CROONIAN LECTURE:

—

ON THE MECHANISM OF HEREDITY

T. H. MORGAN

For.Mem.R.S., Professor of Experimental Zoology
Columbia University.

(Lecture delivered June 1, 1922.—MS. received August 3, 1922.)

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Several years ago I ventured to state that with the demonstration of the wide applicability of Mendel's two laws, and with the later discoveries of linkage and of crossing-over, the traditional problem of heredity had been solved. On account of this statement, I have been rebuked for arrogantly affirming that there was nothing more to be learned about heredity!

Perhaps I had given less offence if I had made clear that I realised, as fully as another, that these discoveries in heredity were of such a sort that a whole new world for investigation opened before us. My critics, however, attempted to put me in the wrong by pretending that I implied that there were no new worlds to conquer. It may, therefore, not be out of place to attempt to show how the solution of the traditional problem of heredity has led to further discoveries, and has given us a glimpse at least of still newer problems that may in time lead to even more far reaching consequences.

Mendel's principles of heredity may be said, I think, to be mechanistic in principle, by which I mean that the coming together and separating of specific elements are concepts characteristic of physical events.* It is true that Mendel did not state that the elements that he

* It is interesting in reading Mendel's paper to note how, in almost every instance, when the opportunity arises for stating that the members of a pair

postulated as coming together and separating are material particles. For all that we know to the contrary, the good abbot may have had something more spiritual or mystical in mind. Nevertheless, whatever it is that meets and separates, whether spiritual or material, the *process* is in the nature of a physical event.

The new data relating to heredity might be treated as a series of purely statistical problems without regard to any special mechanism that has given the data. There would be admittedly a certain security in treating the problems in this way; but there are several reasons for hesitating to divorce the results from the animal and plant that supplies them. In the first place experience has shown that many suggestions of value have come from a knowledge of what happens in the egg and sperm. For example: occasionally, in the ripening of the germ-cells specific irregularities are known to recur. It is, therefore, instructive to find that certain rare events in Mendelian inheritance — events that are not immediately deducible from these principles as such — that these events are expected as necessary consequences of such irregularities; or, stated conversely, there are some types of genetic events that have been shown by actual cytological demonstration to be connected with exceptional behaviour of the chromosomes.

In the second place, need I urge that, as biologists, we are curious to find out how Mendel's principles tie up with the rest of our

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of allelomorphs *separate* to pass to their respective germ cells — it is interesting to note that he does not say this explicitly, but gives rather the result of such a separation. His usual method is to state that the number of germ cells containing the one element is the same number as that containing the other. At the end of his essay, however, the following statement occurs: "Since in the habit of the plant no changes are perceptible during the whole period of vegetation, we must further assume that it is only possible for the differentiating elements to liberate themselves from the enforced union when the fertilising cells are developed. In the formation of these cells all existing elements participate in an entirely free and equal arrangement, by which it is only the differentiating ones which mutually separate themselves. In this way the production would be rendered possible of as many sorts of egg and pollen cells as there are combinations possible of the formative elements." There is another procedure that Mendel follows that obscures the clarity of his view as to the nature of the process of "segregation." The dihybrid formula of an individual homozygous in one of the pairs of elements in question is written *a*AB instead of *a*ABB as we write it to-day. The latter presents to the eye two pairs of elements, each of which segregate; the former formula (*a*AB), if taken literally, seems incomplete from the point of view of segregation. In practice, however, Mendel did not fail to introduce B into every gamete (*a*B,AB) of such an individual.

knowledge concerning the changes in the germ-cells, the agents through which heredity takes place.

I will even venture to suggest that it is not a bad plan, for biologists at least, to maintain a guarded attitude towards abstractions that ignore the sources of the data from which the abstractions were made. Experience has shown, I think, that in an undeveloped subject such as ours progress has come less from unverified speculations about living things than by putting every new idea to the test of a critical experiment on the material itself before regarding a new idea as a serious contribution to science.

I.

During the thirty-five years that followed the publication of Mendel's paper (1865 to 1900) (while it still remained unnoticed) students of the germ-cells — cytologists — found that some extraordinary events take place at the time of maturation of the egg and the sperm. Weismann made use of some of the newly acquired facts in his well-known attempt to explain both development and heredity, but, except for the idea of the continuity of the germ-plasm, his attempt was little more than ingenious guessing and could scarcely have succeeded so long as the fundamental principles of heredity were unknown; and to Weismann, as well as to the rest of the biologists, Mendel's results were at that time a sealed book.

Mendel's paper was discovered in 1900, and even then for two years no one seems to have clearly realised that a mechanism had already been found that furnished an explanation of Mendel's laws. A young student, William Sutton, working in the Columbia Laboratory with Prof. E. B. Wilson, first stated clearly in 1902 the application of the then known facts of cytology to Mendel's laws.

I beg that you will allow me at this point to review rapidly certain rather well-known discoveries relating to the ripening of the germ-cells — discoveries familiar to biologists, but perhaps not so familiar to physicists and chemists.

In any cell about to divide (fig. 1), whether body-cell or germ-cell, the nuclear wall disappears, the chromosomes are resolved into threads, and a spindle appears in the protoplasm. The chromosomes move to the equator of the spindle. Here each splits lengthwise into daughter halves, one daughter chromosome moves to one pole of the spindle and the other daughter chromosome moves to the other pole. Two new resting nuclei begin to form and the protoplasm constricts into two parts. By this process, repeated over and over again, all the cells of the developing animal or plant are produced.

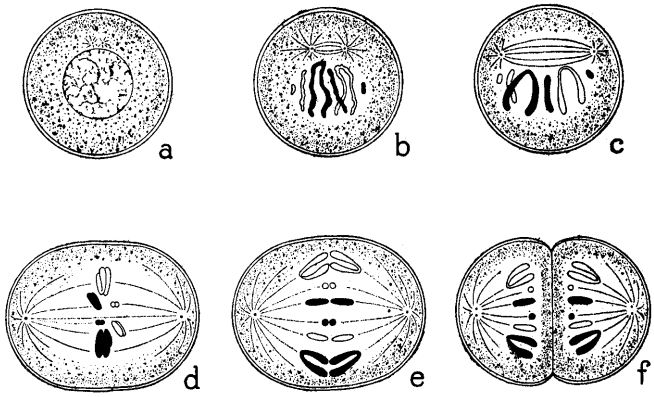

FIG. 1.—CELL DIVISION.

In these familiar processes of cell-division, there are two events of paramount interest to those students of genetics who hold that the chromosomes are the carriers of the hereditary elements; first, that every cell in the body contains the sum total of all of the hereditary elements; and second, that at every division, both in the early development, when the organ-forming regions are being developed, as well as in the later development when the specific tissues have appeared, the chromosomes divide lengthwise into daughter halves that are always exactly equivalent. It is obvious, therefore, that any theory that relates to the chromosome mechanism must recognise that no appeal can be made to a sorting out of the hereditary elements in the fertilised egg during its development — that every part of the body at all times must contain the entire hereditary complex. This means, of course, that development and differentiation must take place in the presence of all the hereditary material at every point (nucleus) of the embryo. The necessity of such an interpretation of the facts of cytology, while generally admitted, is seldom carried to its logical conclusion, with the result that the problems of heredity have become lamentably confused with the problems of embryonic development.

For each species there is a definite number of chromosomes (fig. 2). In every cell the chromosomes are duplex, that is, they are in pairs. One member of each pair has come from the father and the other from the mother. There is also a considerable body of evidence showing that these chromosomes persist from one cell-generation to another, although in the resting phase they cannot, as a rule, be identified. While observation cannot be said to have established the continuity of the chromosomes, the evidence from genetics is overwhelmingly in favour of such an interpretation, but adds one important qualification, that, at one stage at least in the ripening of the germ-cells, an interchange may

take place between the members of the same pair. I shall come back to this point later, for it is this interchange that has opened up to us far-reaching possibilities.

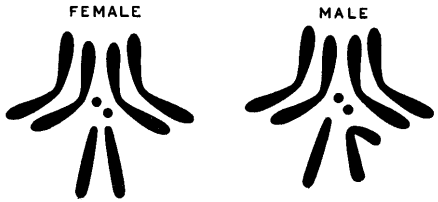

FIG. 2.

Two peculiar divisions take place in the germ-cells just before they become transformed into ripe sperm or into ripe eggs, and leading up to these divisions a process occurs that is unique, something that never happens in any other cell, either in the body or in the earlier cells of the germ-track itself. The chromosomes conjugate in pairs — each paternal unites with the corresponding maternal chromosome. The number of chromosomes appears to be reduced to half. In reality the chromosomes of each pair have come to lie side by side throughout their entire length.* Their reduction in number is apparent, not real. When this conjugation is accomplished the nuclear wall disappears, a spindle develops, the chromosomes pass to its equator, and then each double chromosome separates into its component halves. This process, except for details, is the same in the sperm-cells and in the egg. Let us follow each in turn.

In the sperm-cell the conjugated chromosomes separate and move to one or to the other pole of the spindle, and the cell divides (fig. 3). Then, without a resting stage, another spindle develops, the chromosomes pass into it, and now each splits lengthwise as in ordinary cell-division. The daughter halves move to opposite poles and the cell divides. After these two maturation divisions have been accomplished four sperm cells are produced, each with half of the total number of the original chromosomes.

When the *egg* matures (fig. 4) the conjugated chromosomes pass to the spindle, and the spindle then moves to the pole of the egg. At one division, the members of each pair separate and move towards the poles of the spindle. A protrusion of the protoplasm takes place near the pole into which the outer set of chromosomes passes. The protoplasm

* The possibility that end to end conjugation may take place in certain plants does not affect the question here at issue, except possibly the interpretation of crossing over.

constricts and the first polar body is formed. A new spindle develops about the chromosomes left in the egg, and each chromosome splits lengthwise. The daughter halves separate, half going into the second polar body, half remaining in the egg. The latter pass into a resting stage to produce the mature egg-nucleus. The first polar body also divides at the same time. Here also four cells are produced, but only one, the egg, is functional. It contains the half number of chromosomes.

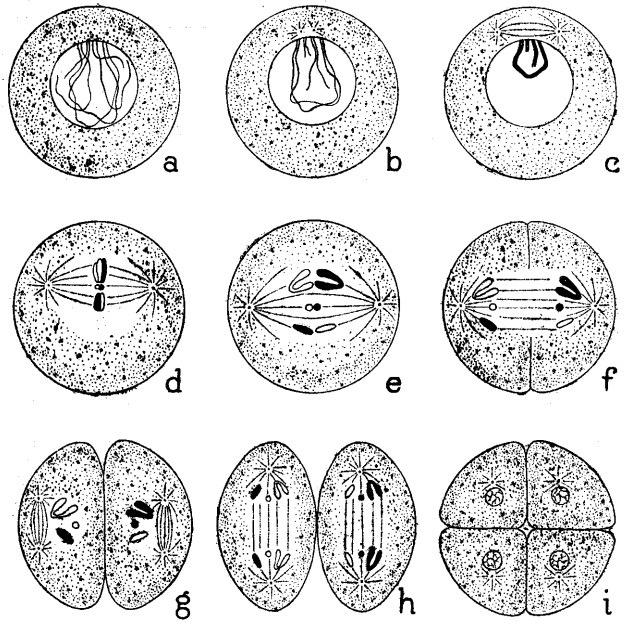

FIG. 3.—CONJUGATION OF CHROMOSOMES IN SPERM CELL.

