THE MECHANISM OF NON-RANDOM SEGREGATION OF SEX CHROMOSOMES IN MALE DROSOPHILA MIRANDA*

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INTRODUCTION

MEIOTIC segregation of a specified chromosome is said to be random when the spindle pole attained and the particular non-homologous chromosomes with which the specified chromosome assorts both appear to be a matter of pure chance. Such randomness of segregation is easily understood, for it is generally a simple consequence of bivalent formation and structure. Since the coorientation of homologues in a bivalent is fixed only with respect to the longitudinal axis of the spindle, not the poles, and because the particular orientation attained by one bivalent is ordinarily independent of the orientation of any second bivalent, disjunction and the subsequent second meiotic division must result in random segregation.

Such is the case in most animals and plants, for they have only bivalents at meiosis. But some organisms possess more than two sex chromosomes (reviews in SCHRADER 1928; WHITE 1940), and in these exceptional forms a non-random segregation of the sex chromosomes regularly occurs. Elucidation of the mechanism involved in such atypical cases promises to be of considerable aid in the construction of a general theory of chromosome mechanics. It is the purpose of the present paper to give a cytological account of the mechanism of non-random segregation of the three sex chromosomes in the male of *Drosophila miranda* Dobzhansky.

Before entering upon a description of the meiotic events in *Drosophila miranda* males, it may be helpful to point out that not less than four principal and perhaps wholly discrete types of segregative behavior of compound sex chromosomes may be recognized when descriptions of cases are reviewed. First, there are those forms with a multivalent that gives a determinate orientation of its constituents (as in some male mantids, HUGHES-SCHRADER 1943; in male Humulus, KIHARA and HIRAYOSHI 1932; etc.). Second, tehre are those forms in which no physical connections appear to be formed between the segregating members. Here there is a distance conjugation, an approximation, or "touch-and-go" behavior on the part of alternative sets of sex chromosomes (as in some male heteropterous bugs, PAYNE 1909; in male Frullania, LORBEER 1934; etc.). Third, the compound sex chromosomes may all be of the same type and, in spite of the absence of a partner or partners of opposite kind, all segregate to the same pole (for example, in X^1X^2 OO males of spiders, PAINTER 1914; in the bug Syromastes, WILSON 1909; etc.). Fourth,

a sex chromosome bivalent and a univalent, or two or more sex chromosome bivalents, although totally unconnected with one another, nevertheless segregate in such fashion that two mutually exclusive sets of sex chromosomes are regularly sorted to opposite poles of the spindle.

In all these types, so far as is known, it is a random matter to which pole of the meiotic spindle the X- or Y-complex of the compound sex chromosomes migrates. These segregations, therefore, are non-random purely with respect to the particular numbers and kinds of chromosomes separated from each other. Collectively they are in contradistinction to cases of non-random, *polarized*, segregation in which a particular chromosome migrates to a specified pole more or less frequently than would be expected on chance alone (as in maize, RHOADES 1942; in a moth, SEILER 1920; etc.).

Now an understanding of cases of the first type, in which multivalents are formed, would allow a generalization of the largest and least specialized class. The second and third classes are of considerable interest in connection with temporally displaced pairing and peculiarly specialized behavior on the part of spindles. But the most puzzling and, if real, theoretically most significant are those of the fourth type in which unconnected bivalents and chromosomes show correlative orientation and consequent non-random segregation. Unfortunately, none of the few cases inferred to be of the latter type are established beyond doubt. The sex chromosomes of *Gryllotalpa borealis* Burmeister (PAYNE 1912, 1916) have long been considered to undergo a non-random segregation of an unequal bivalent and a univalent. This they may in fact do, but a reinvestigation employing modern techniques must be carried out before the essential features involved become clear. From the account of DOBZHANSKY and PAVAN (1943) of compound, multiple sex chromosomes in *Drosophila prosaltans* Dobzhansky and Pavan, it would seem that correlative orientation of two independent bivalents may regularly occur in the male of this fly, but the essential facts are still unknown.[2]

Perhaps the most widely recognized case of such correlative orientation is that of *Drosophila miranda* as first described by DOBZHANSKY (1935). At meiosis in the male of this fly, according to DOBZHANSKY, an X^1-Y bivalent and an entirely separate, independent X^2-chromosome regularly occur. X^2 may lie anywhere on the spindle at first metaphase and early anaphase, it is said, but nevertheless it goes to the same pole as X^1 after the latter disjoins from the Y. The validity of this case was immediately challenged on theoretical grounds by DARLINGTON (1936), but DOBZHANSKY (1937) and KOLLER (1939) both reaffirmed the correctness of the original description. Nevertheless DARLINGTON's suspicions are correct, even though his own interpretation (1936, 1937) is wholly wrong, for MACKNIGHT and COOPER (1944) have shown that X^1 and X^2 both pair with Y at meiosis in the male of *Drosophila miranda*,

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[2] Two principal criticisms may be made of the cytological details of DOBZHANSKY and PAVAN's account. The figures of "diakinetic" chromosomes are quite possibly drawn from some stage other than the first meiotic prophase, perhaps either somatic prophase or meiotic interphase. Furthermore, the "thinness" and "paleness" of the polytene strands may suggest male haploidy for these elements, but, as MACKNIGHT (1939) showed for the X^2 of *Drosophila miranda*, they cannot be considered conclusive evidence for such an interpretation.

and thus regularly form a trivalent. Although the work here reported was originally undertaken to elucidate what appeared to be an authentic case of correlative orientation of unassociated chromosomes, it may now be offered as a fairly detailed analysis of the mechanics of a sex chromosome trivalent in male Drosophila. As will become evident, the clarity of the case and the peculiar construction of the sex chromosomes involved make segregation in this fly of exceptional interest.

MATERIAL

Five strains of *Drosophila miranda* have been used in this study, all of which are from the stockroom of the CALIFORNIA INSTITUTE OF TECHNOLOGY. In each case the strains are descended from single females captured in nature, and derive their names from the place and order of capture. The strains used and their respective histories are briefly as follows:

(1) Olympic-1 was originally collected by DOBZHANSKY in 1934 in the Olympic Mountains near Brinnon, Washington. Olympic-1, along with Cowichan-1 and Cowichan-7, served as type material both for the description of the species and for DOBZHANSKY's (1935) account of determinate segregation of the sex chromosomes.

(2) Whitney-60 was also collected by DOBZHANSKY, having been taken in 1937 on the eastern slope of Mount Whitney, California. KOLLER (1939) presumably used this strain and Olympic-1 in his cytological studies confirming DOBZHANSKY's account of determinate segregation.[3]

(3) Big Basin-2.

