ANALYSIS OF MEIOTIC PAIRING IN OLFERSIA AND CONSIDERATION OF THE RECIPROCAL CHIASMATA HYPOTHESIS OF SEX CHROMOSOME CONJUNCTION IN MALE DROSOPHILA[1]

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INTRODUCTION

STEVENS' (1908) initial study of spermatogenesis in male flies disclosed that the mechanism underlying somatic pairing apparently leads directly to parasynapsis of autosomal homologs at the beginning of the spermatocyte growth period. STEVENS emphasized that this course of meiosis in male brachycerous flies is exceptional, for the customary sequence of pre-diakinetic stages appears to be absent and "bivalents" rather than ordinary cross and ring tetrads are formed. Although MORGAN (1912, 1914) shortly thereafter discovered that genetic crossing over is absent in the male of Drosophila, the full significance of STEVENS' discoveries was first appreciated by DARLINGTON (1934a), who clearly demonstrated that the structure of the autosomal bivalents in the male of Drosophila corresponds with a non-crossover tetrad. As STEVENS (1908) and METZ and NONIDEZ (1921) before him suggested, DARLINGTON holds that the autosomes in the male fly conjoin at meiosis by means of exaggerated forces of somatic pairing (a view which is questioned by BAUER [1939] and WOLF [1941]). On the other hand DARLINGTON maintains that unlike the autosomes the sex chromosomes remain paired until meiotic metaphase only because they invariably form reciprocal chiasmata between their homologous inert regions. There is thus envisioned a mechanical dualism in the modes of conjunction of sex chromosomes and autosomes.

The evidence for such reciprocal chiasmata between sex chromosomes in the male of Drosophila, as will be shown in this paper, is both indirect and susceptible to alternative interpretation of less elaborate nature. The purely hypothetical nature of DARLINGTON'S interpretation is only too often overlooked, and it is not uncommon for geneticists to express their belief that reciprocal chiasmata between X and Y have been cytologically demonstrated in the male of *Drosophila pseudoobscura* at the least (for example, PHILIP 1935; MATHER 1936; WHITTINGHILL 1937; BROWN 1940; WHITEHOUSE 1942). The fact that in many organisms non-disjunction follows failure of chiasma formation has been generalized into the "chiasma hypothesis of metaphase pairing" (DARLINGTON 1929). But this generalization, which appears to make acceptance of the reciprocal chiasma hypothesis more ready in spite of the obvious and acknowledged absence of chiasmata in autosomal bivalents, is by no means universal in its application. There is now good evidence from mantids (WHITE

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1938; HUGHES-SCHRADER 1943a, b), Lepidoptera (*vide* BAUER 1939), a mite (COOPER 1939), a scorpion (PIZA 1939), and a fairly large number of bugs (SCHRADER 1940, 1941) that chromosomes can and do conjoin at meiosis by mechanisms other than chiasmata. To base the means of sex chromosome conjunction in male Drosophila on the chiasma hypothesis of metaphase pairing is no more than to assume a primary mechanism (the chiasma) in Drosophila males which is as much in need of proof as the conclusion (reciprocal chiasmata) itself.

It has been shown that in the fly *Melophagus ovinus* the autosomes of the male physically conjoin by relatively small pairing segments (COOPER 1941). There is no evidence that these conjunctive segments form chiasmata, yet they result in configurations and chromosomal behavior identical with those of the sex chromosome bivalents in male Drosophila for which reciprocal chiasmata have been assumed. Since the comportment of the autosomes of Melophagus indicates that the properties of the sex chromosome bivalent of male Drosophila are not characteristic consequences of chiasma formation alone, the reciprocal chiasmata hypothesis becomes suspect. The following study of a close relative of Melophagus—*Olfersia bisulcata* Macq., a Panamanian fly parasitic on the black vulture—was undertaken with the hope of further elucidating the properties of the conjunctive mechanism apparently common to autosomes in the Melophagus male and the sex chromosomes in male Drosophila. The startling meiotic phenomena discovered in male Olfersia, supplemented by the detailed analysis given below of cytogenetic data on Drosophila, show that there are no longer sufficient grounds for adhering to the reciprocal chiasmata hypothesis proposed by DARLINGTON. Apart from the obvious importance of this conclusion for geneticists working with Drosophila or on problems of crossing over, the present study provides new information on a mechanism of chromosome conjunction at meiosis which does not involve chiasmata. Independently of the reciprocal chiasmata hypothesis, it will be seen that DARLINGTON'S (1929) chiasma hypothesis of metaphase pairing now appears of more limited domain and should be invoked only in those cases where antecedent pachytene and diplotene phenomena justify its application.

MATERIALS AND METHODS

Two males and one female of *Olfersia bisulcata* Macq. were collected from a black vulture (*Catharista urubu* (Viellot)) shot on Orchid Island in Gatun Lake, Canal Zone. One male and the female were fixed for several hours in San Felice, whereas the remaining male was fixed in Allen's B-15 for the same period. The gonads were dissected from the flies in a modified Bělař's salt solution in which the proportions of Na^+ to K^+ in Bělař's original formula are nearly reversed. Peterfi's paraffin-celloidin embedding method was used, and sections were cut from 8 to 15μ in thickness. Stains employed were Heidenhain's iron hematoxylin, Feulgen, and OEHLKERS' (1940) modification of the gentian-violet procedure. Erythrosin was used as a counterstain for some of the gentian-violet preparations, for it brings out the spindle structures clearly. Although fixation with San Felice was satisfactory, the B-15 fixation gave such

brilliant figures with gentian-violet that all but one of the illustrations were prepared from the latter.

Observations were made with Zeiss 3 mm and 2 mm N.A. 1.4 apochromatic objectives, with 20× and 15× Kompens oculars, respectively, and N.A. 1.4 achromatic-apochromatic condenser. Sharpest definition and pleasing contrast of the chromosomes stained with gentian-violet, but not counter-stained, were obtained by employing an Aklo No. 396 heat absorbent glass slip (Corning Glass Works) together with Wratten E22 and Wratten No. 61 filters between the light source and condenser.