For our present purposes the important event in the maturation of the germ-cells is the conjugation of the maternal and paternal chromosomes in pairs and their subsequent dispersal in such a way that each germ-cell gets one member of each pair.* By means of this

* In this description of the two "maturation divisions" no account is taken of "crossing-over," *i.e.*, interchange between members of the same pair of chromosomes. The genetic evidence clearly establishes this relation, even though the cytologist has not yet been able to furnish convincing evidence of its occurrence. It is important to emphasise that the genetic evidence has shown how arbitrary was the distinction made by cytologists between a reduction and an equation division (the first and second maturation divisions), for after interchange it is not the two original chromosomes that separate at reduction, but part of each. In other words, while for the tetrad

mechanism Mendel's two laws can be stated in terms of chromosome behaviour. Two examples will quickly show this.

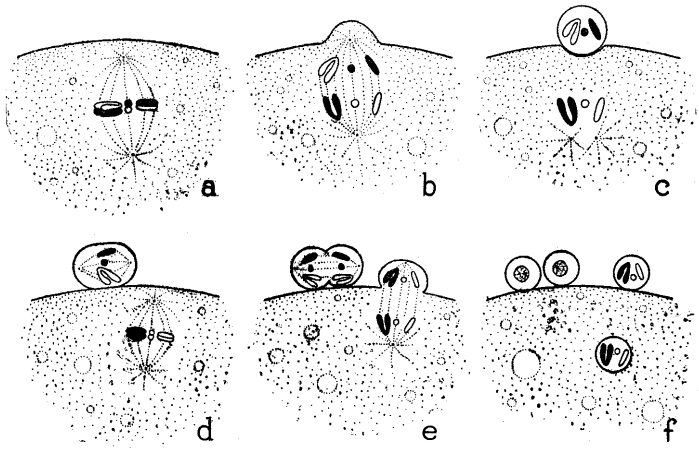

FIG. 4.—MATURATION OF EGG.

If a pomace fly (*Drosophila melanogaster*) of a vestigial winged race is crossed to a wild type (long winged) fly (fig. 5), the offspring (F_1) are long winged. If these are mated, three longs to one vestigial appear in the offspring (F_2).

If a factor for vestigial (*v*) is present in the vestigial race and is carried by each member of a given pair of chromosomes, then after maturation each sperm will have one of these chromosomes. In the long winged fly the corresponding factor (V) is carried by the same pair of chromosomes, and after maturation each egg will contain one of these chromosomes. Hence the hybrid offspring (F_1) will receive one long and one vestigial-bearing chromosome (*v*V), and, since the hybrids have long wings, we say that long dominates. When the hybrids mature and their germ-cells in turn are formed, each egg will contain either the long (V), or the vestigial (*v*) chromosome. Similarly, each sperm will contain either a long (V), or a vestigial (*v*) chromosome (fig. 6). Chance meeting of any egg and any sperm will give the F_2 results, viz.: three long to one vestigial. The genetic and cytological results agree.

there is a reductional and an equational division, the process as now understood relates to genes, not to whole chromosomes.

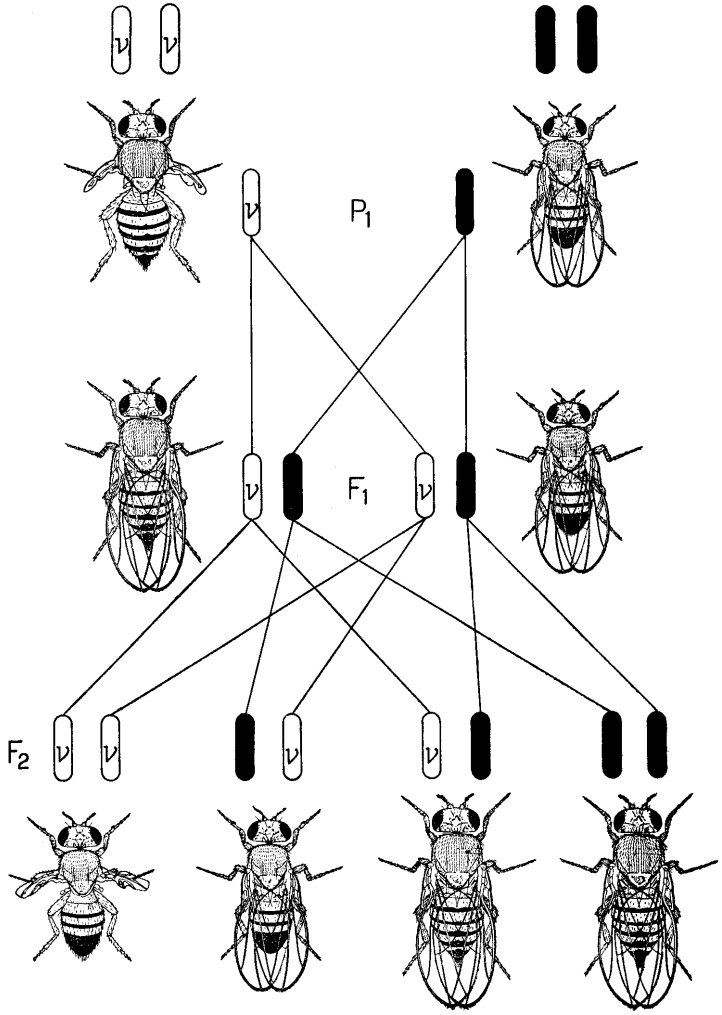

FIG. 5.

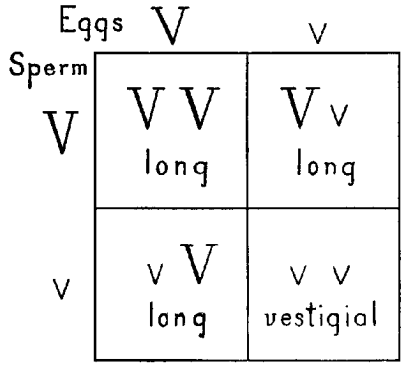

FIG. 6.

The behaviour of the chromosomes gives an explanation of Mendel's law of segregation.

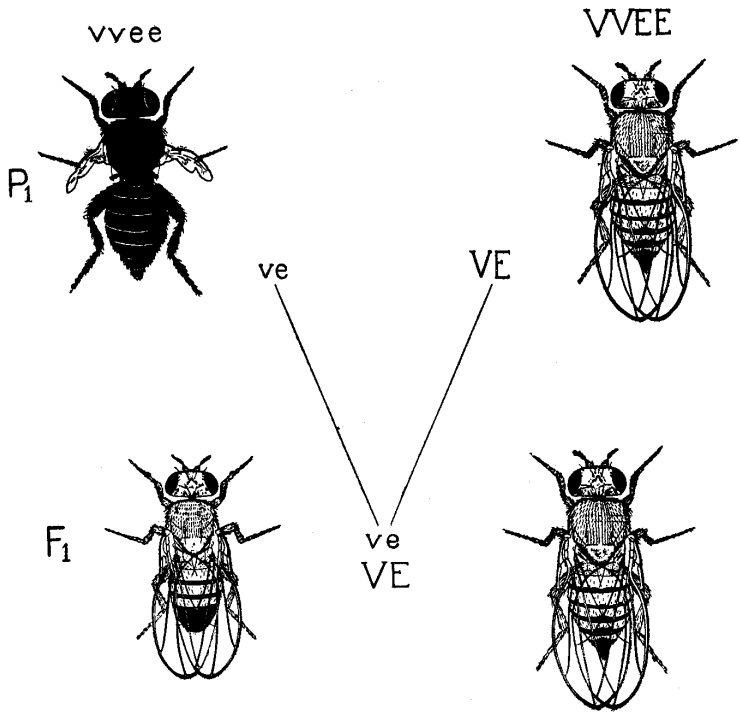

FIG. 7.

When two pairs of factors are present, each carried in a different pair of chromosomes, their inheritance is as follows (fig. 7):— Suppose that a fly that is ebony as to body-colour (*e*) and has vestigial wings (*v*) is used as one parent, and a fly that is grey (E) and has long wings (V) is used as the other parent. Here the two factor-pairs are grey (E) *versus* ebony (*e*), and long (V) *versus* vestigial (*v*). The offspring (E*e*V*v*) are grey long. If the factor-pairs (E*e* and V*v*) are carried in different pairs of chromosomes, as shown in fig. 7, and if each pair is sorted out to the germ-cells of the hybrid independently of the way in which the other pair is sorted out, four kinds of germ-cells result. Chance union of any egg and any sperm (fig. 8) will give the sixteen kinds of individuals which, reduced to classes, are in the ratio of 9:3:3:1. Here, again, the chromosome behaviour is the same as the genetic, provided the pairs of chromosomes are sorted out independently.

Eggs / Sperm	VE	Ve	vE	ve
VE	VE VE	Ve VE	vE VE	ve VE
Ve	VE Ve	Ve Ve	vE Ve	ve Ve
vE	VE vE	Ve vE	vE vE	ve vE
ve	VE ve	Ve ve	vE ve	ve ve

FIG. 8.

Fortunately we have evidence showing that such independent assortment of chromosomes takes place. Miss Carothers has studied in several grasshoppers the behaviour of chromosomes at the reduction division (fig. 9). Slight differences in the mode of attachment of the

chromosomes to the spindle fibres (differences that are constant) enabled her to show that the sorting out of the members of the pairs is, in reality, independent.*

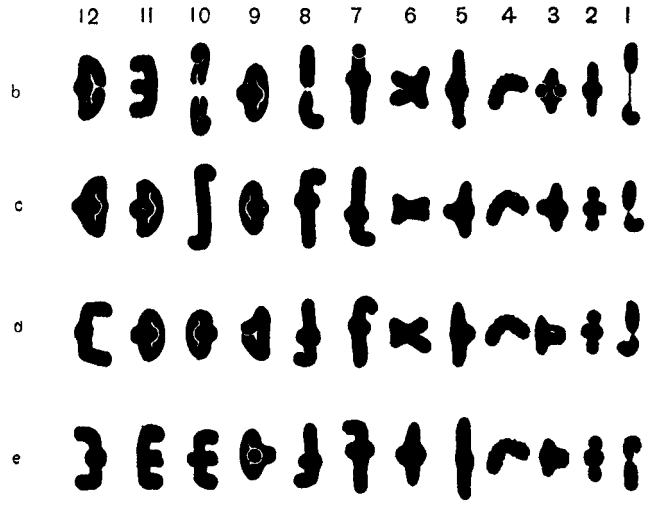

FIG. 9.

