop with the haploid, diploid, triploid, tetraploid, hexaploid, etc., chromosome number. If the diploid parthenogenetic and the bisexual race are crossed, the $F_1$ generation consists of both females and males, the number of the former being considerably higher (ratio 6:1). The major part of the $F_1$ females is parthenogenetic, the minor part resembling the females of the bisexual race. Seiler regards it as probable that these parthenogenetic $F_1$ females have developed from unfertilized eggs.

In the weevils, Curculionidae, the subfamilies Otiorrhynchinae and Brachyderinae comprise several species which have a bisexual as well as a parthenogenetic race. Accordingly a number of the parthenogenetic species correspond with a related bisexual species. Bisexual forms are diploid ($2n = 22$), while the parthenogenetic ones in general are polyploid. Of the seventeen parthenogenetic weevil races and species so far studied, one is diploid (22 chromosomes), eleven are triploid (33 or 34 chromosomes), four are tetraploid (44 chromosomes) and one pentaploid (55 chromosomes) (Suomalainen, 1940a and b, 1947).

The diploid is *Polydrosus mollis*.

The triploids are: *Otiorrhynchus niger* (parthenogenetic race), *O. scaber* (parthenogenetic race from the Austrian Alps), *O. singularis*,

*O. salicis* (parthenogenetic race from the Austrian Alps), *O. sulcatus, O. gemmatus* (parthenogenetic race), *O. ovatus, O. ligustici, Trachyphloeus bifoveolatus, Sciaphilus asperatus* and *Strophosomus melanogrammus.*

The tetraploids are: *Otiorrhynchus dubius* (parthenogenetic race), *O. scaber* (parthenogenetic race from Finland), *O. pupillatus* and *Barynotus obscurus.*

The pentaploid is *Barynotus moerens* (from the Austrian Alps).

In three *Otiorrhynchus* species (*niger, salicis* and *gemmatus*) both the bisexual and the parthenogenetic race have been studied cytologically. The former is diploid in all of them, the latter again triploid. The weevils may, except as a diploid bisexual race, occur as several divergent parthenogenetic races. Of *Otiorrhynchus scaber* a triploid parthenogenetic race is met with in the Austrian Alps and a tetraploid parthenogenetic race in Finland. Of this species, too, a diploid bisexual race is known. The parthenogenesis of the curculionids is apomictic (*cf.* p. 214). In most parthenogenetic species the chromosomes in the maturation division may be arranged in two or even three different plates, each plate containing one or more complete sets of chromosomes. In the thelytokous weevil races and species males are unknown; only in *Strophosomus melanogrammus* have exceptional males been found sporadically. And in *Otiorrhynchus scaber* at least the tetraploid race is of bigger size than the triploid.

The following parthenogenetic animals, earlier regarded as polyploid, have later been established as diploid.

Because the diploid chromosome number of the water flea, *Daphnia pulex,* in some earlier studies had been given as eight, Schrader (1925) regarded the constant thelytokous *D. pulex* race with twenty-four chromosomes studied by him as hexaploid. Mortimer (1936) has however come to the conclusion that the diploid chromosome number of the species is twenty-four, the earlier stated smaller number having evidently resulted from the clumping together of the chromosomes owing to defective cytological technique.

On the basis of chromosome numbers Vandel (1937) has drawn the conclusion that certain parthenogenetic ostracods (*Cypris fuscata*) may possibly be polyploid. Bauer (1940) has, however, shown that at least the parthenogenetic race of *Heterocypris incongruens* is diploid; the earlier determination of the chromosome number was thus proved to be erroneous. In Bauer's opinion the polyploidy of the other parthenogenetic ostracods is open to considerable doubt.

The parthenogenetic cockroach, *Pycnoscelus surinamensis,* supposed to be polyploid, has been shown to be diploid by Matthey (1945). He, on the other hand, assumes the species to be polysomic in that its chromo-

some complement would include seven chromosomes twice and six chromosomes four times (together 38 chromosomes).

### 3. *The Distribution of the Different Races*

Vandel (1928) has been the first to pay attention to the fact that if we have a bisexual and a parthenogenetic form of a single species or two related species, these forms as a rule have at least in part a deviating distribution. Usually the parthenogenetic form is the more northern one. Such cases have been termed geographic parthenogenesis by Vandel. In most of the typical cases the parthenogenetic form has been found to be polyploid.

This rule was established by Vandel when investigating the distribution of the different races of the isopod, *Trichoniscus elisabethae* (*cf.* p. 225). The diploid bisexual race of this species has according to him (1931b, 1933) been found only in the mountains of Southern France, whereas the distribution of the triploid parthenogenetic race ranges in addition to France also to more northern areas as far as the Baltic countries, South Sweden and South Finland. The reason why Vandel earlier (1928, map p. 192) suggested that the distribution of the bisexual race was much more extensive than the one just mentioned, is that he at first erroneously included the bisexual *Trichoniscus provisorius* in the bisexual race of *Tr. elisabethae* as well (*cf.* Vandel, 1931b, 1933). Vandel (1940) has further observed that on the French coast of the Mediterranean the triploid parthenogenetic race of *Tr. elisabethae* thrives in a more unfavorable environment, in this case in a drier climate, than the bisexual forms. The latter chiefly inhabit mountains, which being more damp and forested offer sufficient humidity to these hygrophilous animals. In the surrounding lower and drier areas, which are covered with garrigues vegetation, *Trichoniscus* individuals are only rarely met with and even then are triploid, parthenogenetic females. This last-mentioned race is thus able to live on the one hand in a colder and on the other hand in a drier climate than the diploid bisexual forms.

The different races of *Solenobia triquetrella* have also a distinctly divergent distribution (Seiler, 1943, 1946). The tetraploid parthenogenetic race is spread over Central Europe, in the north to South Scandinavia and South Finland and in the east to Rumania. Diploid races have so far been found only in Switzerland, the diploid bisexual race also in Nuremberg, Germany. The distribution of the different races in Switzerland displays a distinct regularity (Fig. 2). The diploid bisexual race lives only north of the Alps on areas which during the Würm Ice Age were somewhat outside the glacier and thus ice-free. In addition it occurs in Swiss Mittelland (between the Lakes of Geneva and Boden) in regions