THE CHROMOSOME COMPLEMENT

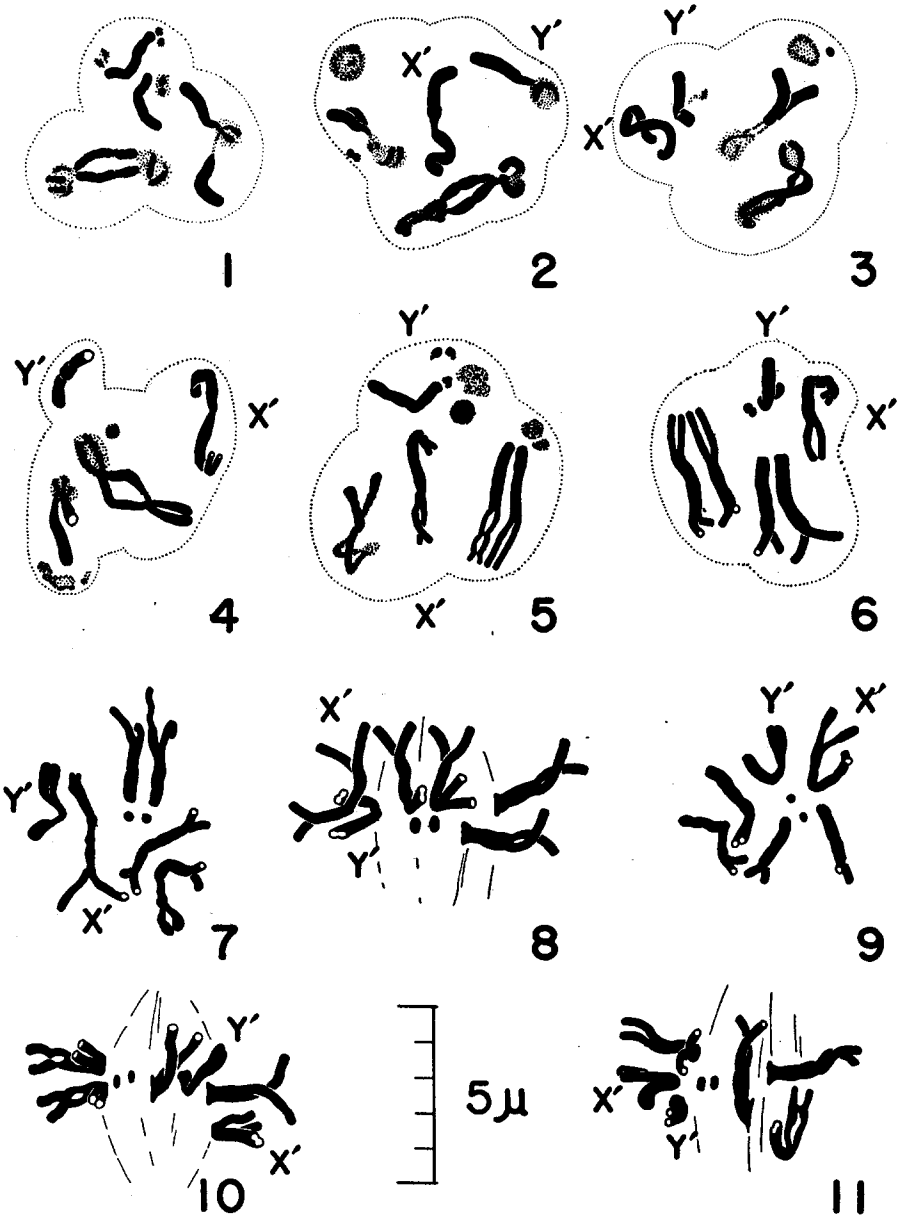
The chromosome garnitures of ten pupiparous flies are now known (COOPER 1941, 1942), and of these Olfersia ($2n=8$) has the smallest number of chromosomes. In the male Olfersia (fig. 7, 9) there are a pair of large V-shaped autosomes with submedian kinetochores, a pair of large and apparently subterminal rods, a pair of small dot-like chromosomes, and a pair of markedly heteromorphic sex chromosomes. The sex chromosomes could not be definitely identified as X or Y because no mitoses were encountered in the ovaries of the female. However, evidence from its pairing behavior at meiosis, as well as heteropycnosis at interphase of meiosis, suggests that the smaller sex chromosome is the Y. Accordingly the large submedian V-shaped sex chromosome will be designated "X'," and the small, nearly median V-shaped chromosome will be denoted "Y'" until such time as the X may be identified with certainty in the female.

It should be pointed out that chromosomes of this fly are especially favorable for the analysis undertaken on two counts. First, the autosomes include the major autosomal types known in Drosophila. Second, the sex chromosome pair morphologically corresponds closely with that of *Drosophila pseudoobscura* race B, the chief difference being that the V-shaped member (Y') is the smaller of the two sex chromosomes in Olfersia.

THE SPERMATOGONIAL PROPHASES

During gonial resting stages the large, slightly staining, spheroidal nucleus contains but few chromatic wisps, a diffuse flocculent mass and a plasmosome. At earliest prophase chromatic segments become visible but show no unequivocal signs of being paired; the flocculency becomes more sparse and the plasmosome dwindles. By mid-prophase (fig. 1) the chromosomes may be individually recognized. The autosomes are somatically paired, whereas the sex chromosomes at most merely occupy the same general region of the nucleus. The sex chromosomes never show close somatic pairing in the gonial prophases while they are condensing or when they have reached the condensed state (fig. 1–6).

The somatic pairing of the large autosomes is quite extraordinary and merits special comment. The rod-shaped autosomes are associated only at their distal regions, the paired regions of the homologs being relatively less condensed than the customarily widely separate medial and proximal portions (fig. 1–4). The



FIGURES 1–11.—Spermatogonial mitosis in *Olfersia bisulcata*. Figures 1–4.—Mid-prophase. Figures 5–6.—Late prophase. Figures 7–10.—Metaphase. Figure 11.—Prometaphase.

