ANOMALOUS CHROMOSOME PAIRING IN THE MALE DROSOPHILA PSEUDO-OBSCURA

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INTRODUCTION

The chromosome complement

The Diptera have not proved to be the most favorable material for the study of chromosome behavior, but the extensive work of STEVENS, METZ, KEUNEKE, and others has shown two exceptional properties in most sections of the order, namely, the pronounced somatic pairing of homologous chromosomes at mitosis, and the apparent lack in the male of the characteristic stages of pachytene pairing and chiasma-formation found at meiosis in nearly all other organisms. Further, GUYÉNOT and NAVILLE have indicated that meiosis is probably normal in the female of Drosophila and Calliphora, thereby showing a difference that may be correlated with the difference in regard to crossing over between the two sexes.

In order to analyze the peculiar character of pairing in the male it seems best to concentrate on those critical stages from diakinesis to anaphase of the first meiotic division when the structure of small paired chromosomes and the mechanics of their changing relationships can alone be accurately described. The object of the present study is to examine the form of the paired chromosomes in the light of those general principles of chromosome mechanics which I have earlier attempted to define, and thus to find out whether these principles may be applied to Drosophila. The processes of inference are therefore necessarily more elaborate than have been attempted hitherto in this kind of work.

For this purpose I have been fortunate in being able to use the admirable preparations of *Drosophila pseudo-obscura* made by Professor TH. DOBZHANSKY in the course of his studies of races and inter-racial hybrids of this species. Various methods have been used by DOBZHANSKY, the most suitable for my observations being fixation in Benda's solution and staining with haematoxylin. All the sections were cut at a thickness of 7μ. *Drosophila pseudo-obscura* Frol. is the North American species referred to as *D. obscura* by LANCEFIELD (1929), KOLLER (1932 a and b) and others, but it has been shown by FROLOWA and ASTAUROW (1929) to be distinct in morphology and in chromosome complement from the European species of this name. *D. pseudo-obscura* has three large pairs of autosomes with terminal spindle attachments and a fourth which is small and spherical: this last corresponds morphologically with the fourth of *D. melanogaster* and is not usually visible at meiosis. The X chromosome has a median spindle attachment and each of its arms is longer than any autosome. Dobzhansky (unpublished) has shown that there are four racial types which differ in the form of the Y chromosome (figures 1 and 2). He has also shown that they differ in the kinds of hybrids they produce *inter se*, both in morphology and in the behavior of the chromosomes at meiosis (Dobzhansky and Boche 1933). I have been able to study meiosis in all the four pure types and their comparison has been of critical value in analyzing the pairing of X and Y.

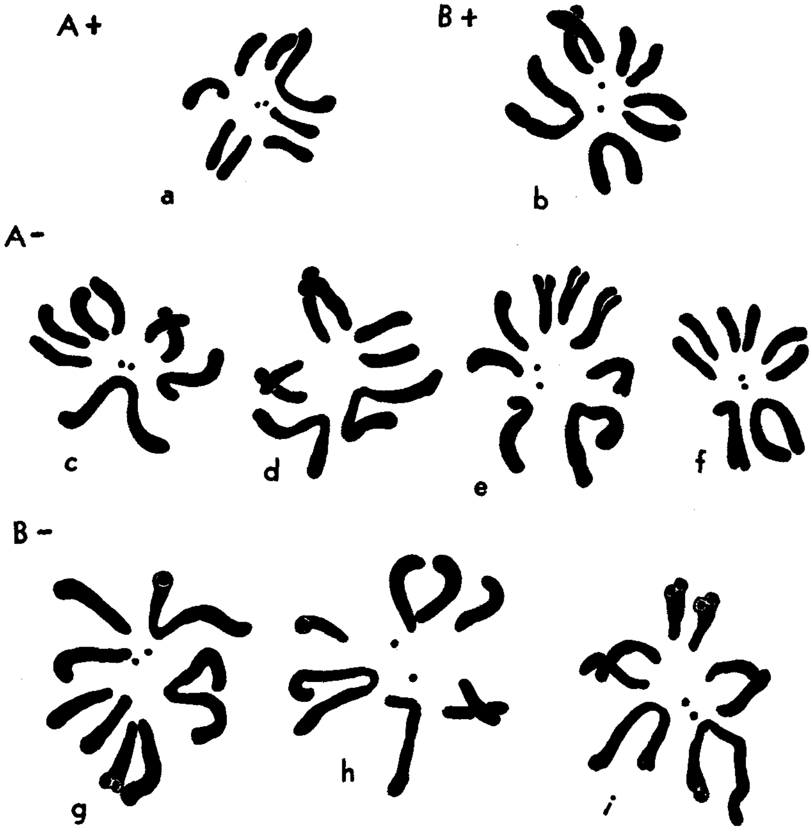

Fig. 1

Figure 1.—Metaphases of spermatogonial divisions of the four racial groups of *Drosophila pseudo-obscura*. Strong A, a (Texas); Strong B, b (Seattle 4); Weak A, c (Seattle 1), d (La Grande 4), e (Pointed), f (Seattle 5); Weak B, g (Seattle 2), h (La Grande 2), j (Seattle 6). (Drawings lent by Prof. Dobzhansky) ×6200.

THE PAIRING OF THE AUTOSOMES

Of the autosomes the three large pairs are alone regularly visible at meiosis. During diakinesis they assume a definite outline. They are rod shaped and their proximal and distal ends are not distinguishable. The bivalents appear from the side to be double, consisting of two clearly separated rods (figures 3–6). In end view, however, they are seen to be quadri-

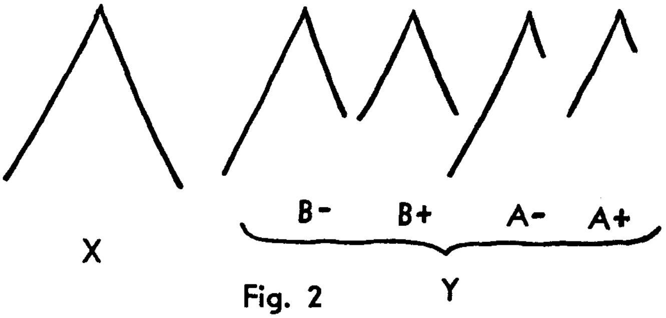

Fig. 2

FIGURE 2.—Diagram to show the relation of the Y, in the four racial groups, to the X chromosome, which is constant.