(4) Big Basin-4 and the preceding were both collected by STURTEVANT in 1941 from a grove of redwoods in Big Basin State Park, north of Santa Cruz, California.

(5) Monterey was taken by DOBZHANSKY in 1940 near Carmel, Monterey Peninsula, California.

Except for the preliminary studies, flies were reared on SPASSKY's (1943) cream of wheat and molasses fly medium upon which they prospered, and cultures were continuously maintained at $19° \pm 0.3°C$. Early experiments were made with flies reared on a cracked-oat, molasses and cotton-seed food at a temperature fluctuating between $16°$ and $20°C$.

CYTOLOGICAL METHODS

Owing to the general unsuitability of paraffin sections and aceto-carmine and aceto-orcein squash preparations for the study of spermatogenesis in Drosophila, all slides were made by fixing nearly unicellular films of the spermatocytes directly upon coverslips. The fixative which gave best results was Allen's B15 made up according to BAUER's (1931) formulas.

[3] DOBZHANSKY and KOLLER (1938) and KOLLER (1939) have described the Whitney strain as a new "race" distinct from the Olympic strains. This seems hardly justified, for the only constant differences between the Whitney and Olympic strains are said to be an inversion in the right limb of X¹, a possible size difference between the Y chromosomes which I have not been able to confirm, and slight mating preferences. Although these authors and DOBZHANSKY and EPLING (1944) seem to consider the Whitney strain as a "disjunct colony" in the Sierra Nevadas, it is by no means clear that sufficient collecting has yet been done to map out the actual distribution of *D. miranda*.

To prepare testis smears of Drosophila a last instar larva and a late male pupa of the same strain were placed on a coverslip in a moist chamber. The moist chamber was designed to allow dissecting and smearing to be carried out within it under the dissecting microscope. The pupa was removed from its puparium and pupal envelope, and the testes quickly pulled from the abdomen. The central area of the coverslip upon which the testes lay was wetted with larval blood. Thereupon the contents of the testes were rapidly smeared directly in the film of blood. The coverslip was immediately inverted onto the surface of freshly mixed B15, where it remained for one to not more than eight hours. Thereafter the smear was stained with Grübler's Gentian Violet according to SMITH's (1934) procedure, and mounted in a 60 percent solution of Clarite "X" in toluene. Successful preparations made in this way are characterized by remarkably well fixed and stained spermatocytes, even those at early diakinesis being exceptionally clear.

Nearly perfect fixation is not all that must be achieved with such difficult material; accurate microscopy is also required. Observations were carried out with Zeiss 3 mm and 2 mm N.A. 1.4 apochromatic objectives, using 20× and 15× Kompens oculars, respectively, and oil immersed N.A. 1.4 achromatic-apochromatic condenser giving a nine-tenths cone. The preparations were not counter-stained, but a combination of an Aklo #396 heat-absorbent glass slip with Wratten E22 and #61 filters gave sharp definition and good contrast. The washes and other drawings were made from camera lucida sketches which in turn were enlarged by pantograph.
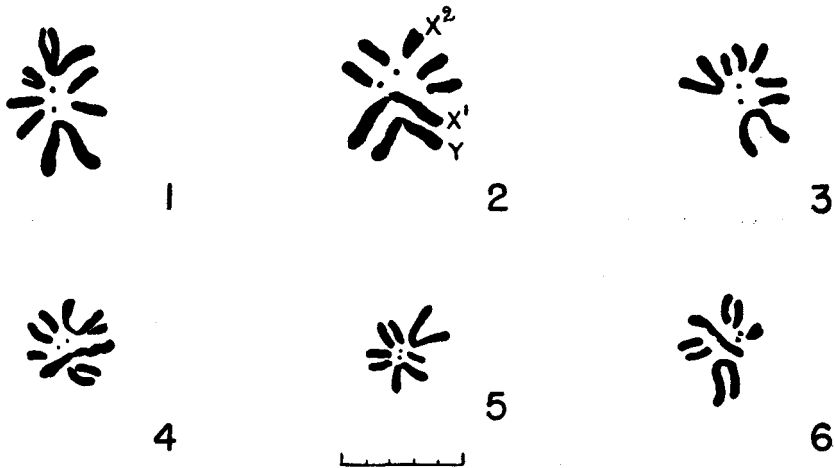
THE CHROMOSOME SET OF DROSOPHILA MIRANDA

The diploid chromosome set of female *Drosophila miranda* corresponds closely in gross morphology with the chromosome groups of race A and race B females of *D. pseudoobscura* Frolowa and Astaurow (DOBZHANSKY 1935, 1937, 1941). There are a pair of apparently equal-armed, V-shaped X chromosomes, three pairs of rod-like chromosomes (that is, chromosomes II, III, and IV), and a pair of dot-like autosomes. DOBZHANSKY and TAN (1936) have shown that each chromosome arm of *D. miranda* is at least approximately homologous (*contra* DOBZHANSKY 1941, see MACKNIGHT 1939, p. 183 *re:* the dot-like pair) to one of *D. pseudoobscura*, the principal differences between them being the result of rather extensive inversions (see MACKNIGHT 1939). This general homology is well shown by the strong somatic pairing of corresponding elements in the smaller neuroblasts of *miranda* × *pseudoobscura* hybrids (KAUFMANN 1940). The claims of DOBZHANSKY and TAN (1936) and DOBZHANSKY (1941) that more or less extensive translocations have occurred within the *miranda*-set relative to that of *D. pseudoobscura* are apparently based on incorrect identifications of chromosome parts (MACKNIGHT 1939; STURTEVANT and NOVITSKI 1941).

According to DOBZHANSKY (1935) the male of *Drosophila miranda* has one less free chromosome in its diploid set (*v.* fig. 1-6) than does the female, or male and female of *D. pseudoobscura*. There are a V-shaped chromosome corresponding with the X chromosome of the female, a somewhat unequal-

armed, V-shaped Y chromosome,[4] two pairs of rod-shaped chromosomes, an unpaired rod, and a pair of dot-like autosomes. The unpaired rod-shaped chromosome corresponds with the third chromosome of *D. pseudoobscura*. Since female *D. miranda* have two of these chromosomes, and the male apparently but one, this chromosome may be designated as a second X chromosome. Thus, if the haploid formula of the female *D. miranda* be written: X^1, X^2 ($=$III), II, IV, V, then the alternative haploid sets of the male gametes are: X^1, X^2, II, IV, V, and: Y, II, IV, V.