V-shaped autosomes likewise show a localization of their somatically paired regions. Their proximal thirds do not pair but form a widely open loop (fig. 1–5). Distal to the central or kinetochore-containing loop the short arms may abruptly twist (from 90° to 180°+) about one another, thereafter somatically pairing more or less along their lengths (fig. 1–4). The long arms likewise twist as they pair for a short region proximal to the central loop. Thereafter the arms once more diverge only to associate again at their distal extremities (fig. 1–4). As in the case of the pair of rod-shaped autosomes, the pairing regions of the V-shaped autosomes appear somewhat less condensed than do the non-paired regions and a flocculent, whey-like coagulum appears to invest the paired regions (fig. 1–4). With the advance of prophase the flocculency vanishes and the paired regions of both the rod-shaped and V-shaped autosomes separate from one another. Correlated with the relaxation of somatic pairing in the rods is the abrupt separation of the chromatids in the distal third to two-fifths of the rod-shaped chromosomes. Ultimate separation and untwisting of the paired regions of the V-shaped autosomes are likewise frequently accompanied by a disjunction of chromatids in both arms but not in the central loop. Somewhat earlier, or at the same time, one or both arms of the X′ chromosome may separate (fig. 4–6). Although a wide separation of chromatids is rarely encountered in Y′, the two chromatids (especially in the long arm) are generally evident in prometaphase and metaphase (fig. 7–11).

The spermatogonial metaphase is like that of most flies, the homologous chromosomes tending to be adjacent to one another on the equatorial plate. Most interesting is the fact that at the equatorial plate stage the sex chromosomes which showed no close association in prophase are as regularly adjacent to each other as are members of the autosomal pairs (fig. 7–11). In anaphase, however, the mitosis is not unlike that of most organisms, for there is no noticeable tendency for homologs to somatically pair. In this respect, as in many others, the spermatogonial mitoses of Olfersia conform to KAUFMANN's (1934) description of mitosis in the ganglion cells of *Drosophila melanogaster* larvae. No differences were observed in the last gonial mitosis, and it would appear that meiotic pairing first occurs in the definitive spermatocyte nucleus following the mitosis. In this respect Olfersia differs from many other flies (STEVENS 1910; METZ 1916, 1926; METZ and NONIDEZ 1921, 1923; etc.).

It is clear that the time of onset and details of somatic pairing are not alike in all Diptera. METZ (1916) generalized the occurrence of somatic pairing at all stages of development and in all tissues examined for many speices, but it is known that somatic pairing is suppressed in the spermatogonia and first spermatocytes of Sciara (METZ, MOSES and HOPPE 1926), is absent in the early oogonia and variable in the early spermatogonia of Tipula, and is not expressed by the supernumerary chromosomes of Tipula (BAUER 1931). SMITH (1942) has recently elaborated a general hypothesis of telophase pairing at the ultimate gonial division. He maintains that a pre-leptotene association in many organisms as well as the somatic pairing of Diptera are consequences of the singleness of chromosomes at anaphase. The assumed singleness of chromo-

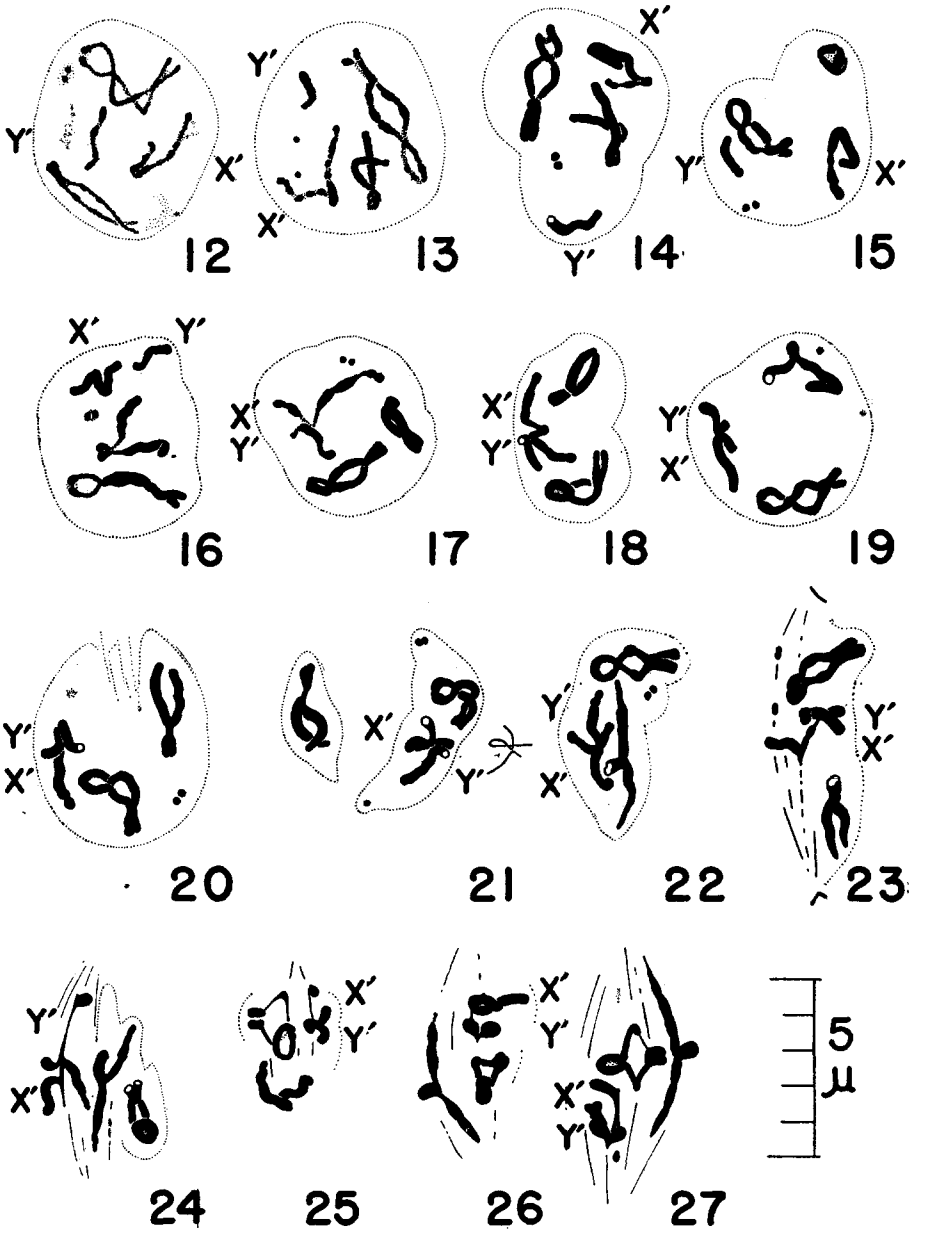
somes and hence pairing are said to occur at the last gonial anaphase in most organisms, but are held a property common to the chromosomes of Diptera "at each and every anaphase." These views are not consistent with the data described above for Olfersia or with published accounts of somatic pairing in many other flies. Without multiplying assumptions, it seems impossible on SMITH's view to account both for the divergence of sister chromatids at late prophase of the gonial divisions and for subsequent failure to pair in anaphase. If the sister chromatids fail to remain associated (and this is a widespread phenomenon in Diptera) at late prophase and metaphase, why do they not reassociate with non-sister homologs? If each chromatid is assumed to be divided and the metaphase chromosome is held to be quadripartite, then somatic pairing should not be found at the succeeding prophase. Without extensive qualification, SMITH's assumptions are likewise out of accord with the facts regarding polytene chromosomes (BAUER 1935; COOPER 1938), multiple association in Culex (BERGER 1937), somatic pairing in polyploid cells (METZ 1916, 1922b, 1925), somatic pairing of chromosomes in ganglion cells of *Drosophila melanogaster* larvae (KAUFMANN 1934),[2] the variable expression of somatic pairing in Tipula (BAUER 1931), the non-pairing of kinetochore regions in Olfersia, Tipula, and Dasyllis (BAUER *op. cit.;* METZ 1922a), and finally the less intense, the frequently variable, or the localized expression of somatic pairing by sex chromosomes (STEVENS 1908; METZ 1914, 1926; COOPER 1941). Whatever the cause of somatic pairing may be, simple non-division of the chromosome does not appear to be the essential factor.