II.

Even if it is admitted from such evidence that the chromosome mechanism suffices to explain Mendelian segregation and assortment, as Sutton pointed out, it still remained to be shown that we are not

* In this figure (fig. 9) each of the four horizontal lines (1*b, c, d, e*) shows the twelve *double* chromosomes (tetrads) present in each spermatocyte, *i.e.*, the twenty-four diploid chromosomes that have conjugated giving these twelve tetrads. The conjugants (paternal and maternal chromosomes) are about to separate in the first maturation division. One of these (No. 4) is the X-chromosome, and has no mate in the male. It is placed in such a position in the drawing that the later migration to the pole would be upwards, *i.e.*, towards the top of the page. The other chromosomes are oriented with respect to this one. Three of these pairs (viz., 1, 7, and B) are each composed of two unlike chromosomes, in the sense that one member of each pair is straight (terminal attachment of spindle fibre), the other, bent (sub-terminal attachment). These differences are found in every pair of these particular chromosomes in this individual.

dealing with analogy or coincidence; but that the chromosomes are specifically related to genetic events. Fortunately we have to-day such evidence. Let me give a few illustrations.

There is a race of Drosophila called "Diminished-bristles" (fig. 10), that was discovered by Bridges, in which all the "Diminished" individuals carry *only one* small, or IVth, chromosome. Half of the ripe sperm-cells carry this IVth chromosome, and half do not carry it. Now if "Diminished" is crossed to a fly from the "eyeless" stock (that carries a recessive gene in the IVth chromosome) there should be two kinds of offspring corresponding to the two kinds of sperm. Such in fact is the case, and the offspring that are "Diminished" have been shown to have only one IVth chromosome. Moreover, since the single IVth chromosome that they carry is derived from the recessive stock, we can understand why these first generation Diminished flies are eyeless; in other words, when Diminished flies are outcrossed to any IVth chromosome recessive stock, the recessive character appears as though it were a dominant.

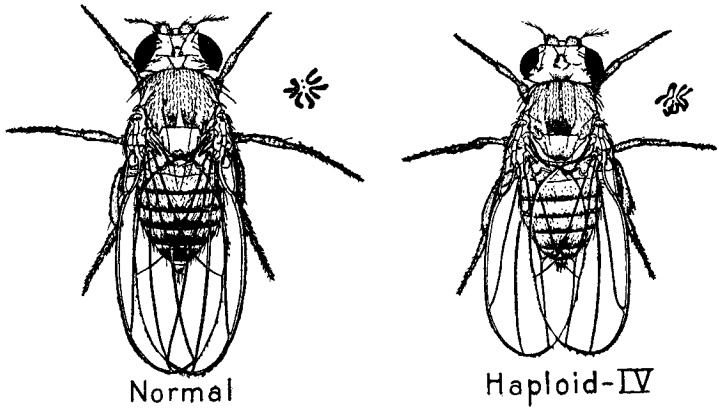

FIG. 10.

There is another stock of Drosophila, also discovered by Bridges, in which certain individuals contain *three* IVth chromosomes. These flies may be distinguished from wild type by their smaller eyes, darker body colour, narrower wings, etc. (fig. 11). Owing to the presence of three IVth chromosomes, half of the matured germ-cells will contain two IVth chromosomes, the other half only one IVth chromosome (fig. 12). If such a triploid-IVth-chromosome individual is mated to eyeless, half of the offspring will be triple IVths. If two such triples are mated to each other they do not give a three to one Mendelian result, but give a

ratio of about 26 to 1, which is the expectation for such a chromosome situation.

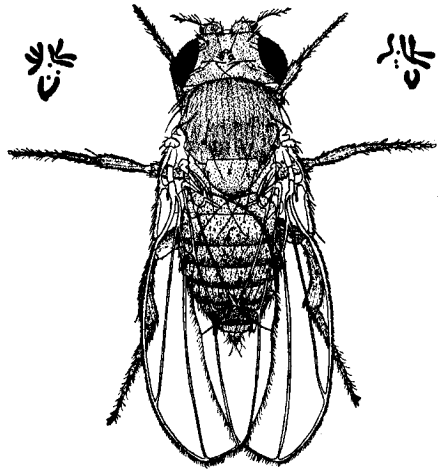

FIG. 11.

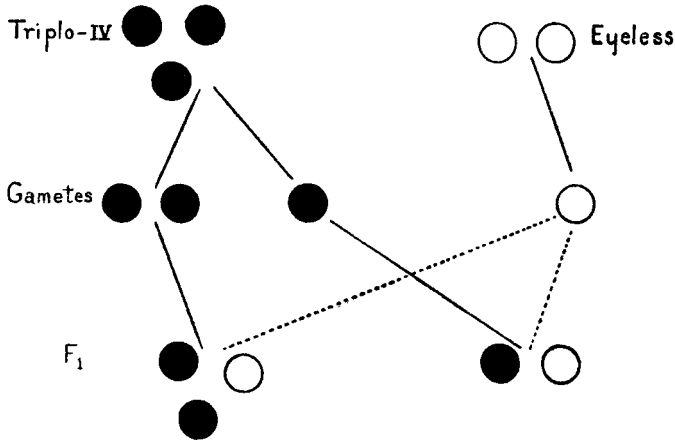

FIG. 12.

In back-crosses such F_1 flies give a 5 : 1 ratio (fig. 13), instead of a Mendelian 1 : 1 ratio.

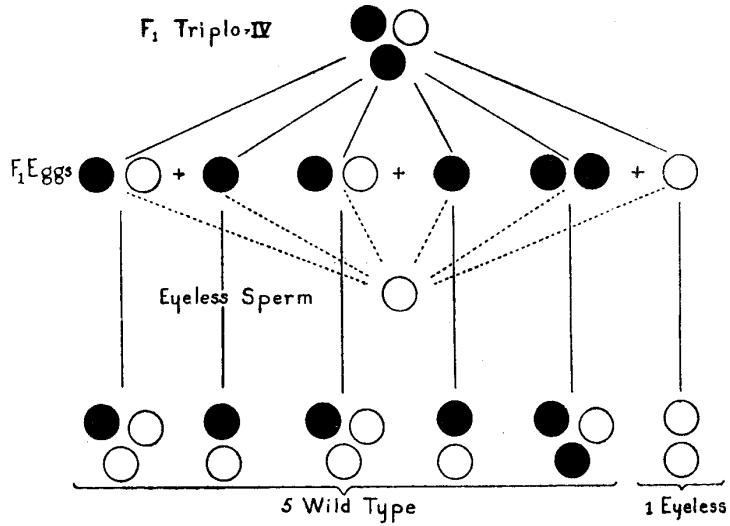

FIG. 13.

Here we have an unusual genetic and cytological behaviour that checks up at every point; I say at every point, because there are several other possible tests, most of which have been made, and the genetics conform to the expectation based on the known distribution of the IVth chromosomes.

Other kinds of irregularities also occur at times in the division of the germ-cells. One kind is called non-disjunction of the Ist chromosome, because at one maturation division the two X-chromosomes do not always *disjoin,* but may both go out of the egg into the polar body or both may remain behind.

If both should remain in the egg and this egg is fertilised by a Y-bearing sperm (fig. 14), an XXY individual will result, having these three sex-chromosomes in all the cells of the body, including, of course, the eggs before maturation. Such XXY females will then exhibit secondary nondisjunction, for when an egg reaches the maturation stage a sort of triune relation will exist in the three sex chromosomes, and since only two can conjugate, the third must do a *pas seul.*

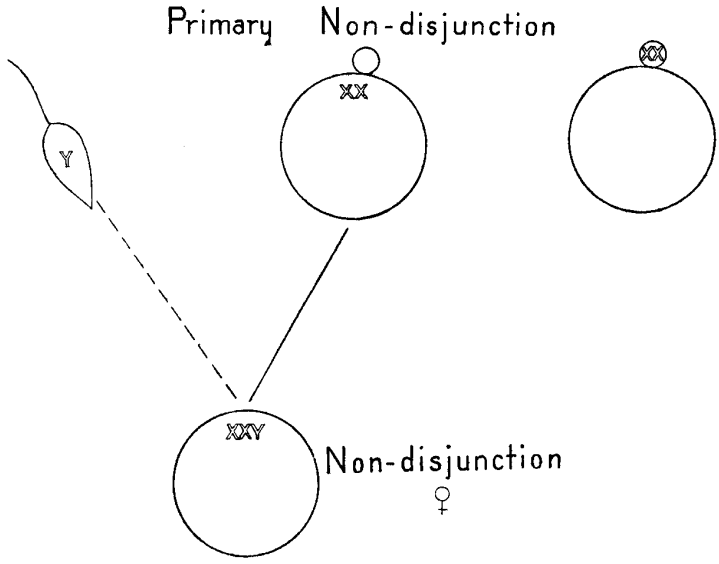

FIG. 14.

Fig. 15 shows the four possible kinds of eggs that result from this nondisjunction. If the four kinds of eggs are fertilised by X-bearing sperms, four kinds of individuals are expected. In order to reveal the nature of these individuals, it is advantageous to use an XXY female in which both X's carry a recessive character, such as white eye-colour, and to cross her to a normal red-eyed male. This combination gives four classes of offspring. One kind of female (1) is XXY, and should show non-disjunction when bred. This has been proven. The next female (2) is normal. The third (3) has three X's, and usually dies, but rarely she comes through, is always sterile and *has* three X's. The fourth individual is a normal red-eyed male. He behaves as such.

If, on the other hand (fig. 16), the four kinds of eggs are fertilised by the Y-sperm of the same male, four kinds of individuals are expected. The first (5) is a white-eyed male with one X and two Y's. If he is tested he is found to produce some XXY daughters that are non-disjunctional. The second (6) is a normal white-eyed male. The third (7) is a white-eyed female that is non-disjunctional. When tested she is found to give such results and has been shown to have two X's and a Y-chromosome. The fourth (8) never appears, because obviously it has no X-chromosome at all.

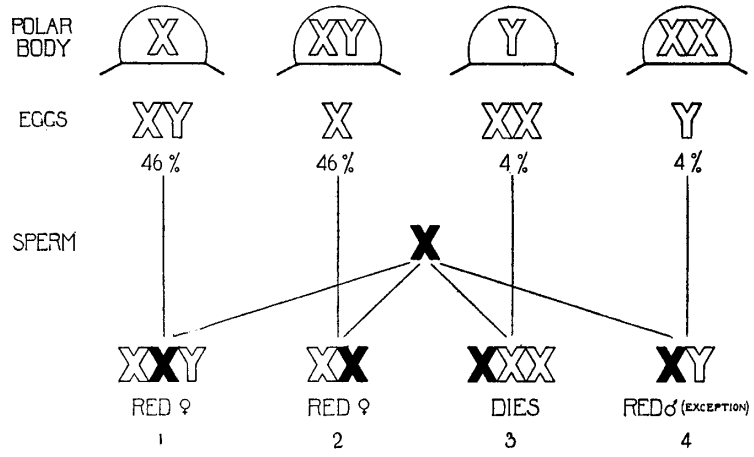

FIG. 15.