raised above the Würm glacier as nunataks. South of the Alps this race has not been found with certainty in Switzerland. The diploid parthenogenetic race inhabits in part the same areas as the previous race. In addition it is found farther away from the glacier edge and also in the Swiss Mittelland on areas which were covered with ice in the Ice Age. The tetraploid parthenogenetic race lives throughout Switzerland both
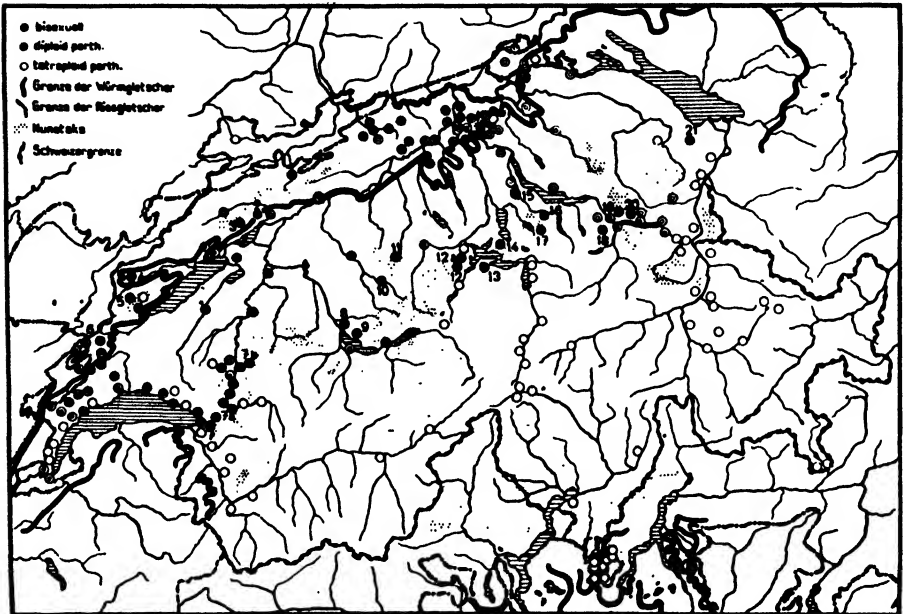


FIG. 2

The distribution of the different races of *Solenobia triquetrella* in Switzerland. ● diploid bisexual race. ◉ diploid parthenogenetic race. ○ tetraploid parthenogenetic race. ⚊ the extreme edge of the glacier in Würm Ice Age (last Ice Age). ⚊·⚊ the extreme edge of the glacier in Riss Ice Age. 🔘 nunatacs. ⚊ the Swiss frontier. (Seiler, 1946)

north and south of the Alps, especially in regions which were covered with ice in the Würm Ice Age.

The different races of the parthenogenetic weevils, too, have an interesting distribution (Suomalainen, 1940b, 1947) *Otiorrhynchus dubius* is a boreoalpine species, having two separate distribution areas, one in North Europe and the other in the mountains of Central Europe. In the latter regions it is diploid and bisexual, while in North Europe it is parthenogenetic and at least in Finland tetraploid. In certain localities in the Eastern Alps *Otiorrhynchus scaber* is diploid and bisexual; in some moun-

tains in Central Europe it is triploid and parthenogenetic, while at least in a part of North Europe it is tetraploid and parthenogenetic.  The deviating distribution of the different weevil races is also encountered in the Eastern Alps (Jahn, 1941; Suomalainen, 1947).  In at least ten Otiorrhynchus species both a bisexual and a parthenogenetic race is found there.  Jahn's map (Fig. 3) shows that the bisexual races of most of these
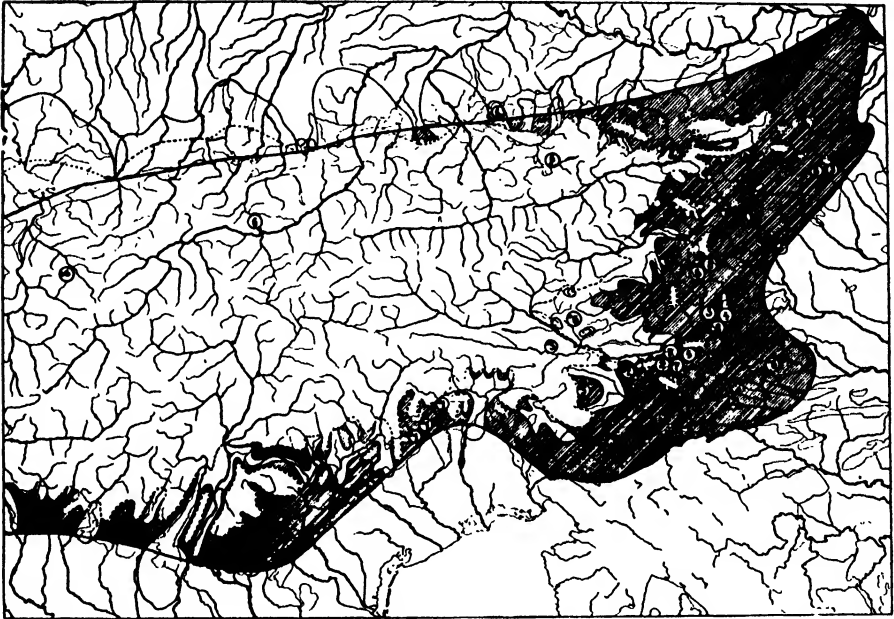


FIG. 3

The distribution of the bisexual races of a number of Otiorrhynchus species in the Eastern Alps.  1. *O. foraminosus*.  2. *O. chalceus*.  3. *O. scaber*.  4. *O. azaleae*. 5. *O. alpicola*.  6. *O. salicis*.  7. *O. auricomus*.  8. *O. chrysocomus*.  The continuous line indicates the edge of the Alps, the broken line the extreme edge of the glacier in Würm Ice Age (last Ice Age).  The parts of the Alps that were ice-free during the Würm Ice Age are hatched.  (Jahn, 1941; somewhat revised).

weevils have spread only to those parts of the Eastern Alps which remained outside the glacier during the Würm Ice Age.  As in recent times, fairly wide, dry and warm (xeroterm) areas were then situated on the east and south edges of the Alps.  These regions were ice-free, and here representatives of the alpine fauna, demanding even a warm climate, could survive during the Ice Age.  After the ice had withdrawn, these bisexual Otiorrhynchus races remained in these, even now, climatically more favorable habitats.  The parthenogenetic races have again been

able to spread to the areas which were later freed from the ice, and occur as a rule in regions which during the Würm Ice Age were covered with the glaciers. All the parthenogenetic races of these weevil species, so far studied, are polyploid.

The tetraploid parthenogenetic tettigoniid *Saga pedo*, too, which is the only parthenogenetic species in its genus, is dispersed farther north (*cf.* p. 226) than any other species belonging to this genus (Matthey, 1941, 1946; Goldschmidt, 1946). Bisexual *Saga* species are confined mainly to Asia Minor and Palestine. The distribution of *Saga pedo* is of theoretical interest also in another respect. As was mentioned above the species is very discontinuous in its dispersal. In White's opinion (1945, p. 293) the distribution area of the species has probably been more continuous earlier, but after a period of successful dispersal the species has apparently become extinct over the greater part of its range. This may at least in part depend on the fact that in more highly polyploid animals with apomictic parthenogenesis mutation will almost have ceased to be an effective agent in evolution since no newly arisen mutations will make their appearance (in presence of several doses of the original allele). Neither will they have any chance of becoming homozygous. Polyploid animals with automictic parthenogenesis are not subject to this disadvantage, as they have a meiotic mechanism which enables new mutations to become homozygous (see pp. 218-222). The fact that *Saga pedo* is now on the verge of extinction does not depend on new disadvantageous mutations, but is rather due to the fact that the species has ceased to develop and thus has failed to adapt itself to changing circumstances.