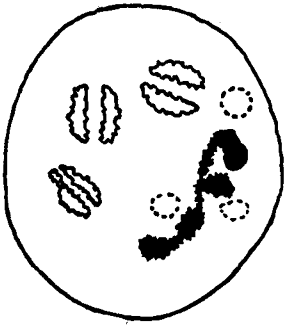

Fig. 3

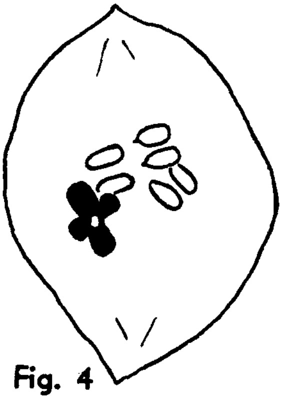

Fig. 4

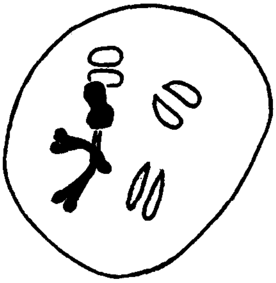

Fig. 5

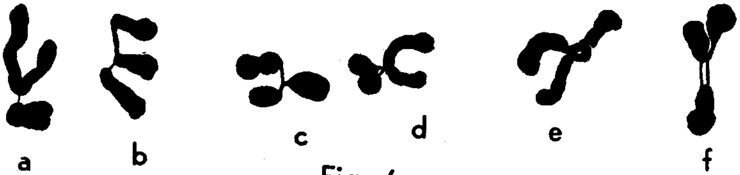

Fig. 6

FIGURES 3–6.—Diakinesis. Figure 3, Texas; Figure 4 and 5, Seattle 5 with long and short arm association of Y; figure 6, X–Y configuration, a–d in Seattle 5, e in Pointed, f in Texas. (In these and succeeding camera lucida drawings of the first division the sex chromosomes are shown solid, the autosomes in outline). ×6200.

partite and the four parallel rods (the chromatids) composing them are equidistant or nearly so (figure 36). The relative positions of the ends of the chromosomes indicate that at this stage the spindle attachments, which are invisible, are not turned away from one another as they are at metaphase. When the spindle develops, the attachment ends of the partners are turned towards opposite poles so that the chromosomes are oriented for regular segregation in the axis of the spindle (figure 9 and following). Anaphase separation, however, often begins before a flat plate is formed (see METZ 1926, on several Drosophila species). At anaphase the sister chromatids, held close together, are gradually drawn away from their partners at the spindle attachments, the length of parallel association decreasing as the spindle attachments move further apart (figure 14 and following). In their behavior during interphase and second division the autosomes, as also the sex chromosomes, are entirely normal (figure 31 and following).

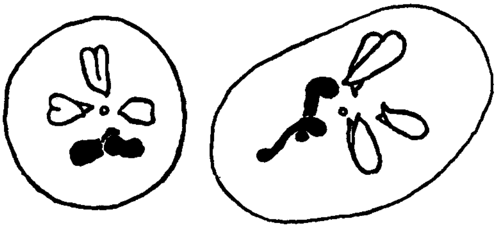
Fig. 7

Fig. 8

FIGURES 7 AND 8.—Polar views of first metaphase in Texas and Seattle, 5, ×6200.

THE PAIRING OF THE SEX CHROMOSOMES

Normal behavior

METZ (1926) and STEVENS (1908) have shown that in various dipteran species, the X and Y chromosomes pair in a short intercalary portion of their length to give a cross-shaped configuration at diakinesis and metaphase. The present observations agree in showing that the X and the different kinds of Y are always associated in the neighborhood of the spindle attachment of the two chromosomes and for a very short length. But the configurations at diakinesis seem to be of two kinds: those showing a close connection between the two chromosomes and those showing a clear space between them. The first kind sometimes appears as a cross with a loop in the middle (figure 4). This difference will be considered in the light of the metaphase observations, which alone give critical results; for with the autosomes, as also with the sex chromosomes, the spindle attachments do not reveal themselves at diakinesis by their mutual repulsion as they do

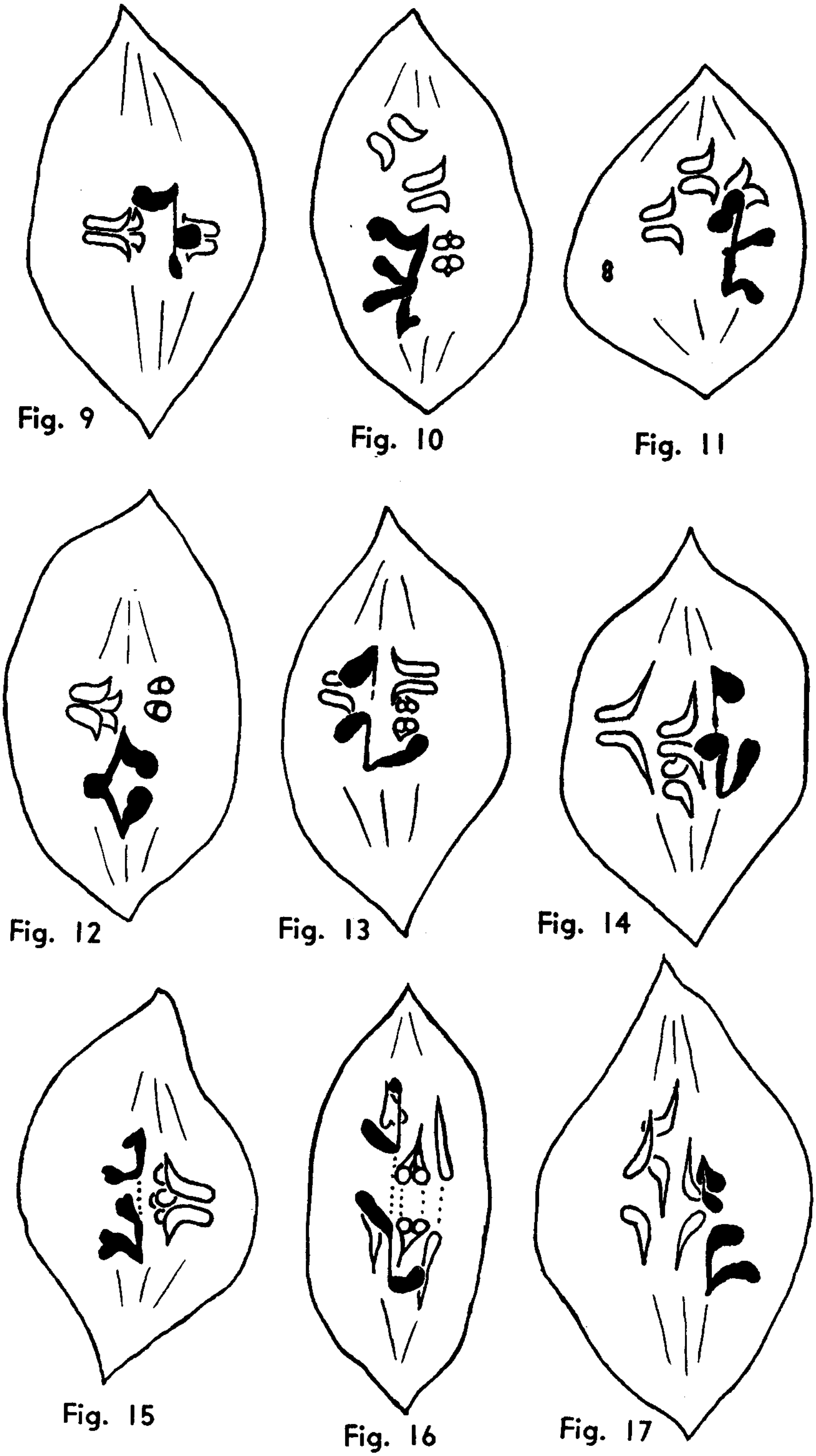

Fig. 9
Fig. 10
Fig. 11
Fig. 12
Fig. 13
Fig. 14
Fig. 15
Fig. 16
Fig. 17

FIGURES 9–17.—First metaphase and beginning of anaphase in side view. Figure 9, Texas (Strong A); figures 10–16, Seattle 5 (Weak A); figure 17, Seattle 6 (Weak B). Figures 9, 11, 12, 13, 14, and 17 show short arm associations, the rest, long arm. ×6200.

in other organisms; they therefore cannot be placed in relation to the point of pairing until metaphase.