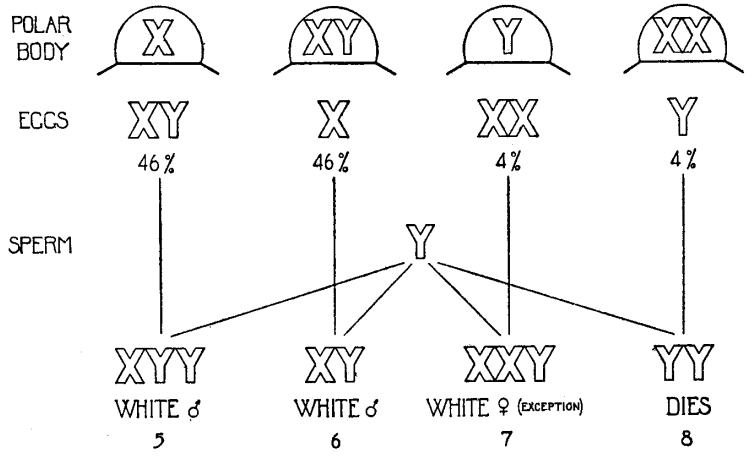

FIG. 16.

My next illustration concerns what we call the double yellow females, recently discovered by Lilian V. Morgan. This stock is descended from a fly that showed a reversal of the usual results in sex-linked inheritance. The double yellow females are now known to

contain two X-chromosomes joined to each other, end to end. Each carries the factor for yellow. When fertilized by a grey male, such females, as a rule, produce yellow daughters and grey sons. Figure 17 shows why this takes place. After the elimination of the polar bodies there are two kinds of eggs. One has the double X the other a Y. Fertilized by a normal male there are four kinds of offspring expected. The first (XXX) has a double yellow-bearing-X and a grey-bearing-X. She generally dies, but occasionally comes through. When she does she is grey, because one grey gene dominates two yellow ones. She is sterile, and cytologically she has been shown to have a double-X (yellow) and a single-X chromosome.

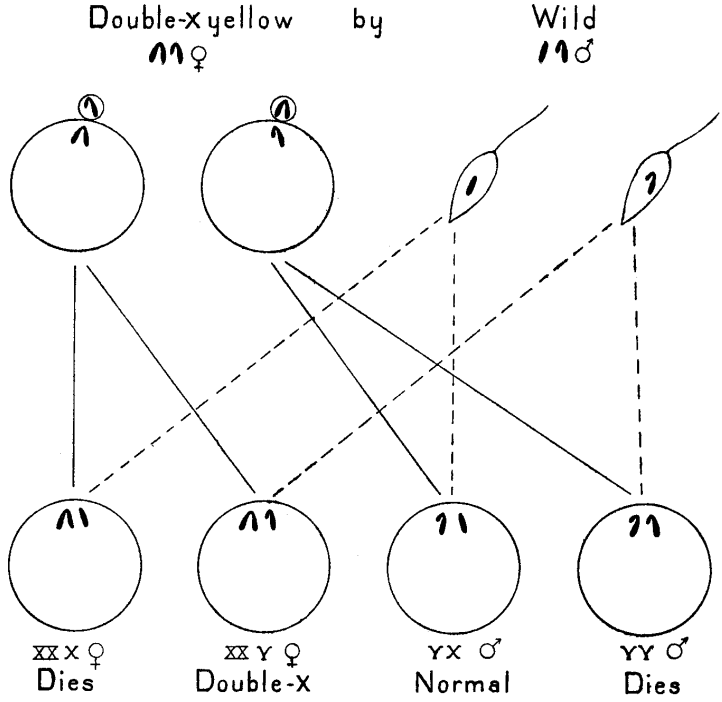

FIG. 17.

The second individual is a double yellow female that repeats the story. The third is an XY male like its father. The fourth, YY, dies.

Thus half the offspring die, and the normal 1 : 1 sex ratio of females to males is maintained. Aside from the great theoretical interest of this case, it has a very practical side also; for by using the double yellow females we can carry on certain stocks of Ist chromosome

characters, which have infertile females. The males of such stock, bred to double yellow females, reappear in the offspring, as well as do double yellow females. The stock is self-regulatory, since the rare XXX females are infertile and require no further attention.*

III.

There is a corollary to the view, that the genetic factors are carried by specific chromosomes, that has far-reaching consequences, which from the first were foreseen.

If there are many factors in the same chromosome, we should expect these characters to be inherited in groups. Such groups are known.

In *Drosophila melanogaster* there are only four pairs of chromosomes (fig. 2). In the female there are two X-chromosomes, two large pairs of autosomes and the minute fourth pair. In the male there is an X and its mate called Y, and the same three pairs of autosomes.

There are about 400 races of *D. melanogaster* whose characters have been sufficiently studied to show how they are inherited. They are inherited in four groups. This means that if two or more members of the same group go in together, *i.e.*, if they lie in the same chromosome, they tend to be inherited together through successive generations.

Fig. 18, shows some of the characters of the first group (I) carried by the X-chromosome. Some of these characters are eye-colours, others are wing, or leg, or bristle characters, etc. In group II we find other eye characters (fig. 19), wing, leg, and bristle characters.

In group III again, we find still other modifications of the same parts of the body (fig. 20). In group IV only three characters have so far been identified (fig. 21). The experimental evidence shows, as I have pointed out, that these last characters are carried by the small chromosome.

* One reservation must be made. Occasionally the two X's break apart in an egg before the polar bodies are produced. Then one goes out, and such a ripe egg behaves like an egg with a single yellow bearing X. If, for instance, it should be fertilized by a Y sperm, it will give a yellow male, as in ordinary sex-linked transmission. If it should be fertilized by an X sperm, carrying wild type genes, it will give a normal wild type (XX) female, that breeds as a heterozygote for yellow.

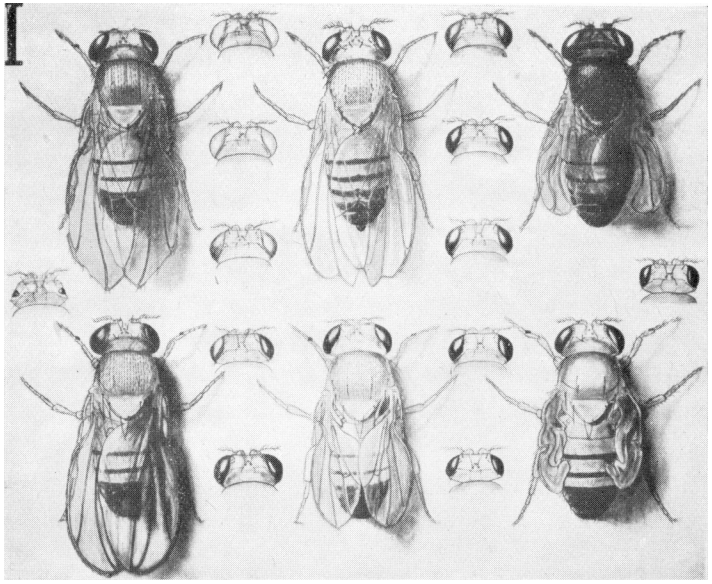

FIG. 18.

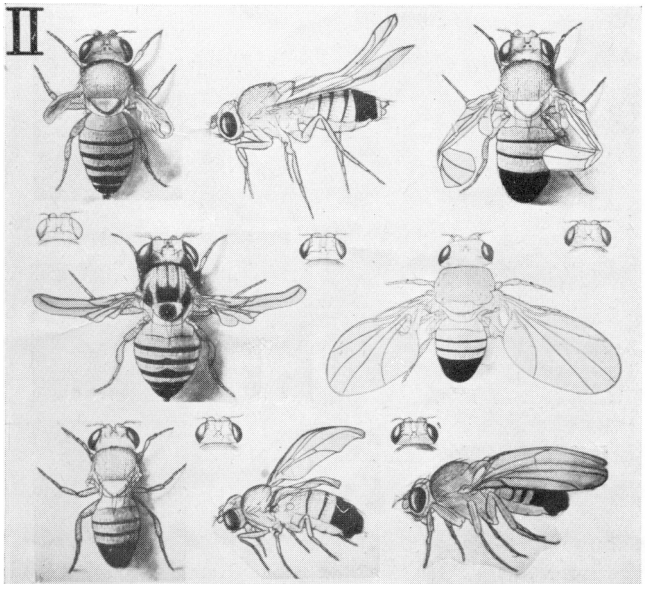

FIG. 19.

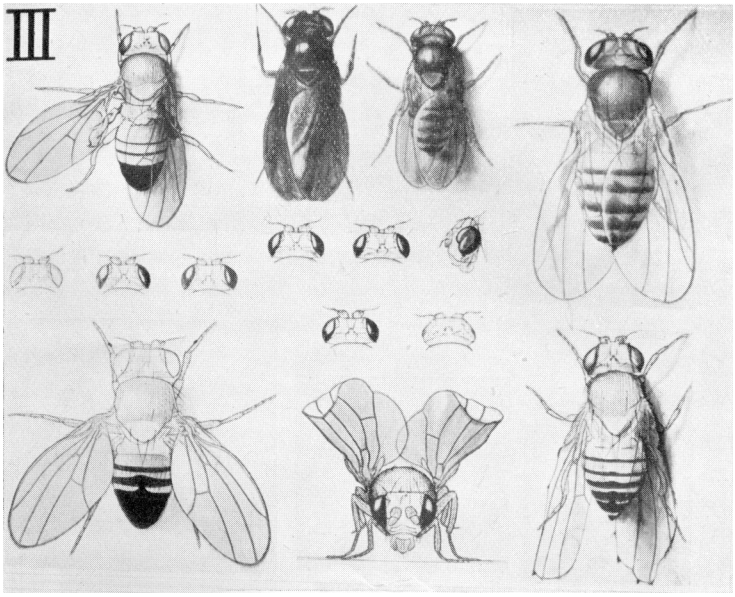

FIG. 20.

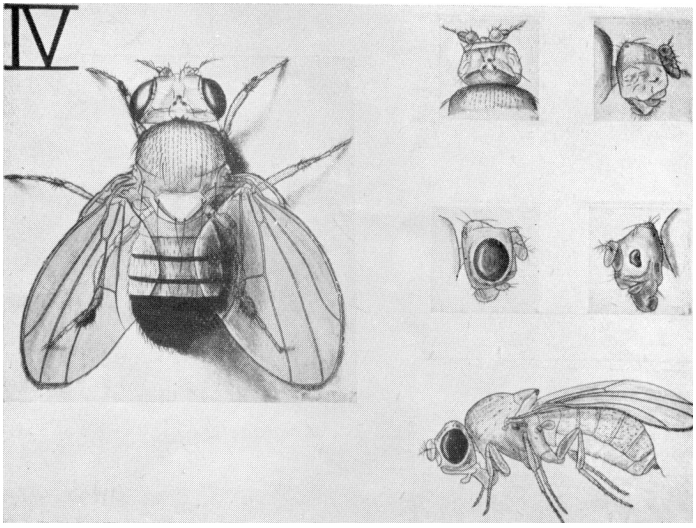

FIG. 21.