For the distribution of the different races of the snail *Potamopyrgus jenkinsi* see p. 224.

The distributive divergence of the races of *Artemia salina* is not so pronounced as in the previous animals; *cf.* the map by Stella (1933, p. 435.) It is true that most discoveries of the bisexual race have been made in southern parts of the range of the animal, whereas the polyploid parthenogenetic races have been found farther north, but no distinct regularity can be established. The diploid, tetraploid and octoploid parthenogenetic races may even inhabit the same place (*e.g.*, in Margherita di Savoia on the east coast of Italy) (Gross, 1932; Barigozzi, 1936, 1946). This may be due to the relative easiness with which the chromosome number of Artemia is doubled (*cf.* below); animals and races of different ploidy may hence relatively easily arise in the same place and even among a single clone.

It may be mentioned that sometimes even the diploid parthenogenetic race may exhibit a different distribution than the corresponding bisexual race. For instance the diploid parthenogenetic race of the psychid

*Apterona helix* is widely spread in Central and South Europe (Narbel, 1946), while the bisexual race is only found in the Mediterranean countries (South France, North Italy, South Tyrol).

In many cases it is difficult to decide whether this deviating distribution of the polyploid parthenogenetic forms, described above, depends on their parthenogenesis or on their polyploidy. We must not forget that the distribution of the polyploid parthenogenetic animals closely resemble the ones of many polyploid plants; in regard to this correspondence *cf.* Suomalainen (1940b, p. 115-116) and Vandel (1940, p. 95-97). Both these animals and plants occur as a rule in areas with more unfavorable conditions (colder and drier climate etc.) than those prevailing in the distribution areas of the corresponding diploid forms. The conclusion is therefore tempting that it is the polyploidy that makes these animals more hardy in unfavorable circumstances and thus enables them to widen their range. The fact that the polyploid parthenogenetic form often occurs in different areas than the diploid bisexual one on the other hand indicates that polyploidy has evidently changed the optimum life conditions of the parthenogenetic form, which change causes the parthenogenetic form to disperse in other and usually climatically more unfavorable regions. Parthenogenesis is naturally important in so far as it renders polyploidy possible, and also favors the spread of the polyploid parthenogenetic form into new areas, as even a single parthenogenetic individual may reproduce and give rise to a new strain.

Seiler (1946) has come to the conclusion that certain historical factors have played a decisive role in the divergent distribution of the various Solenobia races. On the basis of this distribution (*cf.* above) he infers that in the genus Solenobia first parthenogenesis and thereafter polyploidy have developed during the Ice Age. The bisexual race probably gave rise to the diploid parthenogenetic race when the Würm glacier was at its widest, and the parthenogenetic race then followed the retreating verge of the ice, the bisexual race remaining in the regions which had been ice-free. During the retreat of the glacier a tetraploid parthenogenetic race developed from the diploid parthenogenetic race. This tetraploid race in its turn began to follow the retreating ice, thus spreading on to the exposed area. This implies that the tetraploid parthenogenetic race actually would have a greater capacity for competition and dispersal as compared with the diploid parthenogenetic race, this latter in turn having a stronger spreading tendency than the bisexual race.

It is certain that the glacial conditions during the Ice Age in Europe have considerably influenced the distribution of the various weevil races. The significance of the Ice Age in the Eastern Alps in this respect has already been discussed p. 231. The Ice Age has apparently played an

important role in the distribution of the races of the boreoalpine and also
certain other weevil species, though detailed conclusions cannot be drawn
as the distribution of the different weevil races is known too imperfectly.

### 4. *The Origin of Polyploidy in Parthenogenetic Animals*

Regarding this question only hypotheses may be presented, as ex-
periments on the development of polyploid parthenogenetic animals have
not been carried out.  In the following the discussion is restricted to the
animals concerning which fairly reliable conclusions may be drawn.

In the genus Solenobia, as in all parthenogenetic animals the bisexual
race is the original, from which the parthenogenetic races have developed.
Seiler (1943, 1946) assumes that polyploidy in Solenobia has arisen during
the Ice Age (*cf.* above).  The bisexual race of *Solenobia triquetrella* first
gave rise to the diploid parthenogenetic race, and this in turn to the
tetraploid parthenogenetic race.  Parthenogenesis in this genus is of auto-
mictic character (*cf.* pp. 208-209).  In both the parthenogenetic races of
*S. triquetrella* the zygoid chromosome number is restored in that the
nuclei resulting from the second cleavage division fuse in twos.  The
tetraploid parthenogenetic race has probably originated from the diploid
parthenogenetic race, the fusion of nuclei in the first cleavage divisions
having taken place, not only once, as usual, but twice.  Seiler has
observed that this actually happens in the diploid parthenogenetic race,
through which triploid and tetraploid cell generations are produced.
Polyploidy in Solenobia thus has the nature of autopolyploidy.

Similarly the polyploid Artemia individuals are autopolyploid.  In
them too parthenogenesis is automictic.  It was mentioned earlier
that the meiotic mechanism of Artemia is rather labile (p. 210).  Gross
(1932) observed that in a part of the eggs of the parthenogenetic Artemia
races the first polar body is not extruded but remains in the egg which
causes a doubling of the chromosome number.  Gross found further that
this may be achieved experimentally, if the egg is exposed to cold
(+4°C.) or treated with chloroform.  In many such eggs the first meta-
phase spindle is formed, not perpendicular to, but parallel with the
margin of the egg, which induces the first polar body to remain in the egg.

Tetraploidy in other parthenogenetic animals may have its origin in
similar cell fusions, as described above; it may also arise endomitotically.

We must not forget that *Trichoniscus elisabethae* occurs as a triploid
parthenogenetic race and that the majority of the parthenogenetic wee-
vils are triploid, one being even pentaploid.  Triploids and pentaploids
must naturally have a different origin than the one described above.  The
hypotheses concerning the origin of polyploidy in these animals cannot
be discussed here.  Constant triploid and pentaploid parthenogenetic

forms can naturally arise only in animals with apomictic partheno-genesis.

## VIII. CYCLICAL PARTHENOGENESIS

### 1. *General Features*

Cyclical parthenogenesis (p. 196) is characterized by a heterogo-nous alternation of generations, one or usually several parthenogenetic generations alternating more or less regularly with a bisexual generation. Since cyclical parthenogenesis as a rule involves several subsequent parthenogenetic generations, most of these are naturally thelytokous. Only the bisexual generation results from deuterotoky.