THE MEIOTIC MITOSES

In Olfersia the first spermatocytes apparently occur exclusively in nests of 32 cells each, just as in *Drosophila pseudoobscura* (DOBZHANSKY 1934; STURTEVANT and DOBZHANSKY 1936) and *D. miranda* (DOBZHANSKY 1935), although in the streblid bat-flies, close relatives of Olfersia, six gonial divisions giving 64 cells are the rule (COOPER 1942).

The initial stages of meiotic prophase are not resolvable in the material at hand. Lack of the early synaptic stages will not seriously affect the interpretation of the meiotic mechanism, however, for it is becoming increasingly clear that true leptotene stages may be absent in flies whether or not chiasmata are formed (BAUER 1931; WOLF 1941). Bivalents of clearly defined forms are first encountered in Olfersia at a stage which corresponds with late diplotene of most organisms (fig. 12). In such nuclei all the chromosomes may be recognized, and it is of importance to note that from diplotene through early diakinesis the homologous chromosomes simulate the configurations in the early prophases of spermatogonia described above. Thus the rod-shaped chromosomes are closely associated at their distal thirds or fourths whereas their proximal regions appear to be randomly disposed with respect to each other (fig. 12–19).

[2] SMITH reconciles the evidence of doubleness found by KAUFMANN in homologs which are somatically paired by assuming these cells to be tetraploid. The fact remains that KAUFMANN found no striking evidence for somatic pairing at anaphase but rather that somatic pairing reaches a maximum at prophase.



FIGURES 12–27.—Late diplotene to prometaphase of first spermatocyte division in *Olfersia bisulcata*. Figures 12–13.—Late diplotene. Figures 14–17.—Early diakinesis. Figures 18–19.—Mid-diakinesis. Figures 20–22.—Late diakinesis. Figures 23–27.—Prometaphase. In no case is the sex chromosome pair displaced in the illustration, although when necessary autosomal bivalents are slightly displaced so that they do not overlap.

The homologs of the submedian pair form two loops and a more or less closely paired region distal to the median or kinetic loop (fig. 12–19). The dot-shaped chromosomes generally are separated by a "space" rarely exceeding the diameter of a single dot-like chromosome in width. Expecially striking is the fact the sex chromosomes are not paired (fig. 12–13) and may even lie on opposite sides of the nucleus (fig. 14–15). For the sake of clarity the details of definitive bivalent formation in the sex chromosomes and autosomes will be treated separately.

THE PAIRING OF THE AUTOSOMES

The dot-like pair of chromosomes requires no further comment than given above; its formation of a bivalent parallels that of similar small nearly spherical chromosomes in Diptera. The rod-shaped chromosomes are associated at their distal extremities in much the same manner as the small autosomes at diakinesis, or the proximal ends of the large autosomes, in Melophagus (COOPER 1941). In late diplotene and early diakinesis the paired ends may show a ligher staining gap between the conjoined extremities of the homologs (fig. 12, 14, 16). By mid-diakinesis a line of demarcation is no longer visible between the homologous paired segments (fig. 19–21) but end or side views show the homologs still to be parallel in the paired region as late as metaphase (fig. 22, 23, 29, 31). There is accordingly no evidence for the presence of chiasmata in this bivalent, and on analogy with Melophagus the simplest assumption is that we are dealing with a distal conjunctive segment.