It is interesting to note that the number of characters in each group is about proportional to the known sizes of the chromosomes. It is true there are somewhat more in group I than in the other two large groups in proportion to its length, but we understand why this is so, since a new mutant recessive gene appearing in the X-chromosome is more likely to be found than a recessive mutant in any other chromosome.

If we turn to other species of Drosophila we find, as far as the evidence goes at present, that there is in them also a correspondence between the number of chromosomes and the number of linkage groups.

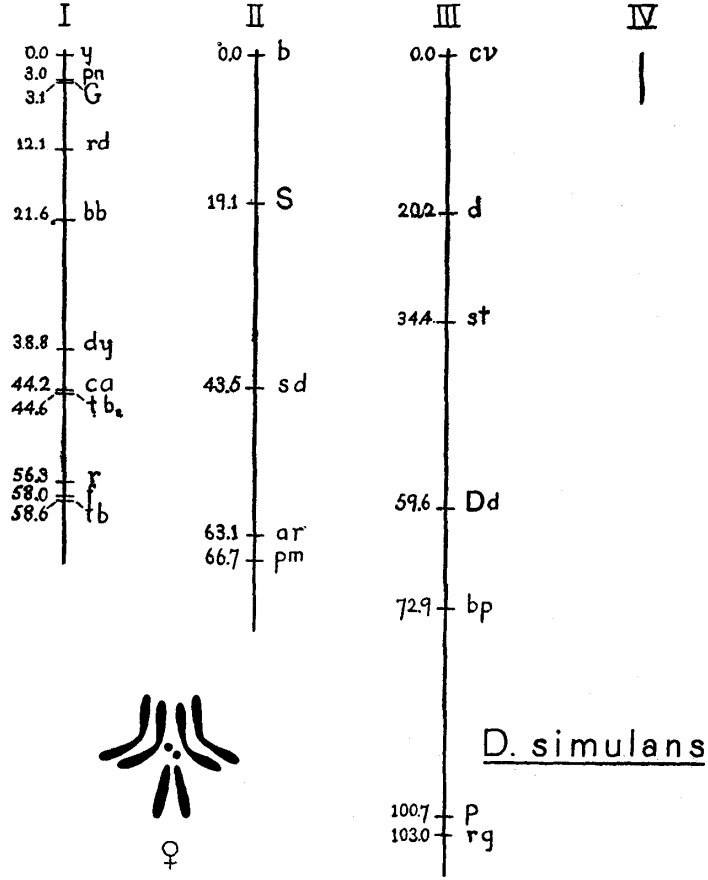

FIG. 22.

The species that is most like *D. melanogaster* is *D. simulans*. So similar are the two that they have until recently been supposed to be the swine species. Now we recognise many minute differences between them, and know that they give sterile offspring when crossed. There are four pairs of chromosomes in simulans (fig. 22), identical in shape and size with the chromosomes of melanogaster. Sturtevant has found, to date, three groups of linked characters in this species.

In *D. willistoni* there are, according to Metz, three groups of linked genes and three pairs of chromosomes (fig. 23).

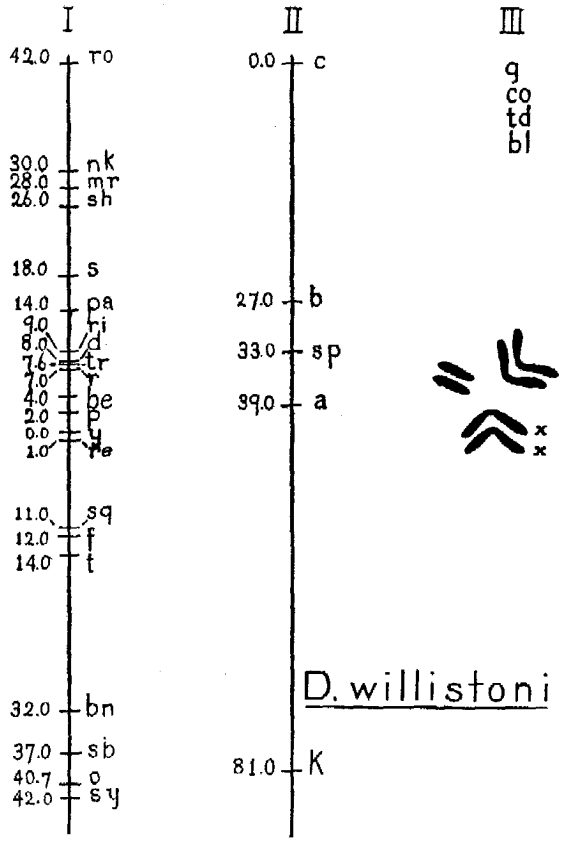

FIG. 23.

In *D. virilis* there are, according to Metz, five known groups of linked genes and six pairs of chromosomes (fig. 24).

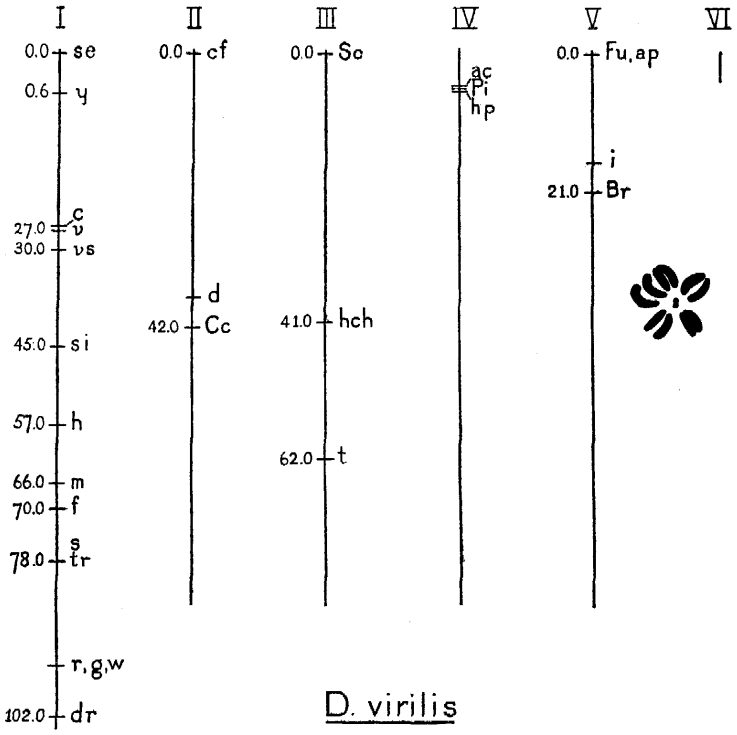

FIG. 24.

In *D. obscura* there are, according to Lancefield, five groups of independent genes and five pairs of chromosomes (fig. 25). The first group is almost twice as long as the first group in melanogaster (the yellow, notch, white loci in the middle instead of at the end), and the X-chromosome is a bent chromosome nearly twice as long as the X in the other species.

In other animals and plants evidence is slowly coming in showing that the linkage groups correspond with the number of the chromosomes. In the edible pea, for instance, there is evidence, according to White, that there are seven independent factors and seven chromosomes.

In a wild California plant, Clarkia, Burlingame has found two linkage groups, and there are only two pairs of chromosomes.

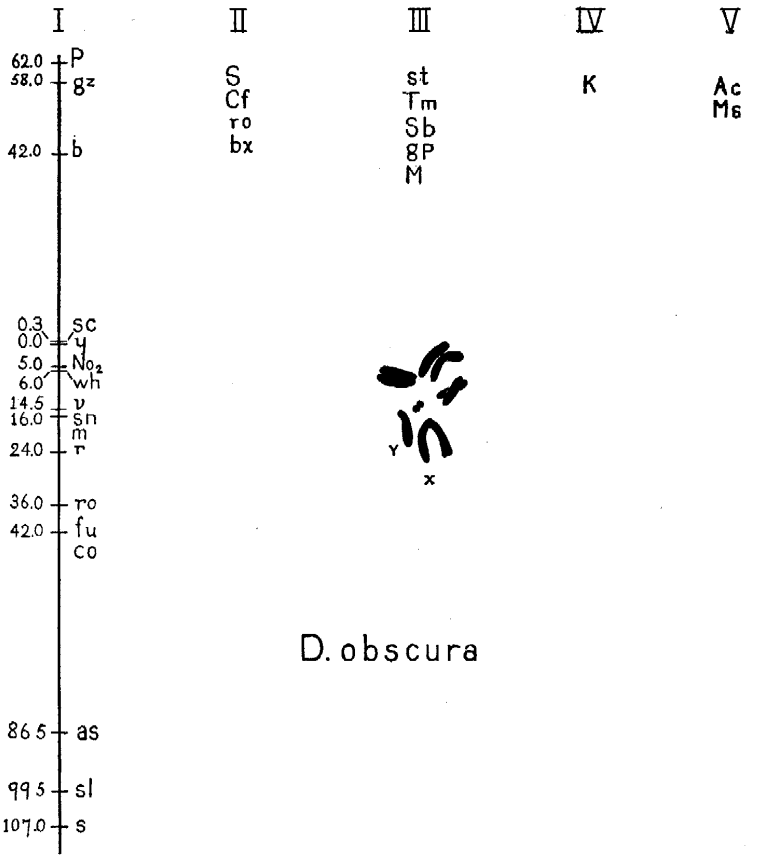

FIG. 25.

In cases where there arc numerous chromosomes it is difficult to find enough independent factors to test out the relation of linkage groups to chromosomes. We shall have to wait for further evidence until more extensive work has been done on such forms. Nevertheless it may, I think, be claimed without exaggeration that the facts so far obtained are consistent with the view that the linkage groups correspond in number to the number of chromosomes.

IV.

The evidence so far considered tells us little or nothing as to how the genes are situated in the chromosomes. Here a new phenomenon enables us to carry our analysis further. The essential evidence can be presented most easily by a few illustrative cases.