The most important for the study of metaphase and anaphase are the weak-A races with the greatest contrast in size between the two arms of the Y chromosome (figure 2). In these races two kinds of association can be distinguished in side view of every complete nucleus. The first kind is that in which the Y is associated with the X in its long arm (figures 10 and 20 a, b, c). The unattached short arm is then lying towards the pole and the long arm is lying in the middle of the figure, balancing the associated arm of the X. The second kind is that in which the short arm of the Y is associated with the X and the long arm is lying free towards the pole (figures 11–14 and 20 *d–j*). This second kind probably corresponds with the diakinesis figures in which no connection between the two chromosomes is visible.

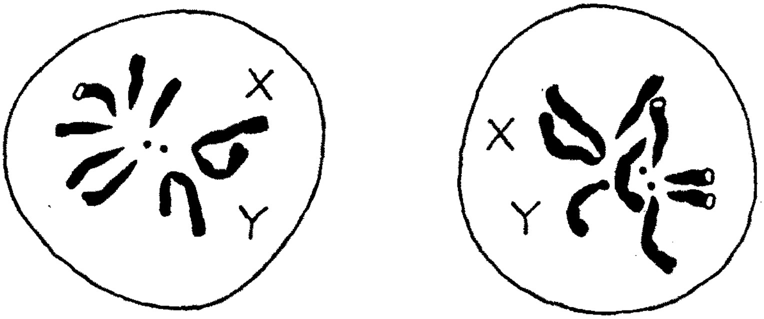

Fig. 18 Fig. 19

FIGURES 18 AND 19.—Spermatogonial metaphases in Seattle 2 and Texas to show the difference in lengths of the two arms of the Y chromosome; this is important in interpreting figure 20. ×6200.

At anaphase in the weak-A races the two kinds of association give characteristic types of separation (figures 16 and 17). In the other races (strong-A and weak-B) the contrast between the two arms of the Y is not so great and the distinction between the two types is more difficult, although it has been attempted. It then appears that, while the weak-A and strong-A races have an equal number of short-arm and long-arm associations, the weak-B races have more in the short arm (21 to 8 in Seattle 6, 8 to 5 in Seattle 2).

As anaphase begins, the portion of each chromosome between its spindle attachment and the point of association is drawn into a fine thread and continues so for a short while after the separation has taken place (figure 16). The lengths of chromosome distal to the point of connection remain unaffected by the separation.

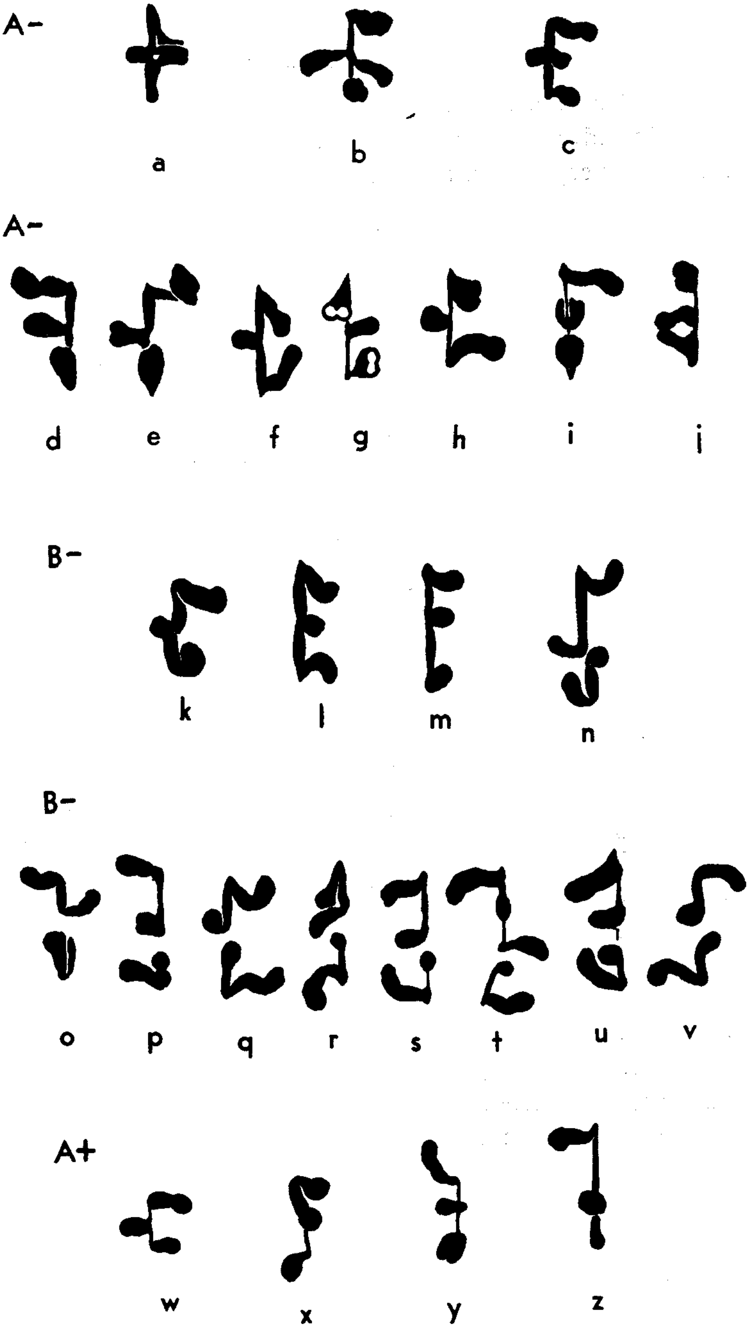

Fig. 20

FIGURE 20.—The XY bivalents to show the distinction between the two positions of association in weak and strong A, and in weak B races. The Y chromosome can only be distinguished at metaphase with long arm association. It is then placed below. a–c, long arm association; d–m, short arm association; n, long arm association; o–z, short arm association (o–v in anaphase, Y chromosome always distinguishable). a, b, e, h, i, j,—Seattle 5; c,—Pointed; d,—La Grande 2; f, g,—Seattle 1; k–r,—Seattle 2; s–b,—Seattle 6. × 6200.

Precocious separation

Metz (1926) has pointed out that the sex chromosomes, especially the Y, are condensed in advance of the autosomes during prophase in Drosophila. In their anaphase separation they also show occasional precocity in relation to the autosomes (figures 22 to 26). In the stock Seattle 6 (weak-B) they separated precociously in eight divisions out of thirteen recorded; in Seattle 5 (weak-A) once in seven divisions. It sometimes happens, however, that the X-Y figure is behind the autosomes in orientation on the plate at metaphase (figure 27, Seattle 6). This has been found to be usual in *Drosophila melanogaster* by Huettner (1930). The irregularity is therefore perhaps best considered as an error in time coördination between the sex chromosomes and the rest.