It is a typical feature in cyclical parthenogenesis that in most groups where it is found it prevails in the whole group. It is true that this kind of alternation of generations is favorable in many respects for the organ-ism concerned. As White (1945, p. 281) has already pointed out, animals with cyclical parthenogenesis seem to have established a compromise which combines the advantages of parthenogenetic and zygogenetic re-production. The species is able to reproduce quickly owing to thelytoky. The speed of reproduction is accelerated in certain forms by paedo-genesis (Trematoda, Cecidomyidae, Micromalthus) and by vivipary (Trematoda, many rotifers and aphids, Cecidomyidae, Micromalthus). In addition, as suggested by Dobzhansky (1941, p. 356), the favorable gene combinations possibly resulting from mutations may be widely spread in animals with cyclical parthenogenesis before they are broken up by crossing over and recombination of genes. In the bisexual genera-tion on the other hand the recombination of genes may take place. This recombination is the more important, as the parthenogenesis of most animals belonging to this group is apomictic, recombination of genes in the parthenogenetic generations being thus impossible. In some repre-sentatives of this group the bisexual part of the cycle fails secondarily, resulting in a purely thelytokous reproduction.

Cyclical parthenogenesis has been established so far for the following animal groups: Trematoda; Rotifera; Crustacea: Cladocera; Insecta: Homoptera: Aphidae; Diptera: Cecidomyidae; Coleoptera: Micro-malthus; and Hymenoptera: Cynipidae.

### 2. *Mode of Cycle in Different Animal Groups*

The cycles of the animals mentioned above differ to a considerable degree. The males of Rotifera and Cynipidae and of the beetle Micro-malthus are haploid and arise through generative parthenogenesis.

The cycle of the rotifers has already been dealt with earlier (pp. 201-202) (for details see Lucks, 1929). From the fertilized eggs parthenoge-

tic females hatch obligatorily after a shorter or longer resting period and these all yield only female offspring. Thereafter follow several parthenogenetic generations of females with apomictic parthenogenesis (*cf.* p. 213). At certain times their eggs also develop parthenogenetically into gamic females. The eggs of the latter which are smaller than the eggs of the parthenogenetic females undergo a reduction. They are facultatively parthenogenetic, *i.e.* they may develop either parthenogenetically or be fertilized. In the former case the egg develops through generative parthenogenesis into a haploid male; in the latter case the egg grows and obtains a thick envelope, giving rise after a resting phase to a female. In the beginning of the bisexual period when males are absent the eggs of the gamic females can naturally develop only parthenogenetically; later when males are present they may be fertilized. The origin of the gamic females and males is determined to a great extent by certain external circumstances (*cf.* below). The period when males occur is very short in many species. It may be restricted to a few days or even a few hours. There are from one to several bisexual periods in the year depending on the species. In the whole order Bdelloidea and in species of several other groups males are unknown so that these rotifers reproduce by constant thelytoky. In the order Seisonida males are fully developed, as abundant as females and can be obtained at all seasons. It has been hence assumed that these rotifers do not possess any parthenogenesis although the question is still open.

A number of gall wasps, Cynipidae, have yearly two differing generations. (The most primitive species have usually only a single bisexual generation a year.) The parthenogenetic generation, consisting exclusively of females, in most cases emerges in spring and the bisexual generation in summer. The eggs of females belonging to the former generation develop parthenogenetically into males and females. The eggs of the females of the bisexual generation again develop only after fertilization, then giving rise to females of the parthenogenetic generation. Patterson (1928a) has found in the parthenogenetic generation of two Neuroterus species (*N. contortus* and *N. rileyi*) sporadic exceptional males also, which however are functionless.

In the more primitive cynipid species with alternating generations (*e.g.*, *Andricus operator austrior*) the eggs of at least most of the females of the parthenogenetic generation develop both into males and females (Patterson, 1928b). In more specialized cases (*e.g.*, *Belenocnema treatae kinseyi*, *Neuroterus irregularis albipleurae* etc.) the eggs of a single parthenogenetic female give rise either to the one sex or the other; the parthenogenetic females are thus of two types, *viz.*, male-producers and female-producers. Sex determination has accordingly in these

species been "transferred" to the previous generation. These types are connected with several intermediate links, as in certain cynipid species the females of the parthenogenetic generation are either male- or female-producers, whereas the eggs of other females give rise to both sexes (Patterson, 1928b). In *Neuroterus baccarum* sex is determined still earlier, as the eggs of the fertilized females belonging to the bisexual generation develop into parthenogenetic females of one kind only, *viz.*, either into male-producers or female-producers (Doncaster, 1916). The question of the nature of the difference between these two types of parthenogenetic females in Cynipidae is so far unsolved.

It is to be noticed that in some of the more highly specialized Cynipidae species males are never found, and reproduction is exclusively thelytokous.

*Micromalthus debilis* (Coleoptera) differs from the previous animals in that it has no distinct alternation of generations because the bisexual generation has probably lost its significance. This beetle combines parthenogenesis with paedogenesis. In North America the species has four different kinds of reproductive females of which three are larvae (Scott, 1936, 1938, 1941). These four types are (a) an adult female, (b) a thelytokous paedogenetic larva, (c) an arrhenotokous paedogenetic larva, and (d) a deuterotokous paedogenetic larva. A young female larva belonging to the offspring of a thelytokous paedogenetic larva may develop into any one of these four reproductive types. In addition to these four female types there are males which are haploid. The adult females as well as the males are found in nature only in August but in the laboratory they can be produced in any month of the year by the proper adjustment of temperature and moisture conditions. The thelytokous and deuterotokous larvae have several offspring, but the arrhenotokous larvae yield usually one offspring. This single male larva devours its mother. If this is prevented a new crop of eggs develops in the ovary of the mother; the eggs develop into females. This shows that the sex of the brood in Micromalthus is obviously determined by environment, intrinsic or extrinsic, and not by the hereditary constitution of the mother. Scott has never observed the adult females copulating. He therefore assumes that the adults are sterile. It is to be noticed that in South Africa (Pringle, 1938) Micromalthus has no bisexual "generation." No males nor larval types giving rise to them have been found there, although adult females are present.

The chromosomal conditions of the animals described above have already been dealt with earlier (*cf.* pp. 201-202, 212). The oogenesis of Micromalthus ought to be subjected to cytological study, as it is so far wholly uninvestigated.