The bivalent formed by the submedian pair of autosomes is most remarkable, superficially appearing to possess at least three chiasmata. Whether or not each of these associations really involves a chiasma is not immediately obvious, but it will be seen that an analysis of these configurations can nevertheless be made. As prophase approaches prometaphase, the bivalent shows three definite loci of association. There is one point of close apposition at roughly the midpoint of each arm. Distal to these two points of apparent contact the short and long arms differ in their pairing. The homologous distal ends of the short arm remain more or less parallel. In the long arm, however, the homologs diverge after their initial contact and form a second loop by pairing once again at their extremities (fig. 12–22). In late diplotene and early diakinesis the loop of the long arm and the paired extremities of the short arm lie at right angles or very obliquely to the kinetic loop (fig. 12–13, 15–18) showing the geometrical relationship so common in bivalents which have an interstitial chiasma in each arm and a terminalized chiasma in one of those arms. But, as is strikingly shown by the data of table 1, the relations of the planes of the loop and paired ends change with the approach of metaphase so that the loop, the parallel arms, or both tend to lie in the same plane as the kinetic loop (fig. 21–23, 25, 29, 31, 33–35). By merging the first two columns (that is, diplotene and diakinesis data) a nine cell table is obtained in which no $m \leqq 5$. There are four degrees of freedom, $\chi^2 = 67.4$, and $P \ll 0.01$. Accordingly we may conclude that the class frequencies of the bivalent configurations for the submedian chromosome pair are not independent of meiotic stage.

TABLE 1

Distribution of V-shaped autosome bivalent-configurations among nuclear stages. Consider the hypothetical axis which passes through the kinetochores and lies in the plane of the kinetochore loop. When the plane of the distal loop in the long arm, or of the paired ends of the short arm, is parallel to this axis it is said to lie in the same plane as the kinetochore loop in spite of obvious lateral flexions of the bivalent concerned. For example, the V-shaped autosome bivalents in figures 25 and 34 are said to have the planes of both arms lying in the plane of the kinetochore loop.

BIVALENT CONFIGURATION	DIPLO-TENE	DIA-KINESIS	PRO-METAPH.	META-PHASE	TOTALS
Class 1: Planes of both distal loops of long arm and distal ends of short arm at right angles or oblique to kinetochore loop (fig. 12, 13, etc.)	24	24	14	4	66
Class 2: Plane of either distal loop of long arm or of distal ends of short arm at right angles or oblique to kinetochore loop, but not both—that is, one of these lies in same plane as kinetochore loop (fig. 22, 27, etc.)	1	1	10	17	29
Class 3: Planes of both distal loops and distal ends of short arm lie in same plane as kinetochore loop (fig. 29, 34, etc.)	0	1	3	18	22

When columns one and two are merged, $\chi^2 = 67.4$, $n = 4$, and $P \ll 0.01$. 117

The simplest interpretation is that both arms undergo a twist at their first locus of association during or before conjunction of their pairing loci. In this respect they correspond with the somatic pairing configurations at prophase in the spermatogonia (see p. 541). With the approach of metaphase, especially as the bivalent orients on the spindle in prometaphase, the divergence of the kinetic loops results in an untwisting moment, so that the planes of the arms come to lie in the same plane as the kinetochore loop. If the average number of twists per bivalent is calculated for each period of prophase, then the relative untwisting of the bivalents with passage from diplotene to late metaphase is given in table 2.

TABLE 2

The relative number of twists per bivalent during meiotic prophase.

MEIOTIC STAGE	NUMBER OF BIVALENTS	AVERAGE NUMBER TWISTS PER BIVALENT
diplotene	25	1.96
diakinesis	26	1.88
prometaphase	27	1.41
metaphase (early)	25	0.76
metaphase (late)	14	0.57

To account for such a reverse rotation of arms distal to chiasmata with divergence of kinetochores would require improbable assumptions regarding the association of strands involved in the hypothetical chiasmata. The interpretation given above is the simplest, and it will be seen that critical evidence from anaphasic disjunction bears out the belief that here we are dealing with pairing segments of chromosomes which do not form chiasmata in the male. Descriptively they may be referred to as "conjunctive segments." Whether or not the twisting is a necessary phase of their function in conjoining the homologs into a bivalent is not known, but it seems unlikely when one considers the similar conjunctive loci of the autosomes of Melophagus (COOPER *op. cit.*).

The Pairing of the Sex Chromosomes

At late diplotene and early diakinesis the sex chromosomes are in most instances undergoing contraction in widely separate regions of the nucleus (fig. 12–15). As contraction progresses, the sex chromosomes come to lie more closely together. At the early stages of their approximation, no pronounced orientation of the sex chromosomes with respect to each other is in evidence (fig. 16). However, in late diakinesis X′ is found adjacent to or actually touching Y′ (fig. 17–19). Such late diakinetic associations as well as those of prometaphase (fig. 20–28) and metaphase (fig. 31–32, 34–35) show that X′ and Y′ conjoin to form a bivalent with regions fairly close to the kinetochore in both chromosomes intimately paired. The pairing segment of X′ appears to be located invariably in the long arm, but Y′ may pair by means of segments in either its short arm (fig. 26, 28, 31, 32) or long arm (fig. 24, 37). X′ long arm by Y′ short arm associations were found in 31 of 41 bivalents which could be analysed with certainty; the remaining ten were X′ long arm by Y′ long arm conjunctions. Quite evidently Y′ possesses a region in both arms homologous with a portion of X′. On analogy with the X and Y chromosomes of *Drosophila melanogaster* (NEUHAUS 1937) and *D. pseudoobscura* (DARLINGTON 1934a), this fact suggests that the Y′ chromosome is the true Y of Olfersia.

Table 3 is a résumé of observations on the progressive pairing of the sex chromosomes at meiotic prophase. $\chi^2 = 57.9$ which, for $n = 4$, corresponds with $P \ll 0.01$. Clearly the relative positions of X′ and Y′ are not independent of nuclear stage. Since no univalent sex chromosomes were encountered at the first meiotic metaphase in an estimated thousand cells, it is justifiable to conclude that the description of sex chromosome pairing given above is correct in all essentials. Namely, the unpaired sex chromosomes at diplotene give rise to sex chromosome bivalents which by metaphase are physically conjoined by short pairing segments located interstitially. As will be seen in the description of anaphase which follows, the conjunctive loci of the sex chromosomes do not differ in their subsequent behavior from those of the autosomes.