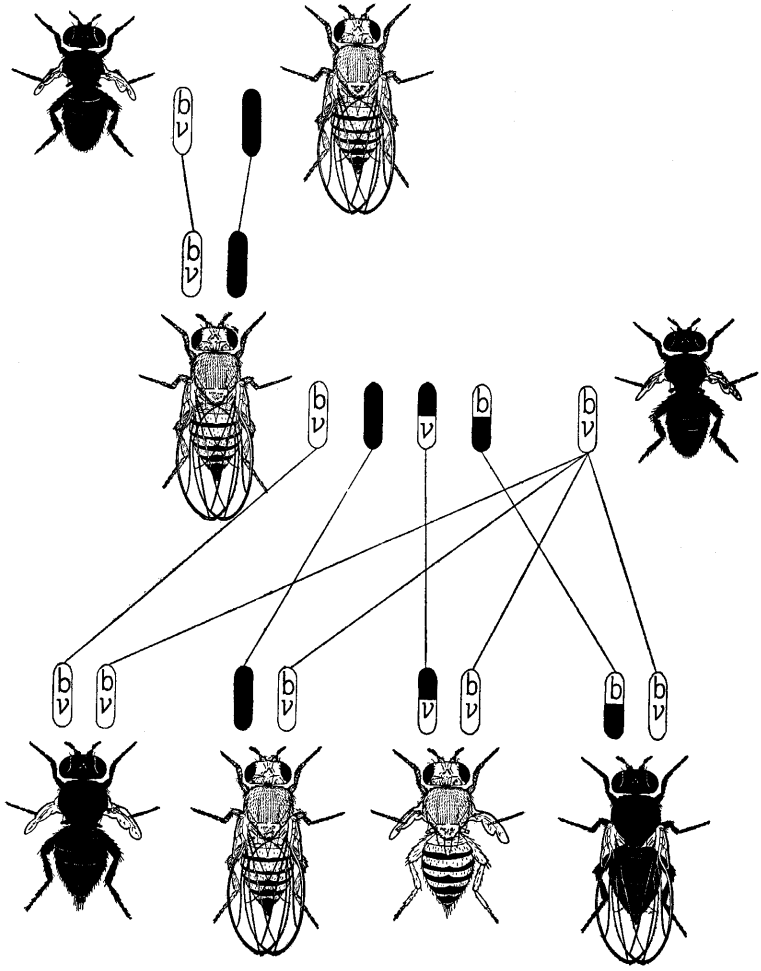

FIG. 26.

As we have seen, there is a mutant race of Drosophila with vestigial wings (*v*). The gene for vestigial is in the IInd group. There is another mutant race called black (*b*), whose gene is also in the IInd linkage group.

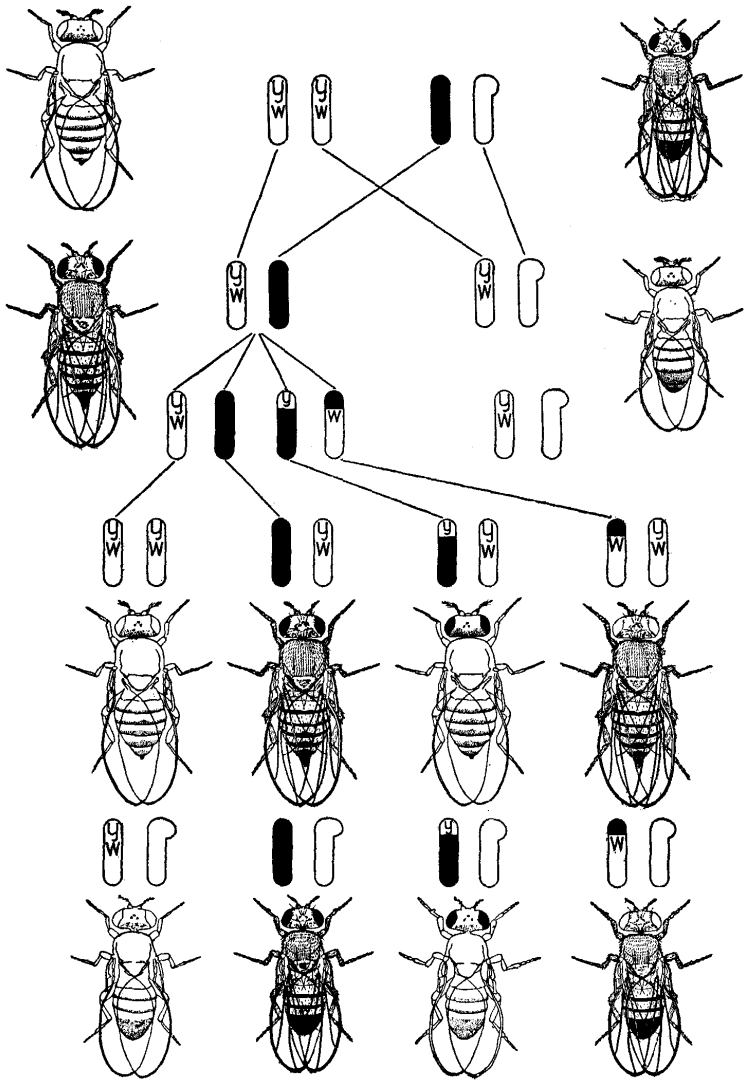

FIG. 27

We can easily make a race that is both black and vestigial. If we cross (fig. 26) a black vestigial (*bv*) to a wild-type fly (BV), we get wild-type hybrid offspring (B*b*V*v*). If now we take such a hybrid female and backcross her to a black vestigial from stock, we get, not two classes of offspring, as expected, if black and vestigial were completely linked; but, on the contrary, we get four classes-two large classes, representing linkage (*bv* and BV) and two cross-over classes (*b*V and B*v*). In other words, while there is still a tendency for the linkage to hold, there is also evidence that it breaks in a certain number of the cases. We may say that black colour has crossed over to long wing in 8½ percent of cases, and reciprocally wild-type colour has crossed over to vestigial in 8½ percent of cases. Taken together we say that there is 17 percent of crossing over.

My second illustration of crossing over relates to the first or X-chromosome. If a fly with white eyes and yellow wings (both characters carried in the X) is crossed to wild type (fig. 27), all the daughters are wild type, and all the sons are yellow white. If we cross the hybrid female to her white-eyed yellow brother (or else to a white-eyed, yellow stock male), we find that 99 percent of the offspring show linkage. In addition there is 1 percent of crossing over.

Hundreds of similar cases could be cited. We find every degree of crossing over, from 50 percent (which approaches free assortment of characters) to cases much less than 1 percent, but there is a constant percentage of crossing over for each two linked genes under the same environmental conditions.

We find such interchanges are always within the same linkage groups. Evidently we must revise our first conclusion that the chromosomes remain intact. We must revise this first conclusion, in so far that members of the same pair sometimes interchange pieces. More important is the fact that the percentage of interchange is constant for each particular combination. It is from such data that we have been able to form a theory of the location of the genes in the chromosomes.

This question of crossing over is so important for what is to follow that the evidence relating to it calls for most careful consideration. I am especially anxious to make clear how far the evidence that is necessary for the construction of the chromosome maps is independent of later attempts to discover the method by which crossing over takes place. To do this, it will be necessary to recall certain general features of the situation:

(1) The chromosome theory, namely, that the hereditary units are carried by the chromosomes, means to-day much more than that the chromatin is the material basis of heredity. The theory carries with it the idea of the individuality and continuity of the chromosomes. The

individuality was held by cytologists largely as a matter of faith until genetics showed that the chromosomes have specific relations to the body characters. But here again the extraordinary exchange of equivalent parts between the maternal and paternal members of a pair demonstrates what was to Boveri only a casual speculation or a possibility with almost no evidence in its favour and no proof at all to support it. As to the continuity of the chromosomes from one cell division to the next — a relation that has proven an almost insuperable difficulty to cytology — it matters not at all to genetics whether in the resting stage the hereditary elements separate (break apart or dissolve) so long as they come together again at the next cell division in the same chromosome and in the same order that they had previously occupied. That the "genes" should actually be dissolved and come together with the extraordinary regularity indicated by all the work on crossing-over is almost beyond belief, yet I repeat it is not essential to the continuity idea to suppose that they remain united.

(2) It seems to me that there is no escape from the conclusion that interchange of equivalent blocks of genes (*i.e.*, pieces of chromosomes) takes place in a perfectly orderly manner for anyone who accepts the view that the "chromosomes" are the bearers of the hereditary units. It shows a failure to grasp the situation, to be willing to accept the chromosome view and assume a critical attitude towards the *evidence* for crossing over — since the evidence for the one is of the same nature and as cogent as it is for the other. By "marking" specific chromosomes (both members of the same pair) it has been possible to demonstrate that when crossing over takes place each chromosome breaks apart at the same level and an interchange takes place. For example, if the loci of a chromosome are indicated by the letters A, BC, D, E, F, and its mate by *a, bc, d, e, f,* then whenever the first series breaks between D and E (let us say) the other one breaks between *d* and *e.* As a result of such a breaking followed by interchange, the two series that result are A, BC, D*, e, f,* or *a, bc, d,* E, F.

(3) Such being the fact, it necessarily follows that the nearer together two units lie the less likely is a break to occur between them; and conversely the farther apart two units lie the more likely is a break between them. It is not necessary to make any further assumption (except that where crossing over occurs at one level it is less likely that another crossover will occur simultaneously near the first) in order to deduce from the situation the arrangement of the genes on the chromosomes.

I should like to emphasise, as strongly as I can, that the chromosome maps, representing the arrangement of the genes, are derived directly from the genetic evidence relating to crossing over. It

is true that we have gone further and have tried to find out how the genetic evidence ties up with cytological processes known or supposed to take place in the germ-cells, but the localisation of the genes has been determined independently of these attempts to discover the mechanism of crossing over. The latter might be entirely erroneous without at all affecting the validity of the methods by which the genes have been located.

It may be best therefore at this point to say something about these maps (fig. 28) before passing to a consideration of possible interpretations of the mechanism of crossing over.

The maps* enable anyone to predict to within a small degree of error how a *new* character that appears in Drosophila will be inherited with respect to the other characters already known. This means that all we have to do is to determine, first, the linkage group of the new character, then the crossing over within this group between the new character and any two other members of its series. After this, by means of very simple calculations that take at most but a few minutes, we can predict how the new character will be inherited with respect to every other known character.†

* In the figure of the maps, giving the principal loci of the four chromosomes of *Drosophila melanogaster,* the more common loci are named, but the others are also indicated by cross lines. The spacing gives the relative position of the loci. "Distance" is used in a figurative sense for crossover value. The distance apart of the loci on the map ("map distance") is only a relative matter, and rests on the assumption that crossing over in one part of the "chromosome" is as frequent as in all other parts-an assumption that we have found reason to think is not entirely accurate.

† Actual length of the section between the loci is only one of the factors determining the amount of crossing over between the loci, and, consequently, the map distance. Both environmental and genetic factors are known to influence the frequency of crossing over within a given length. Therefore, the length of a section of the chromosome represented by a unit distance (1 percent) may be different in different regions of the chromosome. A parallel to the maps is found in a railroad timetable, where the number of minutes between stations is given. From such a table one can judge accurately the sequence of the stations and roughly the actual number of miles between them. Knowledge of the normal speed, of the train and the condition of the road bed and of the grades would make it possible to judge more accurately the number of miles between the stations from the number of minutes between the stations.