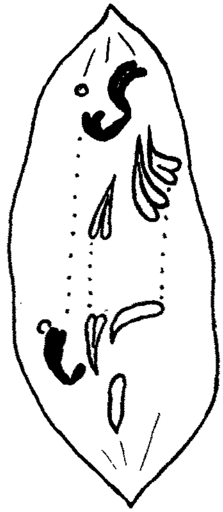

Fig. 21

Figure 21.—Normal first anaphase in Seattle 5. ×6200.

Non-disjunction

Since Bridges' discovery of XXY females with both X's derived from the mother, the so-called "non-disjunction" of the sex chromosomes has been the subject of much genetic study. Females of the XXY constitution have been found in *D. willistoni*, *D. phalerata*, *D. simulans*, *and* (Schultz and Redfield unpub.) *D. pseudo-obscura* itself. It is known also in *D. melanogaster* that the male can produce sperm with both X and Y or with neither sex chromosome, but their frequency is difficult to determine (Stern 1929).

Recent observations, particularly of plants, have made it clear that failure of pairing, not failure of separation, was the most likely cause of the abnormality since such failure is always followed by random distribution of the unpaired chromosomes. In *D. pseudo-obscura* I have found the X and Y unpaired at the first division in three nuclei and the results of

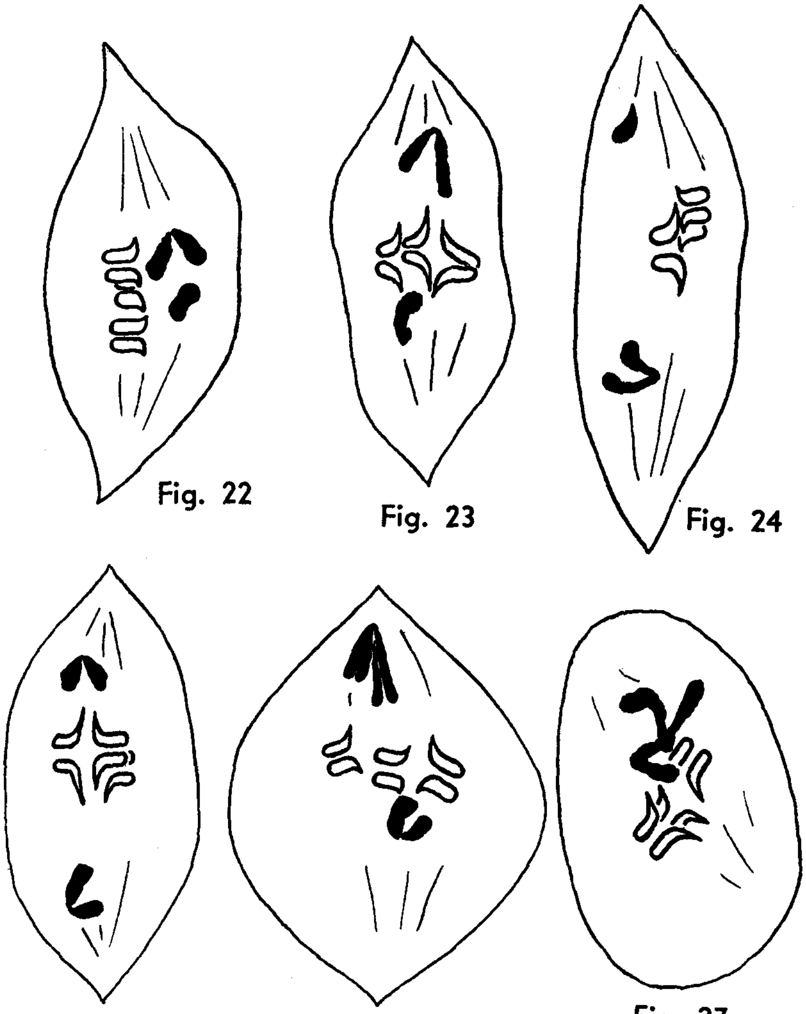

Fig. 22 Fig. 23 Fig. 24

Fig. 25 Fig. 26 Fig. 27

FIGURES 22–27.—Aberrant time coördination of sex chromosomes and autosomes in separation at first anaphase. Figures 22–26, precocity of sex chromosomes; figure 27, delay of sex chromosomes; figures 22 and 24, Texas; figure 23, Seattle 5; figures 25 to 27, Seattle 6. ×6200.

their passage to the same pole in five more (figure 28 and following). In the first divisions the X is lying near to one pole, while the Y lies on the equator. In the second divisions both chromosomes have passed to the same pole.

THE MECHANICS OF PAIRING

The autosomes

I have described the pairing of the autosomes as anomalous because it differs from normal meiotic pairing in three respects: (1) the equal or almost equal association of chromatids in fours throughout their length at diakinesis and metaphase, (2) the sudden change in relationship of spindle attachments between diakinesis and metaphase from attraction or indifference to sharp repulsion; (3) lack of regular orientation on the metaphase plate. With these properties is perhaps associated a reduction of pachytene pairing, in regard to which, however, interpretation is inconclusive.

The first of these abnormalities—the equal association in fours—so far as it concerns diakinesis, may be considered to be, as METZ and KEUNEKE have suggested, merely the final stage of a somatic pairing which is progressively increased during the preceding spermatogonial divisions. In my own words (see DARLINGTON 1932, p. 342) I would say that a specific attraction (A_2) is manifested in somatic pairing. This always reaches an equilibrium with the non-specific repulsion (R_1), due to surface electrical charges, which is a universal property of all double chromatids during the dynamic stages of mitosis and meiosis. The somatic pairing attraction seen during spermatogonial mitoses increases at meiosis until it equals or nearly equals the one-to-one attraction between chromatids (A_1) so that the four chromatids come to be nearly equidistant. In normal meiosis the four chromatids fall apart into two pairs which repel one another and are held together only by the exchanges of partner, or chiasmata, between them. In the male Drosophila there is evidence of very slight repulsion (in equilibrium with attraction) but no evidence of chiasmata. This hypothesis would be tested by observation of meiosis in a tetraploid nucleus in which all eight chromatids of one type should be equally spaced. The variation in the distances apart of the four chromatids between diakinesis and metaphase (top of figure 36) is evidence of the different quality of the two kinds of attraction, the primary attraction within pairs (A_1) and the secondary attraction between pairs (A_2). Whatever interpretation be put upon the observations of the fixed and stained material, this comparison between successive stages must have some validity.