Anaphase of the First Meiotic Division

The V-shaped centrioles of Olfersia (fig. 23, 28) are stained only rarely in the preparations at hand. Accordingly the earliest indication of the onset of spindle formation is the assumption of an elongate and often lobulate shape by the late

TABLE 3

Configurations of the sex chromosomes at different meiotic prophase stages.

SEX CHROMOSOME CONFIGURATION	LATE DIPLOTENE AND EARLY DIA-KINESIS	MID- AND LATE DIA-KINESIS	PROMET.	TOTAL
Sex chromosomes separate and pairing regions not oriented—for example, fig. 12–16.*	16	9	0	25
Sex chromosomes separated by a distance less than length of short arm of Y′ and pairing regions oriented—for example, fig. 17–18, 20–21.	10	11	1	22
Bivalents conjoined—for example, figs. 19, 22–29, 31, etc.	2	15	37	54

$\chi^2 = 57.9$, $n = 4$, $P \ll 0.01$.

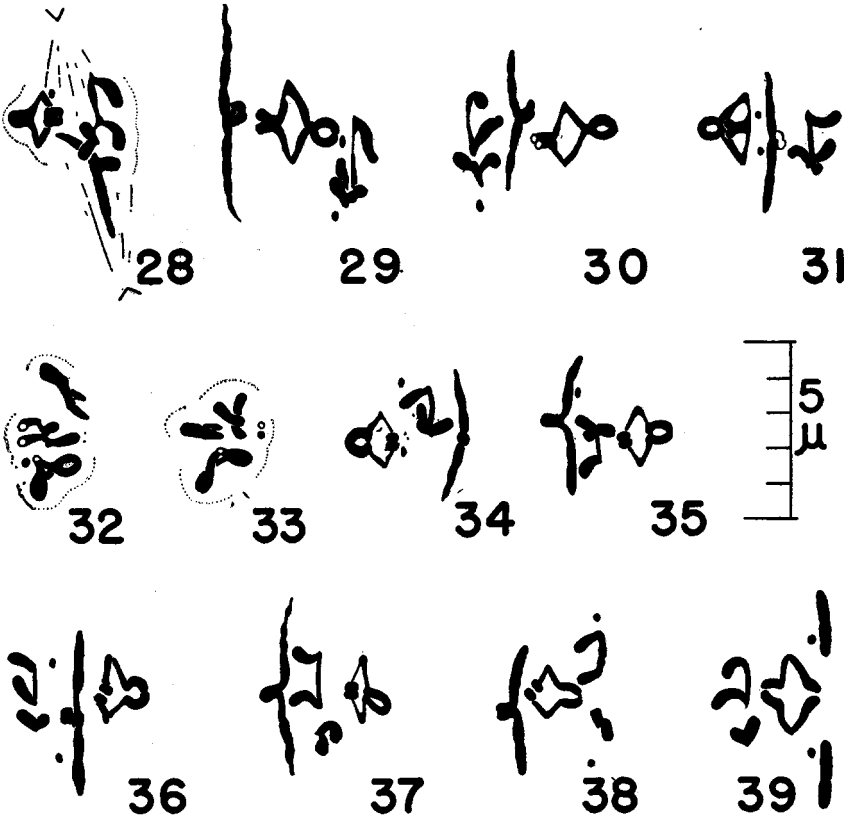
* In cases such as that of figure 16 the two sex chromosomes are separated by less than the length of the short arm of Y′, but the pairing regions are widely separate and not oriented with respect to each other.

diakinetic nucleus (fig. 21–22).[3] The change in nuclear shape during the transition from diakinesis to prometaphase is due to shrinkage of the girth along all but one diameter, not by a process of actual elongation of the nucleus. Nuclear limits vanish wherever they contact the developing spindle, and the bivalents are strewn over the length of the spindle in the initial stages of congression (figs. 23–28).

Although disjunction of the dot-like chromosomes in prometaphase is the rule (fig. 23, 29, 31, 33, 34), a metaphase plate stage for the large chromosomes may be distinguished. At metaphase each bivalent has its cooriented kinetochores approximately equidistant from the equator, this being true also for the disjoined dot-like pair when both its members are visible (fig. 30–36). Apparently metaphase is a stage of short duration, as seems also to be the case in many other Diptera (awakening conflicting reports in descriptions of meiosis in Drosophila males).

The time of onset of early anaphasic disjunction is not synchronous among the bivalents, but seems to be inversely correlated with the absolute physical length of association in each bivalent. Thus the dot-like pair separates in prometaphase and the sex chromosome bivalent generally disjoins while the rod-chromosome bivalent and V chromosome bivalent are still in metaphase (fig. 30, 37). Although the rod bivalent remains closely conjoined during the initial opening of the paired arms of the V chromosome bivalent (fig. 36, 38), it nevertheless undergoes disjunction and poleward movement while the V bivalent is still in its early phases of disjunction (fig. 38–40). Late anaphases show that the chromosomes tend to reach the poles as a group in spite of the

[3] Division of the nucleus into two separated lobules as in figure 21 was noted only infrequently.