Also in those gall flies, Cecidomyidae, which have a heterogonous alternation of generations (at least in Miastor) the males are haploid, though their chromosomal conditions are very exceptional and complicated owing to the polyploidy of the germ-line. These gall flies combine
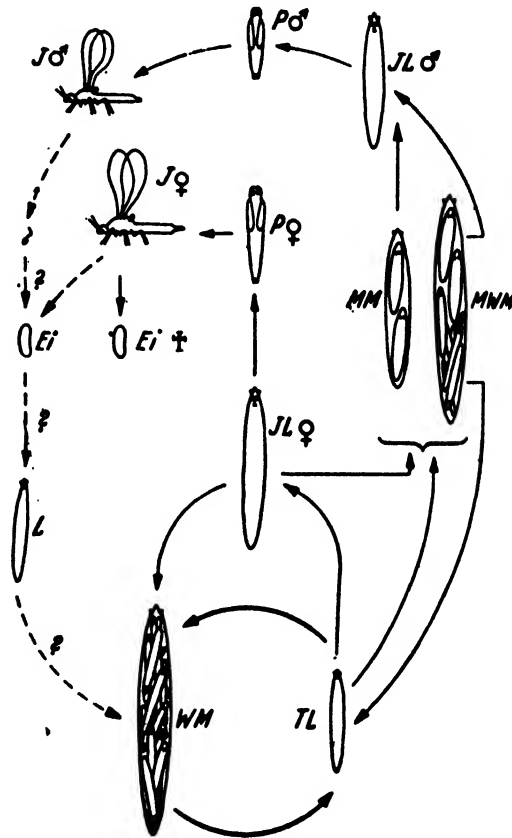


Fɪɢ. 4

Alternation of generations in the gall fly *Oligarces paradoxus*. The details of the cycle are explained in the text. The thick arrows indicate the thelytokous part of the cycle. The part of the cycle represented by broken line is hypothetical, not all the details being cleared up. (Ulrich, 1940).

parthenogenesis with paedogenesis and vivipary. Ulrich (**1936, 1940**) has thoroughly investigated the cycle of *Oligarces paradoxus* (Fig. 4). The species reproduces during the greater part of the year paedogenetically; in summer adult males and females are also found which represent the bisexual generation. As in Micromalthus (*cf.* above) there are

in this species four different kinds of reproductive females. These four types are identical with the ones mentioned above, *viz.* (*a*) an adult female (*J*♀), (*b*) a thelytokous paedogenetic larva (*WM*), (*c*) an arrhenotokous paedogenetic larva (*MM*) and (*d*) a deuterotokous paedogenetic larva (*MWM*). A young female larva (*TL*) given birth to by a thelytokous paedogenetic larva may in Oligarces also develop into any one of these four reproductive types. An arrhenotokous paedogenetic larva produces male larvae which, similarly to a part of the offspring of the deuterotokous paedogenetic larva, pupate and finally develop into adult males. A larva which is developing into an adult female (*JL*♀) may still at a certain stage of fairly advanced growth be converted into another larval type, in which case naturally no pupation takes place. The eggs of an adult female do not develop without fertilization. They give rise most probably to thelytokous paedogenetic larvae but possibly also to other paedogenetic larval types. The cycle of the gall fly Miastor resembles the former (Kahle, 1908; White, 1946a) in its main features, though it has not been subjected to so detailed a study.

The chromosomal conditions of the cecidomyids described above are also rather exceptional (Kahle, 1908; Reitberger, 1940; White, 1946a). It is characteristic of them that the cells of the germ-line are polyploid. The eggs of Miastor are octoploid (48 chromosomes), the eggs of Oligarces dodecaploid (sixty-six chromosomes; five chromosomes are present twelve times, while one is present six times). The parthenogenetic eggs undergo a single maturation division which is equational. The first cleavage divisions are normal mitoses. In Miastor at the third or fourth divisions thirty-six chromosomes are eliminated from the nuclei which are destined to pass into the somatic tissues. Only the germ-line nucleus retains the full octoploid number of chromosomes. In Oligarces two similar chromosome eliminations take place, the first at the third cleavage division, and the second elimination at the sixth to eighth division. In the first elimination fifty-five chromosomes are lost, in the latter only one. The somatic cells of the paedogenetic female maintain thus only the diploid number of chromosomes, in Miastor twelve and in Oligarces ten chromosomes. Individuals belonging to the bisexual generation have been cytologically studied only in Miastor (White, *op. cit.*). The female sexual larvae are cytologically indistinguishable from the paedogenetic ones, *i.e.*, they have a diploid soma and an octoploid germ-line. The male larvae again have a haploid soma and an octoploid germ-line. During spermatogenesis each octoploid spermatocyte gives rise to two haploid sperms and a heptaploid cell which degenerates. There are two meiotic divisions, the first of which is a unipolar division in which a haploid group of six chromosomes is pushed away from the

remaining forty-two. This haploid nucleus undergoes a second meiotic division, which is an ordinary mitosis. In the heptaploid nuclei there occurs no second meiotic division. No sex chromosomes can be observed. The mechanism of sex determination presumably depends on whether thirty-six or forty-two chromosomes are eliminated from the somatic nuclei during the cleavage divisions. The differences between the paedogenetic and the female sexual larvae seem, however, to be determined by the environment (*cf.* below).

In other animals with cyclical parthenogenesis the males are diploid.

In the trematodean order Digenea each species has in its cycle at least two different generations which all are internal parasites. All but one generation are paedogenetic; they are parasites in a snail or other invertebrates. One generation (the hermaphrodite adults) in its turn parasites some vertebrate. As an example we may consider the cycle of the liver fluke of sheep, *Fasciola hepatica* (*cf.* Storer, 1943), which trematode as an adult usually inhabits the bile ducts of sheep. The adults are hermaphroditic. The fertilized eggs pass through the bile ducts and intestine of the sheep and are voided with its feces. From each egg emerges a ciliated larva, the miracidium. If it hatches in water, it penetrates the Lymnaea-snail, living in water. In the pulmonary chamber or lymph vessels of Lymnaea the miracidium larva then becomes a sacklike sporocyst, whose eggs develop parthenogenetically. The parthenogenesis of the trematods is apomictic (*cf.* p. 213). The eggs of the sporocyst develop into another larval stage, the redia, which also is sacklike. These rediae migrate to another organ of the snail, usually the liver. There they produce other rediae parthenogenetically for one or two generations. Finally, each redia gives birth parthenogenetically to several larvae of another type, known as cercaria. They burrow out of the snail to swim through the water using their tails. Subsequently the cercaria settles on a grass blade, loses its tail and produces a tough enclosing cyst. The encysted cercariae get into the intestines of the sheep with the grass and finally into the bile ducts where they end their cycle by becoming adult hermaphrodite flukes.