But when we apply this view to the anaphase separation we meet with a difficulty. Both in mitosis with somatic pairing and in meiosis in polyploid plants with "secondary pairing" we have the same attraction, A_2

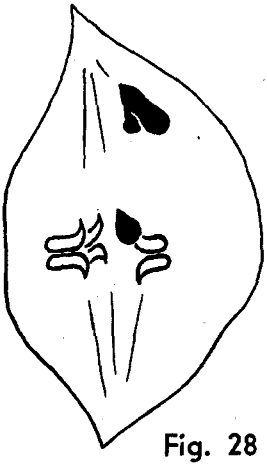

Fig. 28

Fig. 29

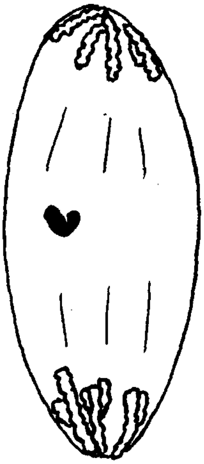

Fig. 30

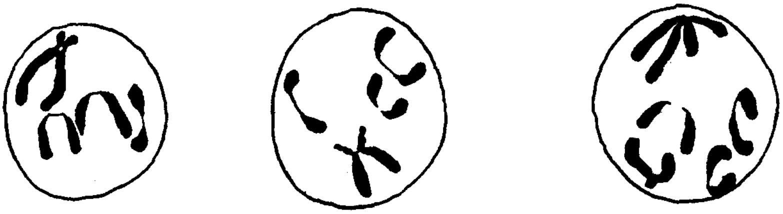

Fig. 31

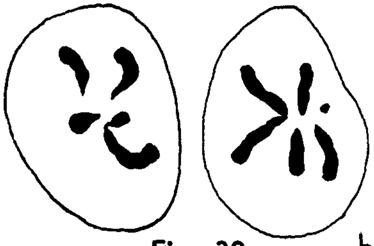

Fig. 32

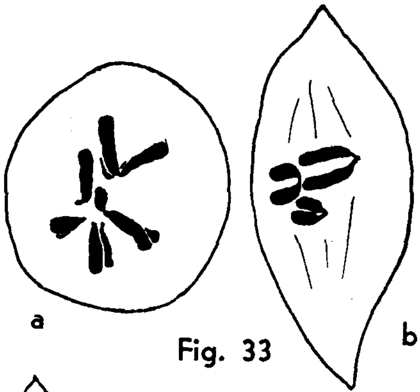

Fig. 33

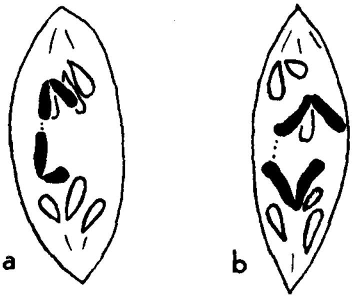

Fig. 34

Figures 28–33.—"Non-disjunction" of X and Y. Figures 28 (Texas) and 29 (Seattle 6), X lying towards one pole and Y on the plate at first metaphase; figure 30 (Seattle 4), X lagging at first telophase after having failed to pair; figure 31, a and b (Seattle 4) normal interphases, each with a Y chromosome, c (Seattle 5) interphase with X and Y; figure 32, a and b (Seattle 5) normal second metaphases, a, with Y, b, with X-fourth autosome visible; figure 33 a and b (Seattle 5), sister second metaphases resulting from "non-disjunction" of X and Y. ×6200.

Figure 34.—Normal second anaphases (Seattle 5); a with Y, b with X. ×6200.

(although in plants it does not manifest itself during prophase). *But this attraction never determines segregation* (see DARLINGTON 1932). Clearly at meiosis in Drosophila something else beyond A_2 is responsible for the regularity of segregation. The second anomaly indicates what this is.

At diakinesis the spindle attachments of the chromosomes are not visible, but the ends at which they lie are clearly not turned away from one another as they are at metaphase. I have suggested that the orientation of the metaphase chromosomes—determining separation at mitosis, segregation at meiosis and the fusion of chiasmata at the ends in terminalization—is dependent on the repulsion (R_2)[1] between the spindle attachments. These are double for each chromosome at mitosis as well as for each bivalent at meiosis so that the orientation in the two cases is the same. The occurrence of segregation therefore demands that the bivalent should have a double, and not a quadruple spindle attachment. The meiotic bivalent must therefore have a delay in the division of the spindle attachment relative to the development of the spindle, in fact a postponement to the second meiotic division. This precocity of the external changes relative to those internal to the chromosomes is, as I have pointed out, characteristic of meiosis.

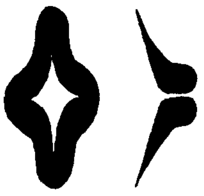

Fig. 35

FIGURE 35.—Right, type of autosome bivalent illustrated by WOSSKRESSENSKY and SCHEREMETJEVA and by HUETTNER in *Drosophila melanogaster* with median spindle attachment. Left, type formed in *D. pseudo-obscura* with terminal spindle attachment, showing that the characteristic cross shape in *D. melanogaster* is misleading since it is not due to a chiasma.

The repulsion of the undivided spindle attachments accounts sufficiently for the separation of the chromosomes at first anaphase in Drosophila. But in normal meiosis repulsion sets in before metaphase, probably as a symptom of the external precocity, and gives rise to greater or less terminalization of chiasmata between diplotene and diakinesis. In Drosophila this repulsion does not appear until metaphase. If it did it would perhaps overcome the attraction A_2, and lead to premature disjunction during prophase. It therefore seems that reduced precocity is a second

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[1] This is the repulsion which I hold to be responsible for BELAR'S "autonomous" separation (HUTH 1933).

characteristic of this abnormal meiosis, and this assumption is reasonable since it also accounts for the failure of prophase pairing and for the rather slight extra contraction of the chromosomes.

There are two points in this argument that require elaboration. My reason for assuming that the beginning of spindle attachment repulsion (R_2) during prophase is externally conditioned is that unless external conditions changed during prophase the spindle attachments should repel one another from the beginning of prophase since their repulsion effected the preceding anaphase separation. And further, only one internal change can be supposed to befall the spindle attachments during meiosis, namely, the splitting which leads to the separation of chromatids at second anaphase. I am supposing, therefore, that the spindle attachments potentially repel one another throughout the meiotic prophase, but that external conditions do not permit repulsion until diplotene in normal meiosis (leading to varying degrees of terminalization) and until metaphase in Drosophila. My reason for considering external and internal changes as two individual systems is merely for convenience of handling. Varying terminalization indicates that spindle attachment repulsions may vary independently of other external factors, but they need not be supposed to do so in the present instance.

I therefore conclude that the special behavior of the autosomes depends on the coördination of two special properties: (1) exaggerated somatic pairing, (2) reduced precocity with a consequent avoidance of pachytene pairing and of prophase repulsion of spindle attachments.

The third anomaly, the failure to form a metaphase plate, may be due either to a failure in time coördination between spindle and chromosomes, which will be referred to later, or to an exceptionally weak polar repulsion (R_3) such as I have assumed to be responsible for arrangement in a flat plate. The latter would be the opposite condition to that giving the "hollow spindle" where the strong polar repulsion forces all the chromosomes to the edge of the plate.

Note on the Classification of Forces. I have in the past looked upon the primary attraction A_1 as different in cause as well as in effect from the secondary attraction, A_2, for this reason: A_1 leads apparently to the most intimate association analogous to the contact of solid bodies, while A_2 merely modifies the equilibrium position resulting from the operation of other forces. The present work on Drosophila, however, does not support or require this distinction. It is possible to consider both the attractions as determining an equilibrium position. It is then possible to suppose (and I am indebted to Dr. G. W. BEADLE and Dr. JACK SCHULTZ for this suggestion) that a common force of attraction exists between identical genes and that this attraction is almost or entirely saturated by the approximation (or association) of genes in pairs. Secondary attraction, A_2, is then a residual attraction and its variation is merely the variation of degree of saturation by pairing. In meiosis in the male Drosophila the saturation reaches its lowest level. Forces of repulsion due to surface charge (R_1) and localized charge at spindle attachment (R_2) and spindle poles (R_3) can likewise all be considered most conveniently as a unit in regard to physical causation. The distinction between them remains, however, convenient in description since their different distributions in space and time lead to their having different effects, and the analysis of these effects must precede the synthetic consideration of chromosome mechanics as a whole.