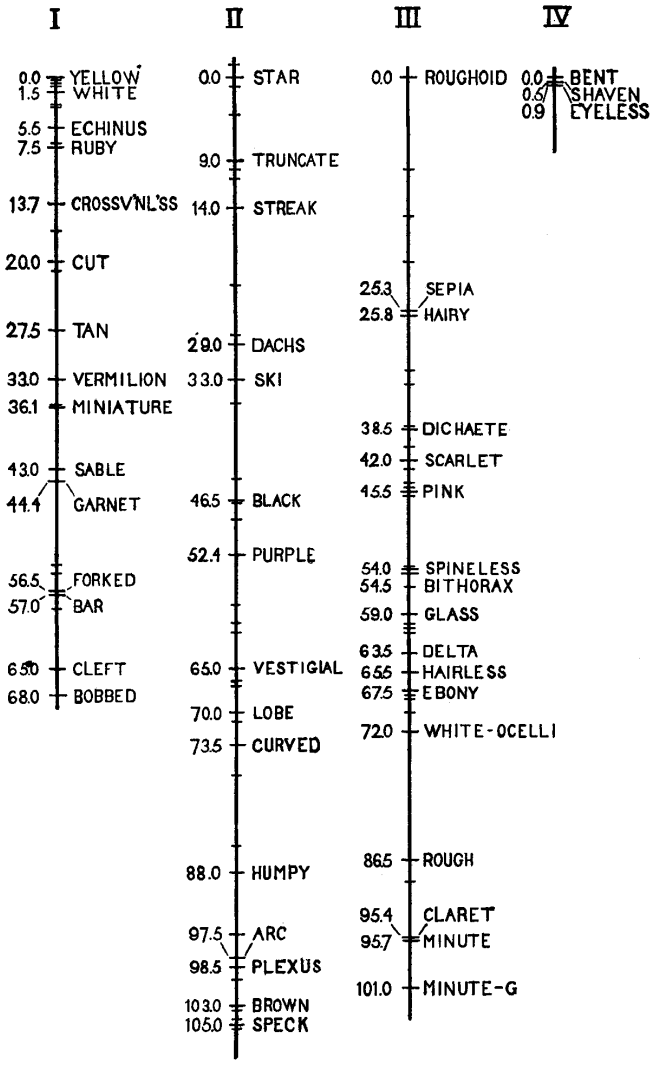

FIG. 28.

This ability to predict would in itself justify the making of such maps. But the maps have for us a somewhat wider interest, because they furnish evidence as to how the hereditary factors lie in the chromosomes. A single illustration will, I hope, make this clear (fig. 29). Two points, yellow and white, in the first chromosome are

represented as 1.2 units apart. Now if a new type, bifid wing, should turn up and its crossover value with white should be found to be 3.5, experience shows that it is expected to give with yellow either the sum of 1.2 and 3.5 (= 4.7) or the difference between 3.5 and 1.2 (= 2.3). In other words, if it lies south of white it should give 4.7 percent crossovers with yellow; if north of white it should give 2.3 percent crossovers. The same principle is known to hold for four, five, six or any number of points in the same genetic series.

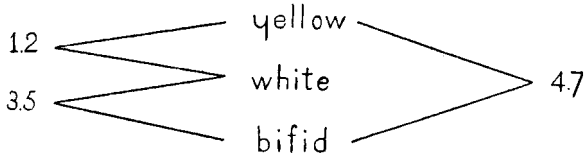

FIG 29.

This relation of three or more points to each other is a relation of linear order, and cannot be represented in space in any other way than by a series of points arranged in a line like beads on a string.*

V.

Once in each generation in the whole course of the germ-track cycle of cell divisions, the maternal and the paternal members of each, pair of chromosomes come together as thin threads and appear to fuse into a single thread. Thus, as has often been pointed out, the conjugation of the chromosomes — the meeting of the maternal and paternal genes — takes place before and not immediately after fertilization.

In searching for a time in the history of the germ-cells when interchanges (crossing-over) might take place, it is to this stage (conjugation) that one's attention would naturally turn. It is very

* It is perhaps hardly necessary to explain that all the results with Drosophila that are given in the text are not the result of one individual's work — certainly not my own. The work has been done by a small band of collaborators, in part under the auspices of the Carnegie Institution of Washington, and in part as members of the Zoological Laboratory of Columbia University. Sturtevant, Bridges, Muller, Metz, Weinstein, have held the advanced line, supported by twenty or more other investigators and students, each of whom has made one or more worth-while contributions to the Drosophila work.

fortunate, therefore, that it has been found possible to obtain experimental evidence in Drosophila that crossing-over does occur at about the time of conjugation of the chromosomes. I refer to Plough's temperature experiments. Plough found that a change in the temperature causes a change in the amount of crossing-over. By subjecting flies whose eggs were known to be at certain stages of maturation to differences in temperature, he showed definitely that crossing-over occurs at this late stage in the history of the egg-cell.

If now we turn to the evidence furnished by cytology no one would fail to have his attention arrested by certain descriptions of an event that occurs when the chromosomes conjugate. At this time, according to Janssens and other observers, the thin threads that are united at one end and extend out into the nucleus sometimes appear to overlap or even to twist around each other (fig. 30). Now if they come together while still in this condition it follows that a maternal thread will lie on one side of its paternal partner for a part of its course, and on the other side for the rest of its course. This gives a mechanical model that fulfils all the requirements of crossing over, *provided* the parts of the thread that come to lie on the same side unite with each other to form a continuous chromosome (fig. 31). In the present stage of cytological research it is extremely improbable that this event — the breaking of the old threads at the crossing over level and the reunion of the ends on the same side — could ever be actually followed, since it would require continuous observation on living material.* At best one might hope to find evidence that crossed threads are sometimes actually present when the conjugation is taking place, and that at the time of separation of the condensed thread (reduction) the chromosome threads are no longer twisted about each other.

* Recently Seiler has found in one of the moths, Solenobia, two pairs of chromosomes that may join temporarily in the male to form only one pair at the maturation stages. If the union is at random the outcome would be the same as for free assortment; but if certain unions are more likely than others, then the results will appear to give the same result as crossing-over in Drosophila. If the union and subsequent separation of the two pairs of chromosomes in question is always at a given point, the phenomena will be quite different from what is observed in Drosophila. Other hypotheses to account for crossing-over are not in harmony with the facts to be explained. For a detailed criticism of this see 'The Mechanism of Mendelian Heredity,' Morgan, Sturtevant, Muller, Bridges.

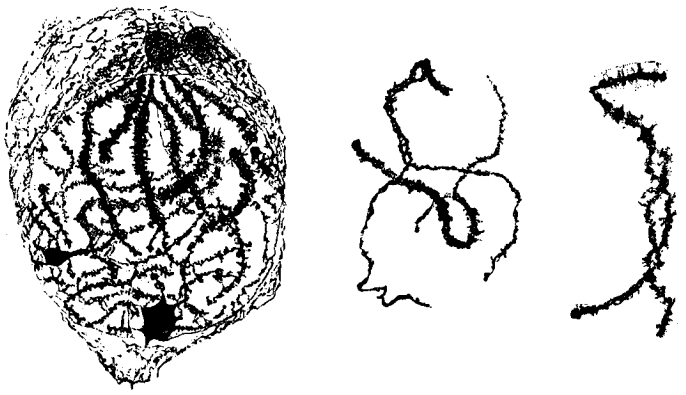

FIG. 30.

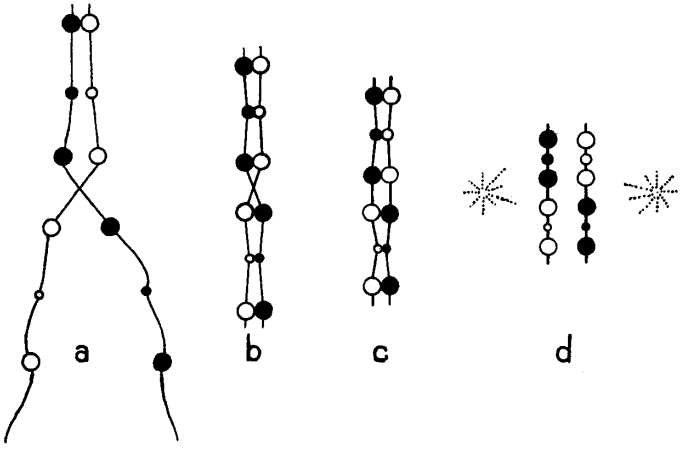

FIG. 31.

There is, it is true, a certain amount of evidence of this kind. For example, Janssens has published certain figures of the separating thread at the reduction division that seemed to him to show that they represented an earlier condition of overlap of the thread with partial fusion at the crossing of the inner threads.* It has been pointed out,

* Crossing-over is here supposed to take place between the two inner threads of the four-strand stage. Janssens has pointed out the necessity of two divisions to bring about the reduction of the diploid number of chromosomes to half, in order that there shall be a single line of genes in each chromosome (*i.e.*, a chromosome not heterozygous). Aside from the

however, that another interpretation of the crossed threads is possible that does not involve crossing-over. Nevertheless, it still seems to me that Janssens' interpretation, while not conclusive, is the more probable interpretation of the later condition that he finds. In addition to Janssens' earlier work a recent account by Gelei (fig. 32) seems to show very clearly the crossed threads at the time of the conjugation of the thin threads.

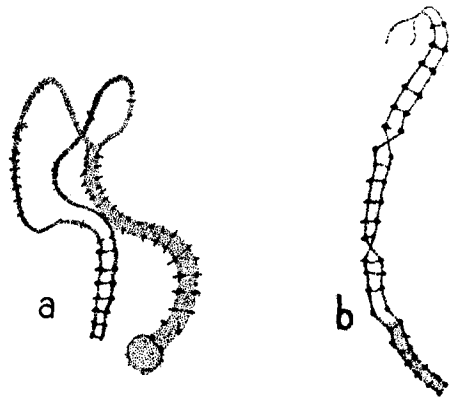

FIG. 32.

Many cases are known in which the chromosomes, *after* they have conjugated and have begun to condense, are twisted about each other (fig. 33); but it is generally supposed that this latter twisting is a secondary process and need not *necessarily* correspond to any earlier twisting of the chromosomes. Such evidence by itself cannot be used either for or against the twisting hypothesis unless it can be shown, as Janssens tried to show, that certain kinds of crossed threads, observed in these later stages, are sometimes traceable to an earlier interchange. Fortunately there is a little evidence that also at the thin thread stage the chromosomes are sometimes twisted around each other. Janssens thinks that his observations show this, but it has been found very difficult to make certain of this relation.