The alternation of generations in the cladocers has been the subject of extensive study for a long time. Though the details may differ to some extent, a common feature is shown by the cladocers in that the reproduction after several parthenogenetic generations becomes zygogenetic (*cf.* Mortimer, 1936). From the fertilized eggs (the ephippial eggs) there emerge, after a shorter or longer resting period, exclusively females, the eggs of which (the subitaneous eggs) develop parthenogenetically in the brood chamber of the female (apomictic parthenogenesis; *cf.* p. 213). Thereafter there usually follow several parthenogenetic generations,

varying in number, which consist exclusively of females, the parthenogenesis being continually apomictic. The female which has laid subitaneous eggs may, however, owing to certain external factors (*cf.* below) later on begin to lay ephippial eggs. (In some cases such a female has later been found again to have laid subitaneous eggs.) The ephippial eggs are bigger and have thicker envelopes than the subitaneous eggs and they are unable to develop without fertilization. In the brood chamber of the female there are usually only one or two such eggs. A part of the shell of the female becomes the chitinous coat of the ephippial eggs (the ephippium), which during moulting is detached from the shell and thus also from the mother, and inside which these eggs remain during the resting stage. At the same time as the ephippial eggs are laid there usually also arise males. The same environmental factors which determine the formation of the ephippial eggs also give rise to the development of the males, though these phenomena are not causally connected. Since the cladocer males originate through a similar apomictic parthenogenesis as the females they are diploid and possess an identical chromosome complement as the females. According to Mortimer (*op. cit.*) sex determination of the cladocers is phenotypical. In certain species males and ephippial eggs are produced only when the unfavorable season (winter) is approaching. In other species they are also met with in summer. In some species no males are known. Such is the thelytokous race of *Daphnia pulex* (Banta, 1925; Schrader, 1925) in which the ephippial eggs also develop parthenogenetically without reduction.

The cycle of the aphids (see *e.g.* Weber, 1930) resembles the one described above considerably at first sight. In spite of the apparent similarity of the cycles in aphids and cladocers they exhibit however certain essential differences. From the fertilized wintering eggs of the aphids parthenogenetic females, so-called stem-mothers hatch exclusively. Like the cladocers, the aphids have several generations, varying in number, which consist of parthenogenetic females, the parthenogenesis being in this case of apomictic nature (*cf.* p. 214). In the families Aphididae and Eriosomatidae (= Pemphiginae) the parthenogenetic females are viviparous, while those in the families Adelgidae (= Chermesidae) and Phylloxeridae are oviparous. The parthenogenetic females are, owing to some intrinsic and extrinsic factors, either wingless or winged. The latter fly to new plant specimens; in the so-called migrating species to a certain new plant species [the intermediate (summer) food plant] where several parthenogenetic generations arise during the summer. When the autumn approaches (in some species even in summer) there develop on the summer food plant winged males and winged parthenogenetic females, so-called female-producing sexuparae, which both fly back to the

primary food plant (winter host). From the eggs of the latter emerge oviparous gamic females, the eggs of which undergo a reduction and are fertilized. The males and the gamic females thus belong in these species to different generations. In other species there develop on the summer food plant only winged parthenogenetic sexuparae which fly back to the winter host and there produce males and gamic females. At least in certain non-migrating aphids the sexuparae produce first parthenogenetic females and later individuals belonging to the bisexual generation. In some species the same sexupara gives birth either to males or gamic females in addition to parthenogenetic females, in others to both (Suomalainen, unpubl.). The cycle of the adelgids lasts two years, and in addition to the main cycle it consists of one or more additional parthenogenetic cycles which makes their cycle very complicated (for details see Börner, 1908). Some phylloxerids have in their cycle a fixed, often small number of generations. So *Phylloxera caryaecaulis* (Morgan, 1909, p. 243-244) has only three generations in the year. The eggs of certain stem-mothers develop into parthenogenetic male-producing sexuparae, whose eggs give rise to males, while the eggs of other stem-mothers develop into female-producing sexuparae. The eggs of the latter again develop parthenogenetically into gamic females. This species has thus separate "male and female lines" which may depend on the fact that these two lines differ somewhat in regard to one X chromosome. Although both the males and the gamic females in the aphids originate from parthenogenetic eggs and are diploid, the male has, unlike the Cladocera males, one half of the X chromosomes of the female. For the peculiar maturation division of the "male-eggs" see p. 215. Two kinds of secondary spermatocytes are formed in the males, but those in which the X chromosome is lacking degenerate (*cf.* Ris, 1942). All sperms are thus similar, resembling the eggs of the gamic females in regard to their chromosomes. This explains why all fertilized aphid eggs give rise to females. In some aphids the bisexual phase fails secondarily, and consequently their reproduction is exclusively thelytokous.

### 3. *The Influence of Environmental Factors on the Cycle*

The cycle of the animals with cyclical parthenogenesis depends both on extrinsic and intrinsic factors.

The great importance of extrinsic factors is seen *inter alia* in that many of the animals described above in certain environmental conditions reproduce exclusively through parthenogenesis, the bisexual phase being wholly absent. For instance, Ulrich (*op. cit.*) has bred paedogenetic lines of the gall fly Oligarces for eight years without the occurrence of

any individuals belonging to the bisexual generation (adult males and females). The number of subsequent paedogenetic generations during this time rose to over five hundred. Similarly some cladocers and aphids have in certain experimental conditions been induced to reproduce exclusively parthenogenetically for several years, although the same species in nature have at least one bisexual generation yearly. These animals reproduce thus in certain conditions illimitably through parthenogenesis. In spite of this no degeneration, no higher death rate nor lower reproductive power can be observed in them. Fertilization is hence in no way indispensable to them.

The dependence of the cycle of the animals with heterogonous alternation of generations on environmental conditions has been much studied. In the present paper it is not possible to recapitulate even the most important studies concerning these questions. In regard to them reference may be given to the review of Shull (1929). In the following only a few typical cases, which are the best analyzed, will be described.

To obtain an exact analysis of the different environmental factors on the cycle, Ulrich (*op. cit.*) bred the gall fly Oligarces in small glass dishes in which a Penicillium species was grown as food for the larvae. The mold grew on agar to which a known amount of a synthetic nutrition solution was added. To obtain identical test animals, larvae of the same age, belonging to a single paedogenetic line, were used. Also the breeding methods were in every case similar.

It was already mentioned that under certain experimental conditions Oligarces reproduces illimitably solely through paedogenesis. It is evident on the other hand that the occurrence of the bisexual generation is also conditioned by certain factors. [The development of the gamic adult females and the males differs, however, in principle. When an undifferentiated female larva develops into an adult female, it does not begin to reproduce at the larval stage (paedogenetically), but undergoes a metamorphosis; accordingly its eggs have a different maturation division from the one found in paedogenetic larva. The occurrence of males is again a question of sex determination. In the eggs giving rise to males chromosome elimination (*cf.* above) takes place apparently differently, since the males have a haploid soma.] Ulrich was able to state that the number of adult females increases considerably due to the following factors: the increase in the number of larvae (on the same area), increase of illumination, and the aging of the nutritive molds. The same thing happens, though to a lesser degree, owing to the decrease in the concentration of the nutritive solution, and the decrease of humidity and temperature. The factors conditioning the occurrence of the males remained obscure to Ulrich.