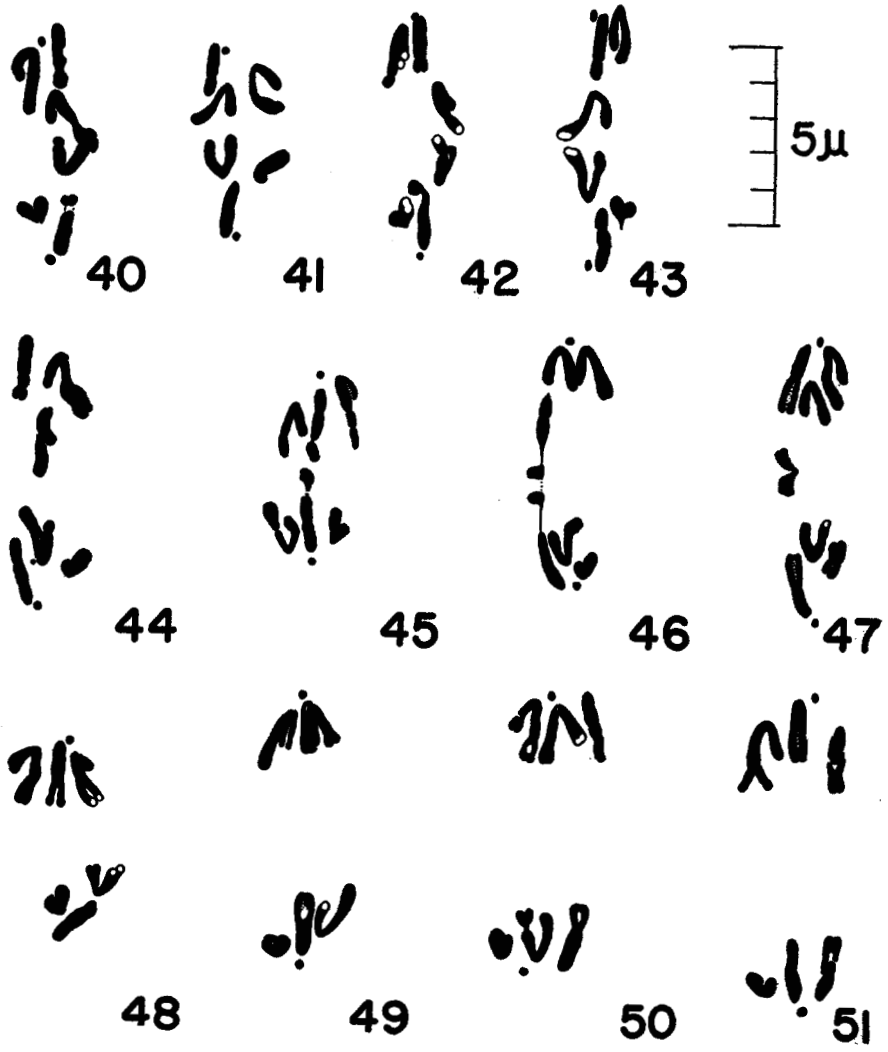
FIGURES 28–39.—Prometaphase to early anaphase of first spermatocyte division in *Olfersia bisulcata*. In all figures, excepting 32, 33 and 35, X′ is directed toward the upper pole. Figures 28–29.—Prometaphase. Figures 30–37.—Metaphase. Figures 32–33.—Metaphase in polar view. Figures 38–39.—Early anaphase. Where necessary bivalents have been laterally displaced to avoid overlaps.

definite order of completion of disjunction (fig. 40–51). At early telophase the distal ends of the arms of the X′ and V chromosomes, and the distal ends of the rod chromosomes show their chromatids to be disjoined (fig. 52, 53). The arms of Y′ occasionally evince a split delimiting the component chromatids of one (fig. 49) or both arms (fig. 47), but the chromatids do not separate widely. No evidence of duality was observed in the dot-like univalents, perhaps owing merely to lack of resolution.

Disjunction of the Large Autosomal Bivalents

Considering the evidence from prophase that the rod, V, and sex chromosome bivalents are not conjoined by chiasmata, the actual disjunction figures of these bivalents merit special attention. For this reason it is regrettable that the precise details of initial disjunction could not be satisfactorily deciphered for the conjoined segments of the rod chromosome bivalent. Whether, as is not infrequently the case for the short arm of the V chromosome bivalent, there is

a regular or occasional twisting of homologs at the proximal ends of the conjoined segments at metaphase could not be decided because of the intimacy of the association. That such may be the case is hinted by some figures (namely, fig. 22, 23, 31, 36), as well as by the fact that the conjunctive segments appear to disjoin first at their distal extremities (fig. 38). In any event final separation is achieved with more or less parallel disjunction of the conjoined segments, between the proximal ends of which there may persist a faint thread of dubious significance (fig. 46). The separating ends of the rod chromosome bivalent show no separation of sister chromatids at early anaphase (fig. 45), but one



FIGURES 40–51.—Anaphase of the first spermatocyte division in *Olfersia bisulcata*. X′ is at the upper pole in all figures. Where necessary, bivalents have been laterally displaced to avoid overlaps.

(about 20 percent) or both (about 13 percent) of the univalents from this bivalent may have split distal ends at late anaphase (fig. 40, 42, 44, 48–51); by telophase all are split (fig. 52, 53). In one instance the conjunctive segments failed to disjoin at what appears to be the distal end. In this isolated case a fracture and separation of both univalents from their conjunctive segments seems to have resulted (fig. 47).[4]

At metaphase and early anaphase the V chromosome bivalent tends to complete rotation of whatever twists are residual in the conjoined segments (fig. 29–35). Disjunction of the short arms results in parallel to oblique displacement of these arms without any evidence of terminalization or exchange of partners (fig. 36, 38, 39). The loop in the long arm similarly undergoes disjunction without terminalization of the proximal association. Characteristically, the proximal association in the long arm disjoins first (fig. 36, 38), followed by separation of the terminal association (fig. 38, 39). In some instances the rotation of one of the conjoined arms is not complete at the time of disjunction, resulting in configurations such as that of figure 40. Customarily there is no visible separation of the chromatids in the univalents of the V chromosome bivalents in early and mid-anaphase (fig. 40–47), but in late anaphase and early telophase the chromatids separate in one or both arms (fig. 48, 51, 52, 53).