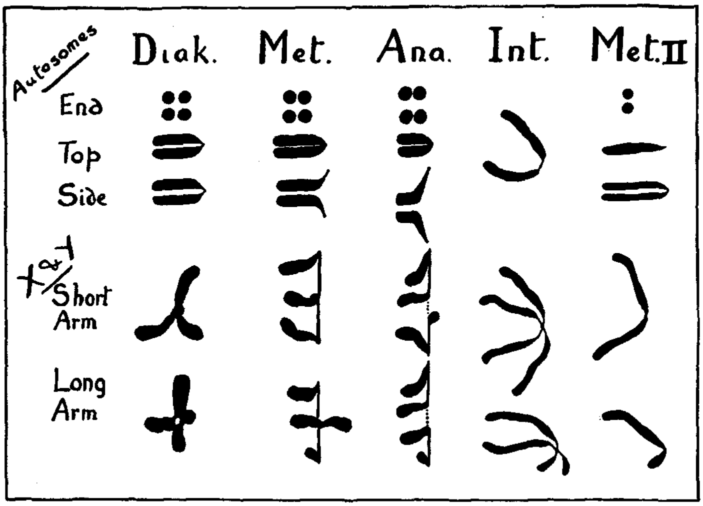

FIGURE 36.—Diagram showing the relationship between autosome and X–Y pairing in a weak A race of *D. pseudo-obscura* during successive stages of meiosis. The interphase column shows merely lateral views of the chromosomes and the last two stages of X and Y are, of course, indifferently derived from long and short-arm chiasma bivalents.

The sex chromosomes

The X and Y chromosomes have an entirely different relationship at the first metaphase, as shown by both structure and behavior, from that subsisting between autosomes. Thus they are only in contact over a short portion of their length and this is evidently not the whole of the homologous segment. In this portion they come into closer contact than the autosomes, sometimes, and at other times rather less close—no visible connection joining them. Other parts, even though homologous, lie apart giving the cross figure clearly owing to their mutual repulsion (R_1). In the short paired region at diakinesis there is sometimes an open space visible between two points of contact. At metaphase a state of tension develops between the spindle attachments and the point of association, and the chromosomes do not separate gradually at this point, but suddenly.

These observations lead to a common conclusion, namely that we are here dealing with a normal case of attraction between pairs of chromatids—not between fours—and that the four chromatids are held together by exchanges of partners between them, that is, chiasmata. Such chiasmata would need to be reciprocal, since both ends of X and Y are dissimilar, and close together as shown in the diagrams (figures 36 and 37, and DARLINGTON 1931, diagram III).

The exchange of partners, being inferred from general considerations, would seem to provide a purely formal explanation of the X-Y pairing in the absence of more specific evidence. Such evidence is provided by other peculiarities of behavior which can be accounted for in the same way. The occurrence of pairing on both sides of the spindle attachment shows not only that the homologous segments extend on both sides, but also that they may or may not be associated at metaphase. The characteristic chance formation of chiasmata on one side, or the other, of the spindle attachment demands this variation in association; A_2 attraction will not account for it. Then the sex chromosomes fail to pair in a proportion of cases, although the autosomes are regularly paired. This also can be accounted for by the chance failure of chiasma formation now known in so many species, particularly among the smaller members of the complement. Again the variation in appearance at diakinesis is accountable if we suppose that the homologous segment extends to the end of the short arm of the Y in weak-A races so that terminalization of the chiasmata in this arm will give a lateral chiasma with no visible connection while in the other arm a connection is always left, complete terminalization being impossible. Finally, the lack of time coördination between spindle, autosomes, and sex chromosomes found also in *D. melanogaster,* is an indication that two independent processes of development are at work in the same nucleus.

I therefore conclude that reciprocal chiasmata are formed between X and Y following normal pachytene pairing of the homologous segments and that these chiasmata are either in the short arm or in the long arm of the Y or—where metaphase pairing fails—in neither.

THE DISTRIBUTION OF ABNORMAL MEIOSIS

The general type of meiosis in animals, plants, and Protista is that in which the chromosomes repel one another at metaphase and are only held together by chiasmata formed during the pachytene pairing. (My application of the term "pairing" to prophase, as well as to metaphase, association has been found ambiguous. But the term synapsis, which I avoid, is even more so. It is used to refer to [1] the act of pairing, [2] the condition of being paired, [3] the period during which pairing occurred, [4] the period when pairing was complete. To use an abstruse expression with an indefinite meaning seems to be an unnecessary offence.) The abnormality described here in regard to the pairing of X and Y chromosomes is relatively slight and is paralleled in other groups. The abnormality in the pairing of the autosomes is profound. How widespread is it? In the first place it is evidently characteristic of Drosophila. In *D. melanogaster*, Stevens (1908), Wosskressensky, Scheremetjeva and Huettner have illustrated autosome bivalents which are quadripartite in side view and correspond with the bipartite bivalents of *D. pseudo-obscura* since the autosomes have median spindle attachments in the one species and terminal in the other (figure 35). The Russian authors describe these as "characteristic tetrads." This they are not for their four parts could not be four chromatids, in view of the position of the spindle attachment, but must be a pair of two-armed chromatids. Further, Stevens' illustrations show the same simple parallel association in Eristalis, Sarcophaga, and Calliphora, and from Keuneke's observations the same method of pairing may be inferred. In these and in some of Metz's work the side views of first metaphase, which are so critical in analyzing bivalent structure, are not illustrated, but the detailed studies of successive stages leave no doubt that an abnormal and probably similar course of development is followed.

The following summary (based on the classification of Imms 1925) includes the most convincing accounts and indicates the extent of the survey of meiosis in male Diptera.

- Orthorrhapha
  - Series I. Nemocera: Culicidae—Culex, Anopheles, Stevens 1911.
    - Choaborus, Frolowa 1929.
    - Sciaridae—Sciara, Metz et al. 1926.
  - Series II. Brachycera: Asilidae—Asilus, Dasyllis, Metz et al 1922.
- Cyclorrhapha
  - Series III. Athericera:
    - Aschiza,
      - Syrphidae—Eristalis, Stevens 1908.
    - Schizophora-Acalyptratae,
      - Scatophagidae—Scatophaga, Stevens 1908, Keuneke 1924.
      - Sciomyzidae—Tetanocera, Stevens 1908.
      - Trypetridae—Tephritis, Keuneke 1924.
      - Ortalidae—Camptoneura, Metz 1916.
      - Drosophilidae—Drosophila (see text).
    - Schizophora-Calyptratae
      - Anthomyidae—Phorbia, Stevens 1908.

Muscidae—Calliphora, NAVILLE 1932.
Lucilia, STEVENS 1908, KEUNEKE 1924.
Sarcophaga, STEVENS 1908, METZ 1916.