The effect of all these environmental conditions on the production of adult females was in the same direction. First the number of these females increases to a certain limit and decreases thereafter steeply. At the same time the paedogenetic larvae become continually smaller. From these data Ulrich drew the conclusion that the factors mentioned above do not directly cause the increase in the number of adult females, but that their effect was based on a single condition, *viz.*, the decrease of nutrition. All these factors have an effect on the growth of the Penicillium mold and thereby on the amount of nutrition, which in the last instance determines whether a larva reproduces paedogenetically or whether it develops into an adult female. When nutrition is abundant, reproduction is exclusively paedogenetic. With the decrease of the nutrition available the number of adult females is increased until at a certain point it begins to decrease. This depends on the fact that metamorphosis cannot take place if the larva has not had enough nourishment. If the extreme limit for this is reached, the larva may still reproduce through paedogenesis; its size and the number of offspring are, however, smaller. In this case we have a kind of hunger paedogenesis.

Ulrich's experiments show that the undifferentiated female larvae of Oligarces have a genotypically determined reaction norm, resulting in the development of a certain percentage of them into thelytokous, deuterotokous, and arrhenotokous paedogenetic larvae and into adult females in every given environment. All these types may be produced in every generation, the various generations being similar in this respect.

The studies on cladocers have given results, agreeing in principle with the previous ones (*cf.* Mortimer, 1936; Banta and Brown, 1939). For the present we have a fairly good knowledge of the effect of environmental factors on their cycle. Also in them certain factors cause the occurrence of males and condition the females, which have laid subitaneous eggs developing through parthenogenesis, to lay ephippial eggs, which are fertilized. Experiments have shown that certain temperatures, lack of available food, and (in mass cultures) overcrowding act as such factors. There are two effective regions in the temperature scale in which male production is favored: at relatively high temperatures (around 30°C.) and at relatively low temperatures (for *Daphnia magna* around 10°C. and for Moina 14-17°C.). The influence of these environmental factors is independent of which parthenogenetic generation we have. Only in regard to exephippio females these factors must have a longer time than usual to act to change the parthenogenetic into zygogenetic reproduction.

The question arises as to what the actual influence of the extrinsic

factors on cladocers is based on. The factors in question often differ in their quantitative influence in that the same factor, when having a weaker effect causes the female to produce males, while a stronger effect conditions the formation of ephippial eggs. Banta and Brown suppose that male production is associated with, and presumably caused by, a lowered rate of metabolism in the mother and in the eggs, which become male-determined. Sexual egg production in its turn is apparently due to the specific effect of marked food scarcity, possibly supplemented by other factors such as metabolic by-products. Presumably it is associated with a rate of metabolism lower than that accompanying male production.

The cycle of the rotifers and aphids has also been found to be affected by extrinsic factors (for details see Shull, 1929; Weber, 1930), although their effect has not been analyzed as extensively as in the former animals. Since the results obtained in regard to rotifers are rather contradictory, they will not be discussed here in detail. As an example may be mentioned that Luntz (1926) and Buchner (1937) have, using the same experimental technique and the same rotifer species, achieved quite deviating results. According to Luntz the most important factors giving rise to gamic females are the following: change of the salt concentration of the culture medium, change of pH, and variation of the nutritive organisms. Buchner again maintains that these factors have no influence on the cycle; in his opinion the art of the food (the different individuals of one nutritive organism) plays the most important role in the formation of gamic females.

In aphids temperature and the periodicity of illumination are important factors in regard to the production of gamic females and males. When the mode of reproduction is changed, there may also arise aphids which are intermediate between gamic and parthenogenetic females, which at least partly depends on the order of embryonic determination (Lawson, 1939). Thus only those organs in the embryo are affected that have not been determined at the time of the environmental change. There are, however, exceptions to this rule.

In most animals with cyclical parthenogenesis the cycle thus depends closely on certain environmental factors. It must be noticed that this influence is, as a rule, restricted to a certain developmental stage of the egg or the individual, which stage varies in different animals.

In some representatives of this type, as in the so-called trimorph aphid species—these have only three generations yearly—(*e.g. Phylloxera caryaecaulis; cf.* p. 242)—and in many gall wasps, having only two generations yearly (*cf.* pp. 236-237), the cycle is constant, and is not influenced by external conditions.

## IX. Origin and Evolution of Animal Parthenogenesis

Thelytoky is found as more or less separate cases in several animal groups (*cf.* above). This shows that the change to thelytoky has occurred several times independently in the course of time. This is indicated also by the great difference in the chromosomal conditions of the thelytokous representatives of the various animal groups (details above). As was mentioned earlier, thelytoky as a rule is restricted to single scattered species and races, and seems never to have become widely established in any greater animal group (an exception is formed by thelytoky connected with cyclical parthenogenesis). This suggests that the cases of thelytoky now met with are of relatively late origin. The same conclusion has been reached in certain cases on other grounds as well. For instance Seiler (1943, 1946) has concluded, on the basis of the distribution of the different Solenobia races (*cf.* pp. 229-230), that thelytoky in these moths arose during the Ice Age. The relative youth of thelytoky may at least in part depend on the fact that, as White (1945, p. 280) has already pointed out, thelytokous forms seem to be frequently successful in the struggle for existence, but sooner or later the inherent disadvantages of a genetic system of this type, representing at least in many cases apomictic parthenogenesis (*cf.* above), must be expected to lead to a lack of adaptability followed by extinction, or perhaps sometimes by a return to zygogenesis.

Arrhenotoky, in the form in which it occurs in connection with generative parthenogenesis, is found only in a few animal groups (*cf.* p. 199). Usually it is, however, characteristic of the whole group, or at least a great part of it. So the males are haploid in the whole order Hymenoptera and probably also in most rotifers and thrips. The same state of affairs prevails in a part of the order Acarina. Among the coccids, the tribe Iceryini is characterized by male haploidy, and as Whiting (1945, p. 253) points out, it is possible that the related homopteran family Aleurodidae, in which generative parthenogenesis is also met with, may have a common derivation with this tribe. Accordingly Micromalthus remains the only separate case belonging to this type, being the only representative of its genus and family. Generative parthenogenesis need not, at least according to the data known at present, have arisen more than six or seven times. Evidently generative parthenogenesis is, at least in most cases, of very old origin. Whiting has hence concluded (*op. cit.* p. 235) that since generative parthenogenesis and male haploidy characterize the whole order Hymenoptera from the primitive horn-tails and saw-flies to the highly specialized parasites and the social wasps, ants, and bees, generative parthenogenesis in the Hymenoptera may

possibly have originated as early as the end of the Jurassic Period, since representatives of several groups belonging to the suborders Chalastogastra and Clistogastra have been found in the Upper Jurassic strata.

Concerning the origin of thelytoky the opinion has been most widely held (*e.g.*, Vandel, 1931, 1936; Whiting, 1945) that tychoparthenogenesis may be regarded as the primitive type from which normal thelytoky has developed. In *Solenobia triquetrella* Seiler (1942, p. 517-518) has actually been able to observe such an evolution. The bisexual race of this moth has even now a slight tendency toward parthenogenesis. If the females belonging to this race are prevented from mating, a part of them lay unfertilized eggs which, however, as a rule do not develop. In some cases they have developed to fairly advanced stages, even to adult larvae. One of them pupated, and from the pupa a female emerged. It in turn laid unfertilized eggs most of which underwent further development. This female thus gave rise to a diploid thelytokous line. A somewhat similar phenomenon has been stated by Nabours (1925, 1929, 1937) to occur in the tettigids studied by him (for details see pp. 210-211), the eggs of which develop parthenogenetically if the female is prevented from mating. It is true that unmated females have less (only about $\frac{1}{3}$) progeny than the mated females, only a part of the unfertilized eggs developing through parthenogenesis. In tettigids also tychoparthenogenesis occurs in several successive generations.