It is unfortunate that it is the figures resembling those of the *later* coiled threads that are beginning to be copied into text books as the stages supposed to furnish the evidence for the twisting of the chromosomes at the time of crossing-over. This is unfortunate because

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appearance of a teleological view implied perhaps in this explanation, it is not obvious why two divisions should be necessary. Only when four strands are present at the time of crossing-over (when two of the strands are interchanged) is Janssens' "necessity" for two divisions called for, but then it would be equally "needed" even if no crossing-over occurred.

these figures do not really conform to the evidence of crossing over furnished by Drosophila. In the first place single crossing-over has been shown to occur for the X-chromosome of Drosophila in only 43 percent of the possible cases, and double-crossing over in about 12 percent, and triple-crossing over (an extremely rare event) in about 2 percent. Yet if these late twisted chromosomes had any relation to crossing-over, it would be expected to occur many times in the length of each chromosome pair. In the second place, it appears that all, or nearly all, of these late coils are straightened out as the two chromosomes condense, preparatory to entering the spindle,

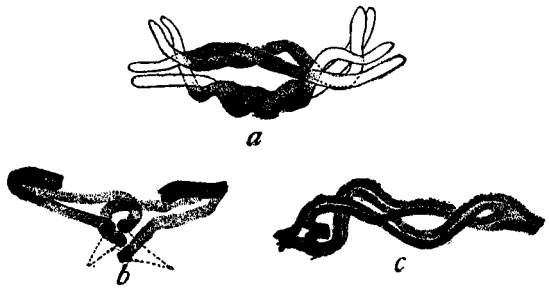

FIG. 33.

While it is evident, therefore, that genetics has far outstripped cytology in regard to the evidence of interchange between members of the same pair of chromosomes, yet cytologists have described a series of events taking place at the time when geneticists expect to get evidence of crossing-over. This creates a very favourable situation so far as genetics is concerned, but, for the present, geneticists may have to wait until the cytologists can make further advance in the study of these stages.

There is one fact about genetic crossing-over that is of prime importance for any interpretation of the process, namely, the fact that whole series of identical (or allelomorphic) genes are exchanged whenever crossing over occurs. In other words, great pieces of the chromosomes are involved and always identical pieces. Moreover, measurements of these "blocks of genes" show that they have a certain modal length, there are very few very short blocks, more larger ones, and very few very large ones, etc. This discovery fits in excellently with the view that crossing-over is brought about by twisting of the chromosomes about each other, for if the chromosomes twist about each other in loops, then, owing to the rigidity of the chromosomes, very short loops will be less likely to occur than somewhat longer ones.

Regions on each side of a crossover are, therefore, expected to be protected from another crossing-over. We call this interference, because a crossover that might otherwise occur is interfered with. Now Muller put this matter to a test by taking into account all those cases in which crossing-over occurs at two points at the same time. For example, if the first crossing-over takes place at the left of the chromosome (fig. 34), the chance that a second crossing over should take place at some other point will be the greater in proportion as the other point is distant from the first. This we find is actually realised.

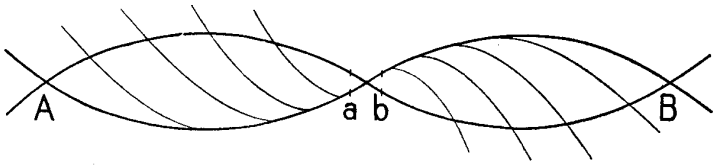

FIG. 34.

Furthermore, on the twisting hypothesis interference decreases until: it vanishes at a certain distance. Beyond that distance, crossing-over should again be interfered with, because the more frequent length of loop has been surpassed* (fig. 35). Weinstein put this possibility to a crucial test. He found that there is a decrease in the amount of crossing-over as anticipated.

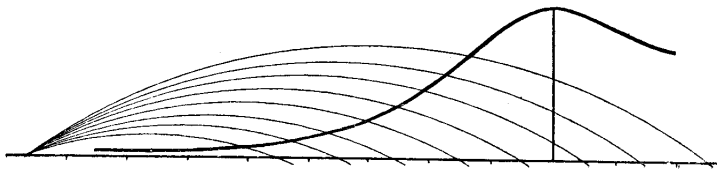

FIG 35.

* Figure 35 is a diagram to show the frequency of interference. The basal line stands for the chromosome; the arcs (arising at one point to the left) are intended to show where a second crossover may fall. The heavy curved line indicates the frequency of a second crossover, or, in other words, the frequency of blocks of different lengths. The modal length is indicated by the distance to the base of the heavy, vertical line. It is evident that, from any given point (crossover level) taken as a starting point, there is first a region in which no crossing over occurs; then the chance of one occurring increases up to the modal length, after which the chance of a second crossover decreases again.

VI.

The evidence from crossing over has led to the conclusion that the hereditary elements, the genes, are arranged in linear order in the chromosomes. If we think of these elements as material particles they muse be supposed to have the power of self-division, and to remain unchanged through long periods. More than this we need not postulate. How they affect the cells in which they lie we do not know. Nor do we know whether they are functioning all the time, or only under certain specified conditions. However they do their work, we must regard the organism in large part as the outcome of the sum total of their activities.

It is, however, important to emphasise that these ultimate units are not necessarily to be thought of simply as the representatives of each part of the organism, for every part of the organism must result from the activity of a large number of the elementary units. But, obviously, if something should come in that brought about a change in any one of these units, the end product made by them all might be affected, and the effect might hit some one particular part of the body harder than other parts. It is this indirect effect that we see when a mutation occurs and we refer it back to the ultimate source from which it arose, that is, to a modification of some part of the germ-material.

I mention this in particular, because I find that many people get the impression that we suppose that each part of the adult organism must have a single representative in the germ-track, and sometimes the impression is produced, unfortunately, that we imagine the chromosomes as representing a sort of miniature of the animal. One of my friends has laboured for years under the impression that our idea of the germ-plasm is that of a mysterious insect in the nucleus, a sort of insectulum, and, under the circumstances, I cannot blame him for thinking that we are on the way to the madhouse.

It is hazardous to make any statement as to the size of these ultimate units, because, in the first place, we do not know how long the chromosomes are at the time of crossing-over, and also because we do not know how near the nearest genes may lie to each other. We have, however, speculated a little about them; and, with the understanding that it is largely speculation, I may venture to state what the outcome has been.

Several methods have been tried. One of these may be illustrated by the following example:— If there were a known number of balls in a bag, say 5000, and one was taken out and put back, and this was done

over and over again for 1000 times, it would sometimes happen that the same ball was drawn again or several times over. It is obvious that the greater the number of balls the less likely is the same one to be drawn more than once. Conversely, if we know the number of times balls have been drawn (twice, three times, etc.), we can find out how many balls there must be in the bag. Now in Drosophila most of the mutations have appeared only once, a few twice, fewer still three times, etc. From these data we have calculated that there are, roughly, more than 2000 genes in Drosophila. Since each individual has two sets of genes, there will be 4000 in all.*

If we take the number of loci at 2,000 (which is a low estimate), and calculate the volume of the four chromosomes (whose total length in preserved material is taken at 7.5 microns in length and 0.2 in breadth), the total volume will be 0.236 cubic microns. If we divide this volume by 2000 and estimate the diameter of the resulting gene, it turns out to be 60/1000 of a micron in diameter.

In this calculation the measurements of the chromosomes were made when they are condensed in the equatorial plate stage. Now, since it is more probable that crossing-over takes place when each chromosome is fully extended (thin-thread stage of conjugation), it seemed better to make another estimate on this basis. If we assume that the second chromosome of Drosophila expands as a loop, its length must be about twice the diameter of the nucleus. Let us allow 5.5 microns for the diameter of the nucleus of this stage, and then double it for the length of the thread. There are 108 genetic units in this second chromosome. If we allow 1/5 of a unit as near the minimum cross-over value, there will be room for 540 genes in this chromosome,† which makes each gene 20/1000 of a micron in diameter.

In still another way an attempt was made to get some idea of the size of the gene. Since the head of the spermatozoon is commonly

* This method of calculating the number of genes was first suggested by Muller for the X-chromosome, where data were then most easily obtainable. Here the calculation is based on data for mutation in all of the chromosomes. Multiple allelomorphs are counted as so many identical (and repeated) mutations of the same locus; but the reversion in Bar has been omitted as exceptional. No doubt many repeated mutations have been overlooked, and the error of random sampling is large, which reduces the reliability of the figures. Moreover, all lethal mutations have been left out of account, because one cannot be sure of their recurrence.

† This calculation assumes that the shortest distance between the genes, treated as points, is 1/5 of a unit, but we have data for smaller distances even than this; in other words, 540 intervals is a relatively low estimate of the number of genes. It also assumes that the genes are evenly spaced, and there is some evidence that this is not the case.

supposed to contain only chromatin material, it would be possible to determine from the volume of the material in the sperm-head how large each gene might be (the maximum limit) by dividing this volume by the postulated number of genes.* From this volume the diameter of the gene could be determined. The result of such a calculation gives the diameter of the gene 77/1000.

The three estimates give 77, 60, and 20 thousandths of a micron respectively for the size of the gene. Different as they are, it is still surprising that the range is not wider when the many possible sources of error are considered. They may at least have an interest as the first crude attempts to get some idea of the size of the material elements postulated by genetics.

It is not without interest to compare these estimates with the estimated sizes of organic molecules. The molecule of haemoglobin has been given at 2½/1000 of a micron, and that of casein is almost the same. The size of the gene on the basis of these tentative estimates seems to be larger, but not much larger than that of some protein molecules.

VII.

We have covered a good deal of ground, and I realise and regret that the mass of experimental data on which most of the conclusions rest has necessarily been left in the background. The data are, however, published and accessible. Except for the questionable attempts to estimate the size of the gene, the main conclusions concerning the mechanism of heredity and the orderly arrangement of specific genes in the chromosomes rest on quantitative data and on analytical deductions that are tested by further experiments wherever possible. The theory that the chromosomes carry the hereditary factors, and that these factors lie in linear order in the chromosomes, enables us to predict, with a high degree of certainty, how any new character will be inherited with

* This calculation assumes that the sperm head is composed of chromatin alone, for which there is no real evidence. If other material is present, then the calculated size of the gene is too large, *i.e.*, its true value will be closer to the results of the other calculations. On the other hand, if the sperm head is composed entirely of chromatin, then the value obtained for the size of the gene 77/1000 is a maximum, since the number of genes used in the calculation is a minimum. It should be stated that the measurements of the sperm head were made from preserved material (sections of the testes, mounted in balsam), and no allowance was made for shrinkage. The calculation should be made again on living sperm, if possible. The sperm head measured about 7.0 microns in length, and 0.3 microns in breadth.

respect to all the other 300 known characters of Drosophila. If the accuracy of prediction is a test of the usefulness of a theory, then we may claim some justification for our view. More than this, I think, it is not necessary to claim for a scientific theory.

The evidence has given us a glimpse at least of processes that are, so orderly and so simple as to suggest that they are not far removed from physical changes; and the order of magnitude of the materials is so small as to suggest that its component parts may come within the range of molecular phenomena. If so, we may be well on the road to the promised land where biological results may be treated as physical and chemical events.