The five strains of *Drosophila miranda* here investigated have chromosome

FIGURES 1-6.—Polar views of spermatogonial metaphase plates in five strains of *Drosophila miranda*. Figure 1.—Big Basin-4. Figure 2.—Olympic, after DOBZHANSKY (1935). Figure 3.—Whitney-60. Figure 4.—Big Basin-2; right arm of uppermost sex chromosome separated into two halves. Figure 5.—Olympic-1. Figure 6.—Monterey. The scale represents five micra.

sets corresponding closely with one another and with the foregoing description (fig. 1-6). Indeed no constant difference between strains could be discerned in the chromosomes, although KOLLER (1939) states that the Y "seems larger in the Whitney race." DOBZHANSKY's (1935) belief that the spermatogonial chromosomes tend to form a specific pattern on the metaphase plate (shown in fig. 2) cannot be confirmed, as KOLLER (1939) also points out. Metaphases do occur in which X^2 lies opposite X^1 and Y on the equatorial plate as described by DOBZHANSKY, but they are by no means the rule and are never unduly frequent. Most usually X^2 lies adjacent to one of the V-shaped sex chromosomes, often the smaller of the two.

SEGREGATION IN THE MALE

Since DOBZHANSKY (1935) could find no evidence of extensive zygotic inviability, and inasmuch as X^1X^2A (A$=$chromosomes II$+$IV$+$V) and YA male gametes alone give normal, fertile combinations with regular X^1X^2A

[4] The *miranda* Y is incorrectly figured by WHARTON (1943, fig. on p. 307).

eggs, we appear to be confronted with a paradox. It would seem that either X^2 must segregate regularly from a chromosome with which it shares neither homology nor special attributes, namely the Y, or else X^2 must invariably assort with the equally non-homologous X^1. The cytological account by DOBZHANSKY (1935, 1937) does, in fact, portray the meiotic behavior of the sex chromosomes as anomalous in exactly this way. X^2 is said regularly to show a pattern of segregation related directly to that of the X^1-Y bivalent, although no tangible connection at any meiotic stage seems to bring about this result. Acceptance of this curious meiotic behavior at first required the analogical support of the possibly similar case of *Gryllotalpa borealis*, but KOLLER's (1939) cytological confirmation of DOBZHANSKY's description, although somewhat ambiguous, seemed to place the general account beyond doubt.

TABLE 1

The sex chromosome configurations at meiosis in male Drosophila miranda in 2035 decipherable nuclei from the following strains: Olympic-1, Whitney-60, Big-Basin-2, Big-Basin-4.

CONFIGURATION OF SEX CHROMOSOMES AT M 1	DIAKINESIS	PROMETAPHASE TO EARLIEST ANAPHASE	TOTALS
X^1YX^2 trivalent completely decipherable	110	697	807
Trivalent not completely analyzable, but no free X^2	53	1,085	1,138
X^1Y bivalent, but X^2 possibly free	8	63	71
X^1Y bivalent, X^2 definitely univalent and free	0	19	19
Total	171	1,864	2,035
Unclassifiable, all chromosomes badly overlapping or matted together	22	471	493
Grand Total	193	2,335	2,528

Be that as it may, the mechanics of segregation became openly suspect when MACKNIGHT (1939) discovered that DOBZHANSKY and TAN (1936), relying upon paleness and thinness of polytene strands as evidence of their initial haploidy, had completely overlooked the actual homolog of X^2 in the male. MACKNIGHT clearly demonstrated that most, if not all, of the euchromatin of X^2 is intercalated piecemeal into the substance of the Y chromosome, and hence the *miranda* male is not haploid for the third chromosome as was earlier believed. Now, since Y and X^1 possess a pairing segment in common (DOBZHANSKY 1935), and since Y and X^2 are partially homologous (MACKNIGHT 1939), it follows that Y may be expected to pair with both X^1 and X^2 simultaneously, forming a trivalent. This is in fact what happens at the first meiotic division (table 1), and the occurrence of such trivalents was briefly reported by MACKNIGHT and COOPER (1944).

The data of table 1 were collected by counting every nucleus in the fields analyzed. The fields chosen for analysis were selected purely on the basis of

their excellence of fixation and sharpness of staining. Of the 2035 decipher-
able nuclei, no more than 90 cases in which X^2 might be unpaired were found,
and of these only 19 were without question cases of a univalent X^2. Since
there is no reason to suppose that any different proportion of free X^2 chromo-
somes or of trivalents would be found among the 493 unclassifiable nuclei, it
is concluded that X^2 succeeds in entering a trivalent formation with X^1 and
Y in not less than 95 percent of the cases, more probably in 99 percent of
all meioses. The details of the sex chromosome trivalent and its behavior
will be described in detail in the succeeding cytological account.

It may be wondered how it is that DOBZHANSKY and KOLLER both mis-
interpreted meiosis in *Drosophila miranda* males in the same way. Possibly
their belief that X^2 lacked a homolog in the diploid set of the male uncon-
sciously led to their interpretation of early anaphase figures as those of a
still earlier stage. Not infrequently (i.e., ca 10 percent of the time) X^2 may be
the first element to undergo disjunction from the trivalent at anaphase. Be
that as it may, it is certain from both figures and description, that KOLLER
(1939) did see at least occasional sex chromosome trivalents. But these he
minimized *because* they appeared in his hybrids of Olympic×Whitney strains.
KOLLER further remarks that "Instances when X^2 lies close to the XY com-
plex are none too rare in the spermatocytes of pure races."

THE FIRST MEIOTIC MITOSIS

The first meiotic stage at which nuclear details may be analyzed with con-
fidence in *Drosophila miranda* corresponds with late diplotene or early dia-
kinesis in other organisms. At such an early stage (fig. 7–8) the nucleus con-
tains two relatively compact clumps, each of which proves to be a bivalent
formed by one of the two large pairs of autosomes. Rarely the tiny bivalent
composed of the dot-like autosomes is visible. In addition to these three there
is also present a much larger element having five (rarely six) more or less con-
torted and projecting arms. This last is a sex chromosome trivalent, as no
other body occurs in the nucleus at this time or appears later. The bivalents
and trivalent stain very weakly with Gentian Violet during this period, but
in the more darkly stained nuclei four chromatids may be discerned in each
large autosomal bivalent, and the actual pattern of conjunction between the
two X chromosomes and the Y chromosome is resolvable (fig. 8).