Series IV. Pupipara: (none).

The forms mentioned in this list may be divided into three groups according to the type of meiosis in the male. First, we find in the Culicidae normal types of bivalent chromosomes with terminal and interstitial chiasmata. Secondly, we find in the Sciaridae an abnormal meiosis without any chromosome pairing. Thirdly, we find all the other flies, the more highly specialized Brachycera and Athericera (which fall into one group according to the segmentation of the antenna), agree in having the Drosophila type of meiosis, without chiasmata except in the sex chromosomes.

It may now be said that there are four groups of abnormalities found at meiosis in the male:—Sciara, Drosophila, haploid-male, and coccid, this last showing an evolutionary series of changes from moderate to extreme types (SCHRADER 1931). Since, amongst other reasons, the female is probably normal in all these groups, we must suppose that the various special processes have arisen in the heterozygous sex from the normal meiosis. In the Drosophila group the sharpness of the abnormality, and the clearness and extent of its distribution, indicate that it arose from chiasma pairing at a remote period, while the difficulty of supposing an intermediate condition between chiasma pairing and secondary pairing indicates that it arose at a single step.

THE CYTOLOGICAL THEORY OF CROSSING OVER

The simplest hypothesis to relate the genetically inferred occurrence of crossing over with cytological observation is the simplified chiasmatype hypothesis of JANSSENS (DARLINGTON 1930). This supposes that all paired chromatids are sister chromatids so that (1) all crossing over between pairs of chromatids of partner chromosomes gives rise to an exchange of partners, or formation of a chiasma, between them and (2) chiasmata can arise in no other way. This hypothesis is supported by three kinds of observation; first, the observation that in a series of critical cases the cytologically demonstrable formation of new chromatid combinations, such as can only result from crossing over, accompanies the formation of a chiasma. These cases consist in special types of quadrivalent and trivalent in polyploids, of the figure-of-eight configuration in interchange heterozygotes and of special configurations in inversion heterozygotes and with interlocked chromosomes. The assumptions on which they depend for validity are physiological, developmental, and morphological in the different cases, but naturally require in each case the additional assumption of chromosome continuity which is inherent in the statement of the problem (DARLINGTON 1932); secondly, the observation that the frequency of chiasmata agrees with the frequency of crossing over on this hypothesis, either in a particular segment of a chromosome (BEADLE 1932) or in the complement as a whole (DARLINGTON 1933); finally, the observation that the chiasmata show all the special properties that have been inferred in regard to crossing over, for example, interference, as shown by their numerical distribution (HALDANE 1931), variation in frequency and distribution subject to genetic and environmental conditions, a special relationship with the spindle attachment as shown by localization (DARLINGTON 1932), and a reduction in the heterozygous sex where crossing over is less frequent (CREW and KOLLER 1933, BRYDEN 1933). Chiasmata also show other properties which have not yet been discovered in regard to crossing over, such as indirect length-frequency relationships and this is owing, no doubt, to the restricted number of organisms available for comprehensive genetic study of the problem, and the uneven distribution of genes on chromosomes.

There has been one outstanding objection to this theory, namely that no difference was known between males and females in regard to chiasma formation such as exists in the complete suppression of crossing over in male Drosophila. This objection became particularly serious when it was found that chiasma formation, and therefore crossing over, was the general condition of metaphase pairing. Since critical information was generally lacking in regard to the female, and, in Drosophila, in regard to the male as well, the field was open for numerous speculations. There were two possible explanations of the behavior of the male on the chiasmatype hypothesis. One was that, as the SCHRADERS have found in the coccids, the female was normal—the chromosomes pairing by chiasmata presumably—while in the male, pairing was wholly abnormal, being determined by forces not operative or effective in the female. The other was that chiasmata were formed in the male as well as in the female, but occurred in inert regions and in such a way as to give no visible crossing over. If, as seemed most likely in the sex chromosomes, the region was intercalary, then the chiasmata must be formed by reciprocal crossing over. Consideration chiefly of the evidence of non-disjunction of the sex chromosomes and of the occurrence of the XY change led me (1931) to adopt the second hypothesis which was rendered more plausible by the discovery that the proximal third of the X is an inert region. It now appears that this assumption actually applies to the sex chromosomes, while the first assumption applies to the autosomes.

While, in the male, chiasma formation is suppressed or regulated so as to give no visible crossing over, in the female presumably one or more chiasmata are formed in each pair of chromosomes following and paralleling the genetically observed crossing over. Thus each chromosome will have a minimum of 50 percent crossing over in its total length if it pairs by chiasmata. Observation of the female has so far merely shown the presence of the normal pachytene stages which are absent or abortive in the male (METZ and others 1926, GUYÉNOT and NAVILLE 1929, HUETTNER 1930, NAVILLE 1932).

It was possible earlier in the absence of any cytological evidence, to suppose that chiasmata were formed equally in male and female and that their breakage was, as JANSSENS suggested together with his other hypotheses, responsible for crossing over. Then, by all the chiasmata being equational (DARLINGTON 1929), or by the failure of breakage (SAX 1930), the lack of crossing over in one sex could be explained. Thus would Drosophila have been saved for the chiasma theory of metaphase pairing. But since there are no chiasmata, in the male autosomes these *ad hoc* assumptions are left without theoretical use, as well as without observational evidence, while the requirements of the simple chiasmatype hypothesis are met in every respect.

The flies and rodents show two ways in which differences may arise between the sexes in regard to crossing over. The grasshoppers show yet a third. In these we find various conditions in the male—suppression of crossing over, a reduction of crossing over, or even between particular genes in some species, an increase (NABOURS 1929). This complicated situation becomes intelligible in terms of the chiasmatype hypothesis when we recall that the mapping of the chromosomes is still fragmentary and that in the Tettigidae in question not more than one chiasma is usually formed in each bivalent. If then this chiasma is always or usually formed in a particular region of the chromosome, the occurrence or non-occurrence of crossing over will depend on the position of the known genes in relation to this region. Different types of localization of chiasmata will then account for differences in crossing over frequency. Localization is difficult to identify in the Tettigidae which have a high degree of terminalization, but in the Acrididae it is well known to distinguish the behavior of different genera. In this regard it is important to notice that NABOURS and his collaborators now find (1933) that crossing over, which does not occur between particular genes in Paratettix does occur between their presumed homologues in female Apotettix and, even more frequently, in the same sex in Acrydium (individual experiments being here significant even though different experiments may be inconsistent). Differences between related species in regard to chiasmata and crossing over are therefore of the same kind as those between the two sexes. The position now is that the simple chiasmatype hypothesis, like other hypotheses, cannot be proved. It can only be subjected to the possibility of disproof, by testing the many rigorous predictions that can be based on it. Like previous attempts the present study has failed to disprove it, and it seems that no difficulty now remains in applying it universally. Assuming its validity, we can begin to study crossing over cytologically, and chiasma formation genetically, thereby reducing the limitations of each method. I propose to discuss these limitations elsewhere.