The fact that an egg begins to develop parthenogenetically implies, as Whiting (1945, p. 235) has pointed out, merely that the block to post-meiotic mitosis which is normally established in animals is broken in a different manner from its breakdown in zygogenetic animals through fertilization. In artificial parthenogenesis the activation of the egg is brought about by certain extrinsic irritations; a detailed account of this is given in the reviews of Tyler (1941) and Peacock (1944, p. 112-118). This activating role of the sperm or the extrinsic irritations seems to be of a chemical (enzymatic) nature. We may notice that, of the animals whose eggs have been artificially induced to develop parthenogenetically, certain show a definitely stronger tendency toward parthenogenesis than others. This has been established *inter alia* by Astaurov (1940) in the silkworm moth, *Bombyx mori*, in which it is much easier to induce parthenogenetic development in the eggs of some strains than of others. According to Astaurov (p. 225) this depends on certain genes. It is certain that also in animals with normal parthenogenesis, this phenomenon depends on certain genes and gene combinations. The whole question is, however, so far obscure in its details.

In this connection it may be mentioned that in plants more is known concerning the genes which cause apomictic reproduction (and parthe-

nogenesis); in regard to this we may refer to the reviews by Gustafsson (1947a and 1947b). Thus it is known that the simplest forms of apomictic reproduction are due to one or a few recessive genes. Apomixis in its most complicated form (apospory or diplospory followed by parthenogenesis) on the other hand is of very complicated construction. We must remember that in plants parthenogenetically reproducing species are predominantly polyploid. This depends on the fact that in them parthenogenesis breaks through more easily in the polyploid than in the diploid stage. In regard to the origin of apomixis the number of genes having an influence on it probably plays a prominent part. Since apomixis breaks through only when a certain sufficient combination of these genes comes together, we understand that this number most easily becomes high enough in the polyploid forms. This may also account for the fact that apomixis in many plant genera is combined with hybridization processes of various kinds; in this case those genes which are necessary for the origin of apomixis may more easily come together. In plants both polyploidy and hybrid structure thus facilitate, but do not cause apomixis. Hitherto no certain case of apomixis, caused by dominant factors, has been demonstrated. Müntzing's (1940) studies on Poa in addition suggest that apomixis, at least in a number of plants, "is due to a rather delicate genetic balance. This balance may be upset in various ways, by crosses with other types or merely by a quantitative change in chromosome number either in plus or minus direction" (p. 171).

If we have a case of thelytoky, the animal, developing through parthenogenesis, must naturally have the zygoid number of chromosomes. In automictic parthenogenesis the egg cell undergoes a reduction, but the zygoid chromosome number is later restored, which may happen in several different ways (details pp. 208-212). In apomictic parthenogenesis chromosome reduction is absent, the zygoid chromosome number being retained during all phases. Seiler (1942) assumes that when zygogenesis is replaced by parthenogenesis automixis is usually substituted for fertilization, as the former enables the maintenance of the greater part of the chromosome mechanism characteristic of zygogenetic animals. In automixis the fusion of the nuclei of two different individuals, which takes place in fertilization, is replaced by fusion of the nuclei of a single individual. Seiler presumes that development continues in such a manner that the chromosome mechanism gradually changes towards the apomictic type, the reduction division becoming rudimentary, and the chromosomes ceasing by degrees to pair. Development in this manner ends with apomictic parthenogenesis which involves only one equational maturation division. Seiler regards it as possible that the development need not go through all these intermediate stages, but can overstep cer-

tain of them. It is, however, equally possible that the cytologically differing types of thelytoky have arisen independently of each other. At least in some cases of apomictic parthenogenesis, the automictic phase cannot have been more than at most a short tychoparthenogenetic transitional period, since in certain such animal groups in which we may suppose parthenogenesis to be of relatively recent origin, all parthenogenetic forms display apomictic parthenogenesis. Hertwig (1920) could even state that in the zygogenetic nematode species, *Rhabditis pellio*, an apomictic parthenogenetic race arose quite suddenly through mutation or segregation in a laboratory stock. In this stock parthenogenesis was established as a constant feature, representing, however, pseudogamy as in other nematodes (*cf.* p. 207).

Whiting (1945, p. 253-256) in his survey on generative parthenogenesis has considered the origin of this type of parthenogenesis from many points of view. Schrader and Hughes-Schrader (1931) on the basis of their studies of coccids have presented a theory of the attainment of male haploidy, based on the gradual inactivation or degeneration of one of the haploid chromosome groups. Whiting on the other hand regards this hypothesis as improbable. Though tychothelytoky appears to be the first step toward constant thelytoky, Whiting is of the opinion that it is not probable that generative parthenogenesis and male haploidy would have originated through tychoparthenogenesis (tychoarrhenotoky). Instead he thinks that "a theory involving repeated and extensive race hybridization seems more probable, as furnishing abundant material for natural selection" (p. 254).

Whiting (*op. cit.*) presumes that modifications within the X-chromosome or within some autosome have occurred, to produce a multiple allelic series and the complementary type of sex determination associated with male haploidy. A large population with a strong tendency toward parthenogenesis and with modified chromosomes and normal chromosomes freely recombining and crossing over, thus producing new types, is required for the evolution of male haploidy. Such a population might be found in the borderlands between the ranges of an ancestral bisexual race and its thelytokous offshoot. The latter would have reproduced over a sufficiently long period to accumulate several genetic changes, but still brief enough for retention of crossability with the parental stock. Crossing occurs in the borderland of the distribution ranges of two races, at first between the males of the bisexual race and the parthenogenetic females, then between the former and the hybrid females, and later between the members of the successive segregating hybrid generations. From cytogenetic recombinations a great number of different types result. The viable fertile types may be stabilized at

three stages, (a) zygogenesis of both sexes, (b) parthenogenesis of females with males eliminated, and (c) zygogenesis of females, parthenogenesis of males. The third case would furnish the basic material from which the stable arrhenotokous type might evolve. This third type would be produced from combinations of recessive male-determining factors with genes determining parthenogenesis. Thus the zygotes would tend to include the females, while the azygotes would be male. According to Whiting (*op. cit.*) "the nucleoplastic regulation should be gradually modified by an increase in the size of chromosomes, so that the haploid number might be retained into progressively later embryonic stages, especially in the germ track. In this way meiosis of the spermatocytes should be changed from the diploid to the haploid type" (p. 257).

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