At this early stage the Y chromosome generally appears as a somewhat
flexed, V-shaped element which seems more heavily condensed, or more com-
pact, than the other sex chromosomes. X^1 is slender, has loose gyres in each
arm, and submedially is paired with Y at what appears to be the juncture of
the latter's arms. X^2 is always totally independent of X^1 and conjoined with
the distal half of one arm (always the same arm?) of the Y chromosome. Not
all of X^2 appears to synapse with this arm of Y, for at the earliest recognizable
stages and thereafter the proximal half of X^2 is separate from it (compare
fig. 8 and 9).

From mid-diakinesis on, the trivalent fixes and stains with such excellence
that no doubt can be fostered concerning its principal details. X^1 proves to
be conjoined to Y close to the kinetochore regions of both chromosomes, es-

pecially to that of the Y chromosome. It could not be determined whether X^1 has pairing segments homologous to Y in only one or both of its arms. Nor could it be determined whether Y has two such regions homologous to X^1. The fact that figures may be found in which it appears that X^1 and X^2 have both paired with the same arm of Y (fig. 21, 32), or with different arms (fig. 15g, 35), shows that Y may be amphivalent with respect to at least one of the X chromosomes. But, as would be expected with such small chromosomes, figures are very rare in which the exact locus of conjunction of X^1 and Y, and the site of Y's kinetochore, can both be determined without much doubt. Of 20 such, 15 have X^1 and X^2 definitely conjoined on different sides of Y's kinetochore, while five appear to have X^1 and X^2 conjoined on the same side of Y's kinetochore.

The most surprising feature about X^1 is that it possesses very striking relic coils in both its arms (fig. 8–12, 15a, 15b, 27) until late diakinesis, in spite of the fact that such coils are only rarely, if ever, detectable (fig. 27) at this time in the Y, and have never been discerned in X^2. As with relic coils generally, these gyres loosen and their pitch increases as prophase advances until, finally, the X^1 chromosome can no longer be said to show any vestige of the coiling of the last gonial mitosis.

As in the case of X^1, X^2 likewise proves generally to be conjoined interstitially with Y during diakinesis, for distally and proximally to the region of conjunction it may flare away from the body of the arm of Y to which it is joined (fig. 9–10, 12). Although this appears to be the rule, and many prometaphase (fig. 19–20), metaphase (fig. 21, 30, 33), and anaphase (fig. 25) figures show that such a disposition may persist until disjunction of X^2 from Y, in some trivalents the whole distal half or third of X^2 may appear conjoined with Y (fig. 11, 14, 18, 28). Whether the former configuration is a consequence of pairing with one arm of Y, the latter a result of pairing with the other arm of Y, can only be conjectured. Rare pairing conformations will be mentioned below in relation to their possible rôles in bringing about primary non-disjunction. Finally, in individual nuclei one or more of the sex chromosomes can be seen to be split in prophase (fig. 8–10, 13), so that each member of the trivalent at all of the stages described is composed of at least two chromatids.