TYPES OF DIFFERENTIATION OF SEX CHROMOSOMES

In so far as chromosome pairing is conditioned by crossing over, the type of crossing over in the sex heterozygote must condition the type of differentiation, that is, of development, by mutation and structural change, of non-homologous or "differential" segments in the pairing of sex chromosomes. We now have evidence of the existence of the three simplest crossing over or differentiation types which are, *a priori* to be expected, as follows. (The proximal segment is that which includes or adjoins the spindle attachment):

Type with distal homologous or pairing segments, proximal non-homologous or differential segments

Both ends may have pairing segments and then the differential segments are intercalary. Crossing over is unrestricted, but must be distal; the first division is therefore reductional for the differential segment. This type is analogous to the Oenothera and Rhoeo rings as I conceive them, and can give rise by interchange to the chains of four or five found in Humulus, as well as, by fragmentation of either X or Y, to the chain of three found in Rumex and Phragmatobia. This is the simplest type and it is found in all plants and in most animals with differential sex chromosomes (figure 37I).

Type with proximal pairing segments, distal differential segments

Crossing over is proximal and unrestricted, but if the pairing segment overlaps the spindle attachment, crossing over, occurring either on the near side of the inequality or on the far side, will give a reductional or equational division of the inequality according as 0, 1, or more chiasmata between the spindle attachment and the inequality maintain or break their chromatid relationship. This type is analogous to one kind of unequal chromosome in Phrynotettix and its occurrence may be inferred from genetic observations on *Bombyx mori* (GOLDSCHMIDT and KATSUKI 1931) and possibly from cytological observations of the mouse (CREW and KOLLER 1933, figure 37II).

Type with proximal intercalary pairing segments, distal differential segments in both arms

Crossing over is necessarily reciprocal. The requisite chiasma relationship is found in autosomes in *Fritillaria meleagris* and *Aggregata eberthi.* The sex chromosome type is known only in the genus Drosophila, but may be inferred in others of the Brachycera group. The pairing segments may be restricted to one arm, as in *D. melanogaster,* or extend to both arms, as in *D. pseudo-obscura* and no doubt in other species with both X and Y two-armed (figure 37III).

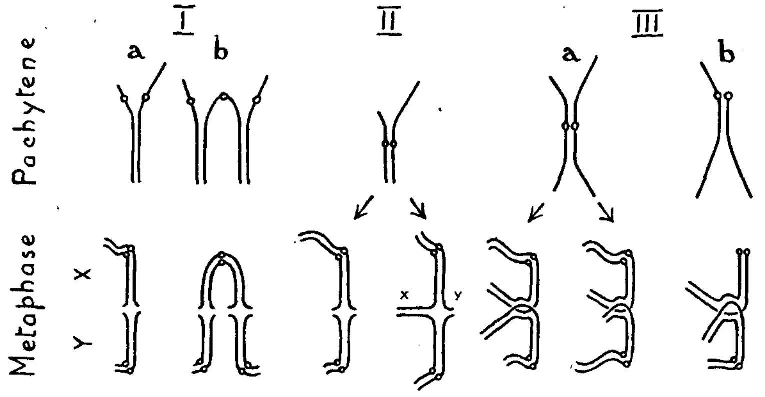

FIGURE 37.—Diagram showing the types of known or inferred relationship of X and Y and of chiasma formation and disjunction related with each. The spindle attachment is represented by a circle. At pachytene the homologous parts are those lying parallel, the X to the right except in Ib. Ia, the normal type in plants and animals with distal crossing over. Ib, *Rumex acetosa, Humulus japonicus, Phragmatobia fuliginosa,* with fragmented Y. II, type with spindle attachment in the homologous segments giving facultative equational first division, that is, with proximal crossing over (as in Bombyx). III, Drosophila type with intercalary pairing segment and reciprocal crossing over. *a, D. pseudo-obscura* with chiasmata in either arm. *b, D. melanogaster* with chiasmata in one arm.

This classification indicates that special mechanical properties of the pairing sex chromosomes have been the condition of various types of evolutionary change of which Drosophila provides a special example.

SUMMARY

1. The autosomes and sex chromosomes of *Drosophila pseudo-obscura* are in different ways anomalous in their method of pairing, as seen between diakinesis and first anaphase of meiosis in the male.

2. The autosome bivalents consist of four chromatids equally paired throughout their length without chiasmata, a condition made possible by exaggerated somatic pairing and changed precocity.

3. The sex chromosomes, as shown especially by their structure and behavior in races with an unequally armed Y, are paired by reciprocal

chiasmata either on one side or the other of the spindle attachment. Failure of pairing leading to "non-disjunction" was found eight times.

4. These two special mechanisms of pairing are compatible with the absence of crossing over in the male on the simple chiasmatype hypothesis. Reciprocal chiasmata between X and Y are a condition of a type of differentiation of the sex chromosomes in which the Drosophila group is unique. The special method of autosome pairing is probably characteristic of all the short-antenna section of the Diptera.

I am obliged to Dr. T. H. MORGAN for the facilities placed at my disposal during this study at the CALIFORNIA INSTITUTE OF TECHNOLOGY. I also have to thank Dr. TH. DOBZHANSKY, not only for the use of his preparations, but also for his experienced advice throughout the study.

APPENDIX I

The effect of the Gowen gene on the male

Since the X and Y chromosomes pair by a mechanism similar to that presumed in the female, Dr. BEADLE suggested to me that the effect of the Gowen gene, a third chromosome recessive which suppresses crossing over as well as pairing in the female, might affect the sex chromosomes in the male, although it is known not to affect the autosomes. I therefore crossed homozygous bobbed females by Gowen males. Normal disjunction of X and Y in these would give progeny with the bobbed character suppressed by either X or Y. Non-disjunction would produce XXY females and "extreme-bobbed" XO males (as shown by STERN). These might be of reduced viability, in which case the sex ratio would be affected. One cross (single male and female) yielded 130 males and 130 females. Others gave 8:5, 55:58 and 25:35. None of the males were bobbed, so that I conclude there is no evidence of non-disjunction of XY in the Gowen stock males.

APPENDIX II

Two recent studies bear on the interpretations and inferences made in the present account.

BAUER (Zeits. Zellforsch. u. mikr. Anat. **14**: 138–193) has described the normal meiosis of the nemoceran, *Tipula paludosa*, and has pointed out that in the males of the short-antenna Diptera, pachytene pairing is found in different degrees of degeneration and that this distinction corresponds with the systematic division of the Diptera. BAUER has thus arrived, on the evidence of pachytene pairing, at the same conclusion that I have reached on the basis of the correlated chiasma structure at metaphase.

GUYÉNOT and NAVILLE (Cellule **42**: 211–230) have studied oögenesis in female *Drosophila melanogaster*. Paired chromosomes, which correspond to those seen at pachytene and diplotene in normal meiosis, pass directly into the diakinesis and metaphase bivalents. We must assume therefore (as I have assumed in the present study) that metaphase pairing in the female is conditioned by chiasmata, as in homozygous organisms generally. The authors consider however that the diakinesis and metaphase stages in the male correspond to those in the female. This I deny on two grounds: first, they are not observably derived from pachytene pairing and chiasma formation (as shown by GUYÉNOT and NAVILLE); secondly, their structure shows no evidence of such a derivation but on the other hand compels the assumption that different forces are responsible for the pairing (as shown in the present study, in contradiction to GUYÉNOT and NAVILLE). The issue therefore depends on the correctness of the one or the other interpretation of meiosis in the male.

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