The end of diakinesis and the onset of prometaphase is heralded by the disappearance of relic coiling in X^1, breakdown of the nuclear membrane, and consequent separation of the kinetochores of conjoined chromosomes as they coorient on the spindle (fig. 13, 14, 16, 17, 19, 20, 22, 28). A metaphase plate stage is generally formed (fig. 18, 21, 24, 29, 30, 32, 33). At metaphase all cooriented kinetochores appear to be in a state of active repulsion, frequently drawing into slender threads the proximal parts of their chromosomes between the insertions of the spindle fibers and the regions of conjunction in both autosomes and sex chromosomes alike (fig. 15d, 18, 21, 29, 30, etc.). Aside from the dot-like pair which may undergo disjunction as early as prometaphase (fig. 28) and, when visible, is generally found to be disjoined at metaphase (fig. 24, 29), anaphase comes simultaneously for the autosomal bivalents and the sex chromosome trivalent (fig. 15f, 15f', 15g, 23, 36, 37, 39, 40). Occasionally large autosomal bivalents appear to enter anaphase before the sex
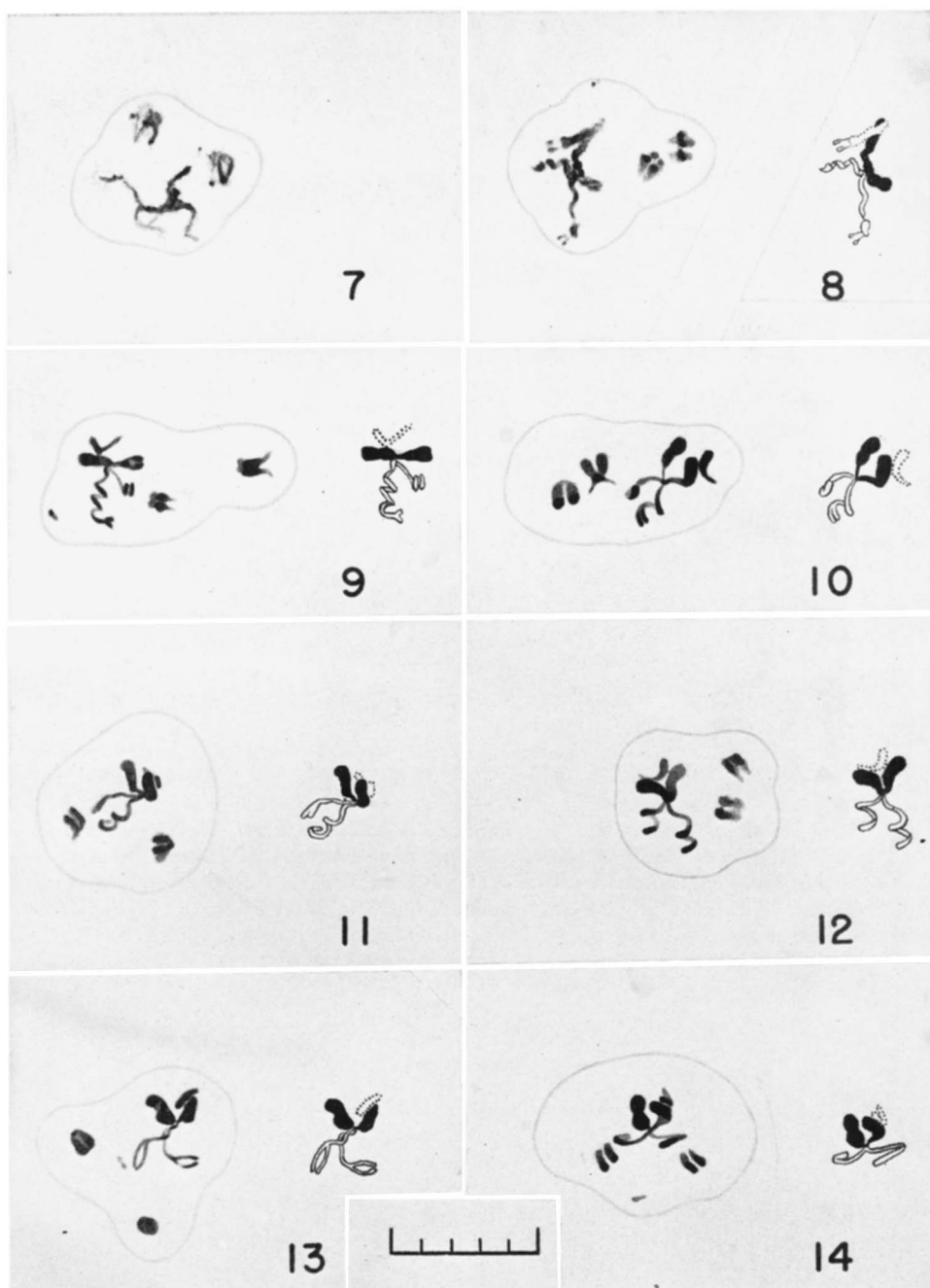
FIGURES 7–14.—Late diplotene to late diakinesis of the first spermatocyte division in *Drosophila miranda*. The diagrams to the right of the figures serve as keys to the elements comprising the sex chromosome trivalent. X^1 is represented by a line figure, X^2 by a dotted outline, and Y by solid black. X^1 is below in each case. Figure 7.—Late diplotene. Figure 8.—Early diakinesis. Figures 9–14.—Mid through late diakinesis. Figures 7–8.—Big Basin-2. Figures 9–14.—Whitney-60. Note that figure 12 is the same as photomicrograph 15b. The scale represents five micra.
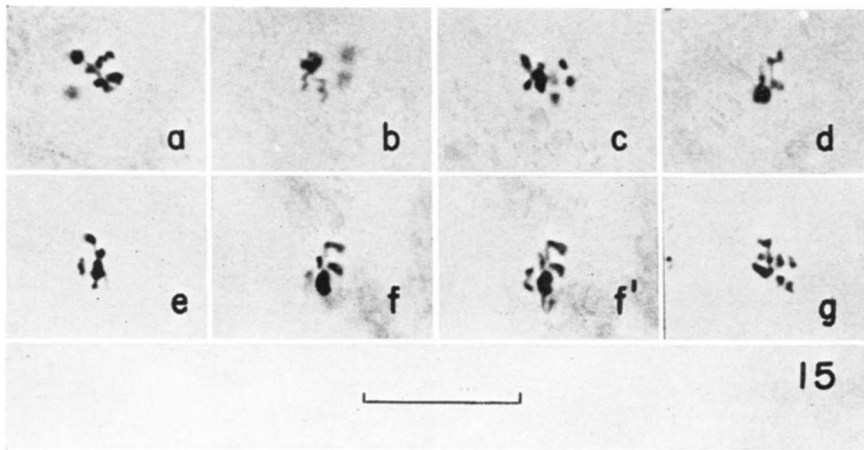
FIGURE 15.—Photomicrographs of the first spermatocyte division in *Drosophila miranda*. Figure a.—Mid-diakinesis (= fig. 27). Figure b.—Mid-diakinesis (= fig. 12). Figure c.—Early anaphase showing the sex chromosome trivalent to left and dividing autosomal bivalents to right (= fig. 31). Figure d.—Sex chromosome trivalent at metaphase; X^2 (to left) and X^1 (to right) above, Y below. Figure e.—Early anaphase, showing a misoriented sex chromosome trivalent (= fig. 38). Figures f-f'.—High and low focal depths of sex chromosome trivalent dividing at earliest anaphase (= fig. 36). Figure g.—Early anaphase (= fig. 37); X^2 is to the extreme left below one arm of X^1 and above Y. The two pairs of large autosomes are to the right. Figures a, d.—Big Basin-4. Remaining figures are Whitney-60. The scale represents ten micra.
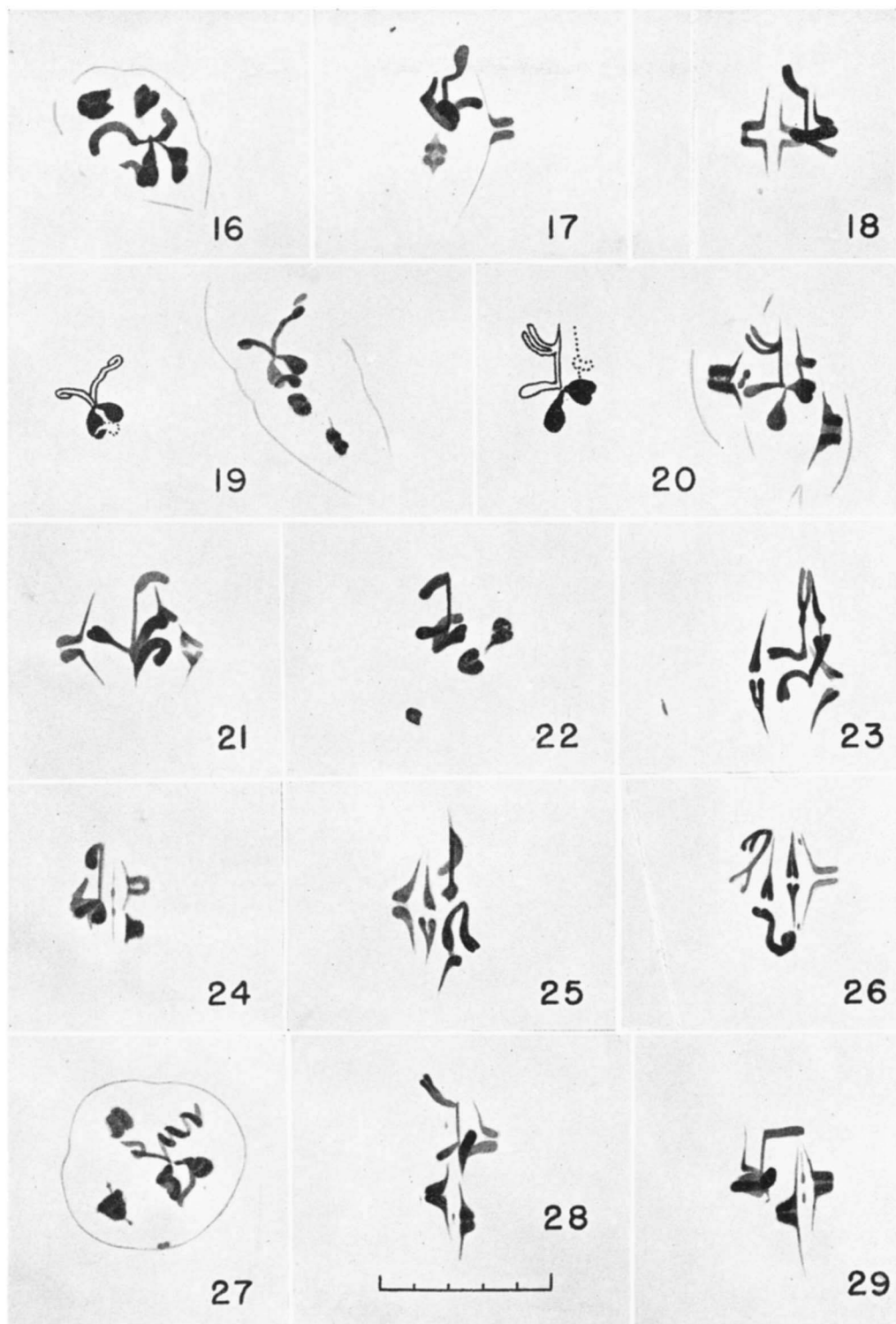
FIGURES 16–29.—Diakinesis through early anaphase of the first spermatocyte division in *Drosophila miranda*. In all cases X^1 is toward the top of the page, as is X^2 in most of the figures; Y is generally below. Figures 16–17, 19–20, 22, 28.—Prometaphase. Figures 18, 21, 24 and 29.—Metaphase. Figures 23, 25–26.—Anaphase. Figure 27.—Mid-diakinesis (= 15a). Figures 22 and 25 are of special interest as they show a univalent X^2 and a misorientated trivalent, respectively. Figures 16–18, 22, 25.—Olympic-1. Figures 19–20.—Whitney-60. Figures 21, 23.—Big Basin-2. Figures 24, 26.—Monterey. Figures 27–29. —Big Basin-4. The scale represents five micra.
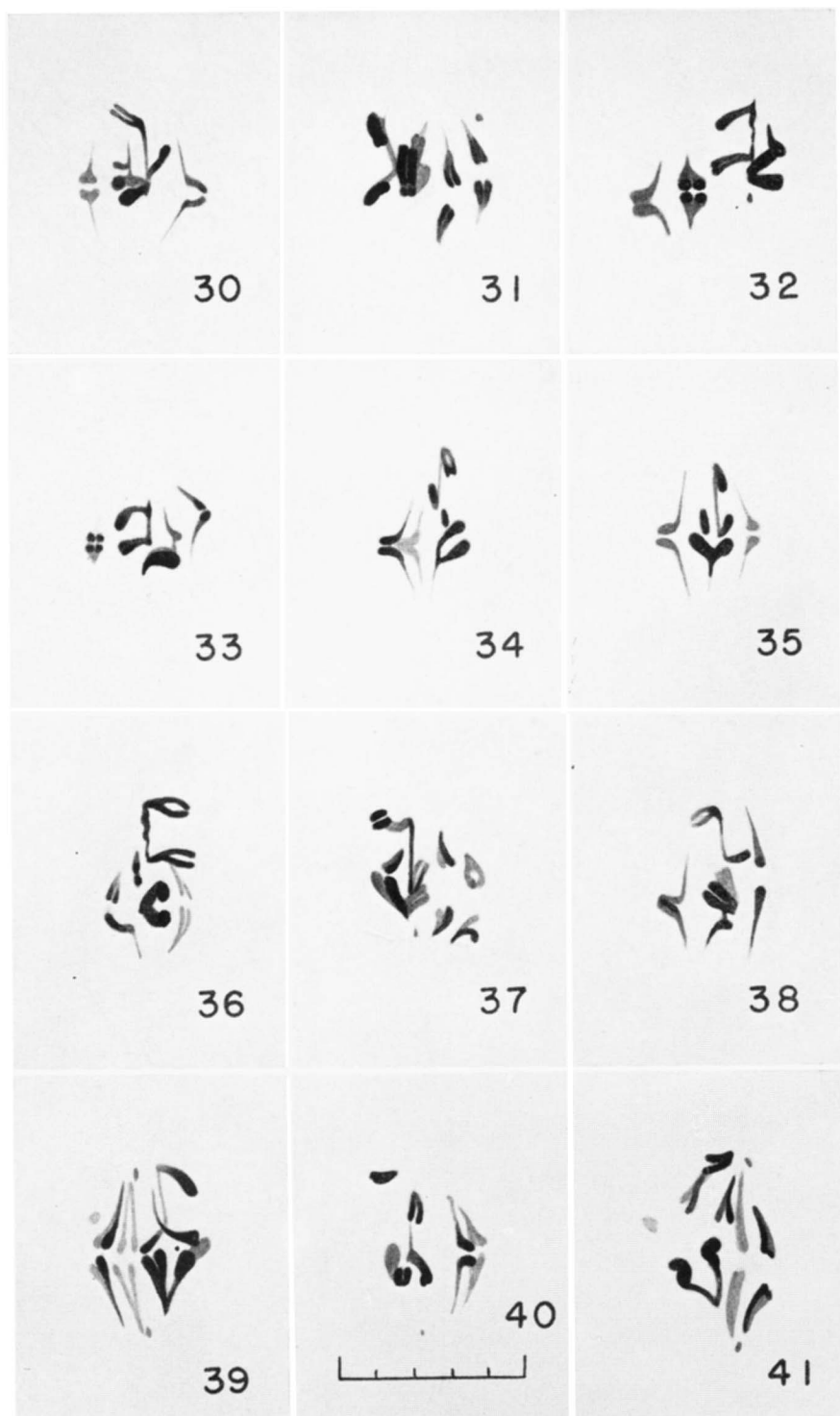
FIGURES 30–41.—Metaphase through anaphase in the first spermatocyte division of
Drosophila miranda, strain Whitney-60. In every case, except figure 38, X^1 and X^2 are above, Y
below. Figures 30, 32–33.—Metaphase. Figures 31, 34–41.—Anaphase. Figure 37 (=15g).
Figure 38 (=15e) represents a misorientated sex chromosome trivalent. Figure 40 shows a
univalent X^2. Figure 31 (=15c). Figure 36 (=15f, 15f'). The scale represents five micra.

chromosome trivalent (fig. 15c, 31), but equally infrequently the sex chromosomes may undergo a precocious disjunction (fig. 26, 33–35).

The actual course of anaphase presents no exceptional features (fig. 15c, 15e, 15g, 26, 31, 37, 38, 41). Even if some of the chromosomes have disjoined precociously, generally by late anaphase all chromosomes going to the same pole have their kinetochores equidistant from the spindle apex. X^2 segregates from Y just as does X^1, and accordingly X^2 is found at a pole with X^1, not at the equator, at the close of the first meiotic anaphase. One last detail from anaphase should be mentioned. At mid and late anaphase Y is often as long or considerably longer than X^1 (fig. 23, 25, 26, 34, 39, 41) when the abnormal stretching of the region of X^1 between its kinetochore and conjunctive locus is taken into account. At diakinesis, metaphase and early anaphase Y is definitely shorter and more "thick-set" than X^1. Whether, as DOBZHANSKY (1935) states, Y is actually smaller than X^1 at mitotic stages can be only conjectured from the evidence so far at hand.

The foregoing account will be found to differ radically from the earlier ones by DOBZHANSKY (1935, 1937) and KOLLER (1939). Thus it must be reemphasized that X^2 does not lie at random on the spindle nor does it lag during anaphase to become located at the equator of the spindle after all other chromosomes have migrated poleward. X^2 forms a constant member of a sex chromosome trivalent and its behavior throughout is typical of all chromosomes which regularly enter into multivalent formation and segregate nonrandomly therefrom. These results are based on the study of about five thousand nuclei and are consistently found in the spermatogenesis of *Drosophila miranda* regardless of the source of the strains which have been available for study.

THE STRUCTURE AND PAIRING OF THE SEX CHROMOSOMES

Drosophila miranda and *D. pseudoobscura* were presumably derived in relatively recent geologic times from a common ancestor. In spite of the radical innovation in *D. miranda* of a second X chromosome conjoining at meiosis with Y, conjunction of X^1 and Y apparently remains essentially the same as in *D. pseudoobscura* (compare figures in this paper with those of DOBZHANSKY 1934 and DARLINGTON 1934). This is an especially interesting circumstance because, as MACKNIGHT (1939) has shown, the Y chromosome of *D. miranda* has been very radically altered from that of *D. pseudoobscura* by having had the euchromatin of what was originally an autosome-III (element C) scattered through its substance. According to MACKNIGHT, this has presumably been brought about by the translocation of the third autosome onto Y (perhaps with the loss of one arm of Y, and in the special way that STURTEVANT and NOVITSKI (1941) conceive such translocations to occur) with subsequent repeated inversion within the III-Y fusion chromosome. The result has been a wholesale disorganization of the translocated element so that today there is but slight sequential homology between any sizeable part of Y and X^2. The differentiation between a part of the new Y and X^2 has therefore been brought about chiefly by a reorganization of Y itself. Nevertheless at least one proximal region of pairing between X^1 and Y has remained functionally unaltered

by the extensive fragmentation, inversion, and relocation that has occurred. Presumably rearrangements disrupting the conjunctive and hence segregative mechanism of X^1 and Y were quickly eliminated by strong selection against aneuploidy.

The lack of gross structural homology between X^2 and Y perhaps accounts for the fact that these chromosomes show little or no recognizable pairing tendencies in polytene nuclei. For this and other reasons, MacKNIGHT (1939) suggested that if meiotic pairing between X^2 and Y occurs, then it may involve a heterochromatic region. While such a mechanism immediately springs to mind, it must be pointed out that inferences regarding meiotic pairing properties of chromosomes have very questionable validity when deduced solely from observations on polytene chromosomes. In any event, this possibility could not be put to direct test in the present study because the slides failed to give a consistent differentiation between heterochromatin and euchromatin. The most that could be made out was that X^1 and X^2 appear never to compete for proximal (presumably heterochromatic) pairing loci in Y, nor ever to hinder one another mechanically by attempting to pair in immediately adjacent segments of Y. Rather, X^2 appeared most frequently to conjoin in the distal half of an arm of Y. Furthermore, conjunction most often seemed to involve an interstitial region in X^2 (fig. 9, 10, 12, 19, 20, 30), although configurations in which the distal length of X^2 paired with that of an arm of Y were not uncommon (fig. 8, 11, 14, 16, 18, 28).

For the most part such restricted pairing opportunities probably tend to guarantee greater regularity of determinate disjunction from a trivalent. Irregularity of meiotic behavior of homologs in trisomics and triploids forming trivalents can generally be ascribed primarily to competitive pairing (DOBZHANSKY 1933), which results in certain percentages of non-conjunction, and to non-convergent or indifferent orientations on the spindle arising very largely from the production of relatively loose, end-to-end associations[5] (DARLINGTON 1937). Both non-conjunction and non-determinate orientation lead inevitably to the occurrence of "non-disjunction" in the genetic sense, and hence to aneuploidy.

Whether the accumulation of such a succession of inversions in the III-Y fusion chromosome of *Drosophila miranda* experienced positive selection because it led to more efficient trivalent structure and segregation by eliminating competition and random orientation can only be guessed. But such a view would nicely supplement MacKNIGHT's (1939) suggestion that the III-Y fusion has selective value because it must tend to prevent the spread of "sex-ratio" (STURTEVANT and DOBZHANSKY 1936) through a population by halving the fertility of those "sex-ratio" males possessing the fusion chromosome.

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[5] That loose terminal associations in a trivalent may still allow a high percentage (ca 97 percent) of determinate disjunction follows from the interesting observations of HUGHES-SCHRADER (1943) on the sex chromosome trivalent in Stagmomantis. The sort of behavior which she has described might be expected where the linearly arranged trivalent is disproportionately long relative to the effective length of the spindle. But such regularity of convergent orientation would not be expected *a priori* in forms with trivalents whose total lengths are short when compared with the spindle axis, as is the case in *Drosophila miranda*.

This would be the case because in "sex-ratio" males the Y is eliminated during the maturation divisions which, in this case, must also result in the simultaneous elimination of the attached autosome. While MacKnight's hypothesis seems satisfactory to account for the persistence of the III-Y fusion chromosome once it has arisen, it nevertheless fails to explain why there apparently has also been a strong selection for cumulative loss of sequential (structural) homology between the free and Y-attached elements C. Certainly it seems hardly likely that the progressive loss of structural homology between X^2 and element C in the III-Y fusion chromosome was purely a matter of chance. Yet it is also clear that trivalent formation in *D. miranda* does not follow an absolutely stereotyped pattern, and that a more rigorous localization of conjunction between X^2 and Y might be expected to improve the overall mechanism of determinate disjunction. Although true end-to-end associations of X^2 with Y have not been observed, in a minority of the cases (less than 26 percent) X^2 may distally overlap the end of Y (fig. 21, 25, 32). Definitely in seven instances, and perhaps in as many as 26, X^2 actually paired in reversed fashion with Y so that the lengths of the respective chromosomes were directed, relatively to their kinetochores, in opposite directions (fig. 38). Both types of pairing occurred indiscriminately among strains and gave rise— perhaps being exclusive antecedents—to the small number of linearly oriented trivalents (fig. 15e, 25, 38) which result in non-disjunction of either X^1 or X^2 from Y. Such irregular types of conjunction are not unexpected if MacKnight's (1939) general interpretation of the *miranda* Y is correct, although they also suggest that meiotic conjunction of X^2 and Y does not necessarily involve heterochromatic regions. Perhaps further selection for disorganization of structural homology between X^2 and the III-Y fusion chromosome may be expected to result in still greater rarity of configurations giving rise to linear orientations.

From the foregoing argument, such reorganizations of sequence would be expected to occur most frequently within Y. Surviving inversions in the free X^2 for the most part should involve only relatively short proximally or distally located segments, as is the case for the two inversions in X^2 which KOLLER (1939) has recorded.

At any rate, the *miranda* X^1YX^2 trivalent is today about 97 percent efficient, as follows from the facts that: (1) non-conjunction of X^1 and Y was not observed in a total of 2035 cells in which it would have been possible to recognize its occurrence. (2) Non-conjunction of X^2 and Y was established cytologically in only 19 of 2035 cells, and occurred in not more than 90 of 2035 meiocytes. This gives a frequency of "non-disjunction" of X^2 and Y lying somewhere between 0.4 percent and 2.2 percent. (3) Only 12 misoriented (that is, linearly oriented) trivalents were found among 1864 first meiocyte divisions in which the pattern of orientation could be made out. Thus the overall failure of determinate X^1X^2-Y segregation lies normally between 1 percent and 3 percent.[6]

[6] MacKnight (1939) was able to detect genetically only one of the four types of gametes resulting from primary non-disjunction of X^1 and Y—namely, X^2A gametes. His data suggest about 1.5 percent linear orientations of the trivalent, a value approximately twice that (0.64 per

CHIASMATA AND CONJUNCTION OF THE TRIVALENT

DARLINGTON (1931, 1934, 1939) has consistently maintained that X and Y conjoin at meiosis in male Drosophila by constantly occurring reciprocal chiasmata. Were it not for the fact that this interpretation has gained a wider acceptance of DARLINGTON's primary "chiasma hypothesis of metaphase pairing" than either facts or theory merit, no further discussion than that already given (COOPER 1941, 1944a, 1944b, 1945) would be called for here. Perhaps it is of some value to point out merely that the structure and behavior of the *miranda* sex chromosome trivalent is in agreement with my earlier conclusion that the reciprocal chiasmata hypothesis is not a necessary inference from the primary data. Thus X^2 in conjoining with and disjoining from Y parallels the behavior of X^1 to a most remarkable degree when association is interstitial for both X^2 and Y (fig. 9, 10, 12, 20, 30, 35–37, 39, 41). The common mode of conjunction of X^2 with Y is therefore cytologically indistinguishable from that by which X^1 forms an association with Y. But the variable patterns of conjunction of X^2 with Y lead to no cytologically discernible differences in actual mode of association. Therefore, either Y invariably elicits reciprocal chiasmata in conjoining with X^2, as it is supposed by DARLINGTON (1936) to do in conjoining with X (X^1), regardless of site or direction of pairing, or else such a pattern of chiasmata is not essential for metaphase association. Since X^2 and Y differ structurally by a multitude of sequences, the former interpretation appears inadmissable without the introduction of a number of special subsidiary assumptions. Accordingly it may be supposed that X^1 and X^2 conjoin with Y by similar mechanisms and that the general mechanism involved is independent of crossing over—hence of chiasmata.

The above conclusion is in accord with that derived from comparative studies of the flies Melophagus (COOPER 1941), Trichobius (COOPER 1942), and Olfersia (COOPER 1942, 1944a, 1944b), as well as from a review of the relevant literature (COOPER 1944b). Since MATHER's (1944) recent genetic evidence for the regular occurrence of reciprocal chiasmata between X and Y in male *Drosophila melanogaster* is based upon faulty experiments (see COOPER 1945, pp. 479–480), there can no longer be said to exist any published data uniquely requiring such an interpretation of meiotic conjunction in male Diptera. This is not surprising, for the primary "chiasma hypothesis of metaphase pairing" itself (which alone demands the reciprocal chiasmata interpretation) has been shown not to be of general application. Thus sex chromosome bivalents devoid of chiasmata nevertheless undergo normal segregation in *female Drosophila melanogaster* (COOPER 1945). How widely the chiasma hypothesis of metaphase pairing may be applied remains to be demonstrated, because, although widely employed, it has been little subject in the past to unbiased experimental test.

cent) for the cytological data presented here. MACKNIGHT's estimate may well be distorted by viability complications, since it is derived from the hybrid progeny of *Drosophila pseudoobscura* females by *D. miranda* males. In any event there is fair agreement between the independent genetic and cytological estimates.

SUMMARY

Contrary to the earlier descriptions by DOBZHANSKY and KOLLER, an X^1YX^2 trivalent is regularly formed at the first meiotic division in male *Drosophila miranda*.

The non-random segregation of X^1 with X^2 is explained by the fact that both these chromosomes conjoin with Y and segregate from it.

The X^1YX^2 trivalent is not less than 97 percent efficient in giving euploid gametes, and more probably is nearer 99 percent efficient.

Failures in segregation are related to non-conjunction of X^2 and Y, and to aberrant modes of association of X^2 with Y leading to linear orientations of the trivalent.

It is suggested that the loss of sequential homology between X^2 and the III-Y fusion chromosome may be accounted for by its possible rôle in the elimination of pairing competition and truly terminal associations. These two factors seem to be primarily responsible for randomness of segregation in trisomics and triploids.

X^1 and X^2 conjoin with Y by similar mechanisms not involving crossing over, hence independent of chiasmata.

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