Disjunction of the Sex Chromosome Bivalent

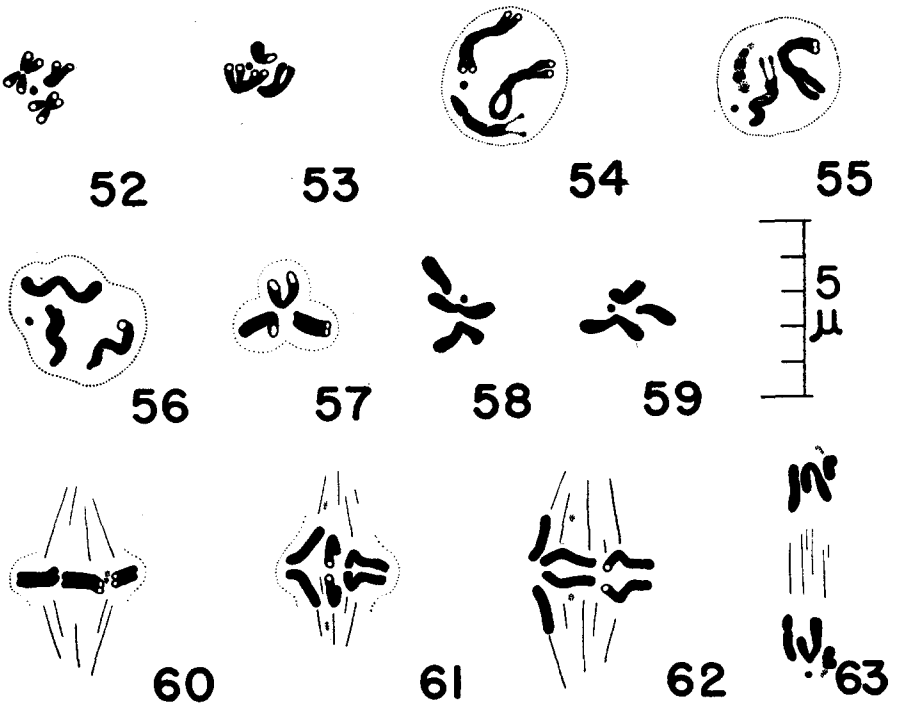
The fact that X′ and Y′ pair in prophase at a time when they are already markedly condensed may be taken as conclusive evidence that they are not conjoined by chiasmata. Nevertheless, the disjunction of the sex bivalent follows in detail the course of events described by DOBZHANSKY (1934), DARLINGTON (1934a), and KOLLER and TOWNSON (1933) for the sex chromosome bivalent in *Drosophila pseudoobscura*, and by DOBZHANSKY (1935) and KOLLER (1939) for the X^1Y bivalent of *D. miranda*, where reciprocal chiasmata are supposed to occur. During congression and metaphase the kinetochores of the X′Y′ bivalents appear to be under tension, as evidenced by the attenuation of the points of apparent spindle fiber insertion (fig. 23, 24, 26–29, 31, 34, 35). The limb of X′ between the kinetochore and the locus of conjunction with Y′ becomes drawn out (fig. 23–25, 27–29, 31, 34–35), although the portion of this arm distal to the locus of conjunction remains unaffected (figures cited). Only rarely does the proportionately stouter segment between the kinetochore and conjunctive locus of Y′ show pronounced attenuation (fig. 24). At early anaphase there appears to be a parting of the conjoined segments without any appreciable alteration in the relative dispositions of the limbs distal to the loci of former conjunction (fig. 30, 36, 37, 39). As anaphase progresses, the attenuated arm of X′ shortens, and the general appearance of the poleward moving sex chromosomes becomes normal in all respects (fig. 38, 40–46). By late anaphase one or both arms of X′ show a separation of chromatids to have occurred (fig. 44, 48–51). In telophase the chromatids of both arms distinctly

[4] True non-disjunction has been observed in Melophagus where similar conjunctive segments are involved (unpublished data).

separate (fig. 52, 53), and it is in this condition that X′ enters interphase. As noted above, Y′ rarely shows more than a line of separation between its component chromatids at late anaphase (fig. 47, 49).

Interphase and the Second Meiotic Division

At early interphase, separated chromatids show characteristic patterns in the rod and V chromosome and X′ univalents. Thus the distal extremities of the rod univalents often appear negatively heteropycnotic, the chromatids in this region being divergent or parallel, and the ends bead-like (fig. 54, 55). The V chromosome univalents show both arms divided, and frequently the chromatids of the long arm form a loop through contact of their ends (fig. 54). This condition is not infrequently noted during late anaphase, although it is only poorly represented in one of the figures illustrated (fig. 51). As noted above, X′ has both arms prominently split at early interphase (fig. 54). The structure of Y′ is difficult to decipher, but it may be stated generally to show differential heteropycnosis (fig. 55). The dot-like autosome remains plainly visible throughout interphase, but shows no eccentricity of behavior. The chromosomes do not fade from view during interphase. Onset of the second meiotic division is



FIGURES 52–63.—Telophase I, interphase, and second spermatocyte division in *Olfersia bisulcata*. Figures 52–53.—Equatorial views of polar telophase I groups. Figures 54–55.—Interphase nuclei. Figures 56–57.—Second prophase. Figures 58–60.—Second metaphase. Figures 61–63.—Second anaphase. Univalents have been laterally displaced to prevent overlaps only in figures 60–62.

forecast by the shortening of the chromosomes and the parallel reassociation of their chromatids where formerly separate (fig. 56, 57). The univalents form a flat metaphase plate (fig. 58–60) and initiate anaphase more or less simultaneously. The chromatids of the dot-like chromosome, as would be expected, complete their separation first. The other chromosomes appear to achieve their early anaphase separation by both pronounced parallel displacement of sister chromatids and an increasingly more rapid separation of kinetochore regions (fig. 60–63).

Chiasmata and Conjunction of the Large Autosomes

The data of anaphasic disjunction are in agreement with the conclusion that in Olfersia conjunction of the rod chromosomes in meiosis is independent of chiasmata. Suppose, for example, that the associations in this bivalent were due to a single, sub-terminal chiasma. The disjunction at anaphase should then result in terminalization with consequent separation of chromatids distal to the point of exchange. Actually, however, disjunction does not result in terminalization, for the conjoined chromosome segments part distally prior to or simultaneously with disjunction of the most proximal point of association. Furthermore, separation of sister chromatids distal to the supposed point of exchange, resulting in bifurcation of the distal ends of the chromosomes, does not occur in the univalents at the time of disjunction. The chromosomes are not so small as to make observation of such a separation of chromatids impossible if it occurred, for later in anaphase such a separation of chromatids is characteristically found. However, separation of chromatids at this time does not show this to be the result of disjunction of conjoined segments, for at late anaphase (fig. 49) and early telophase even the short arm of X′ may have its chromatids separated. This is an important and relevant point, for the short arm of X′ has never been observed to conjoin with Y′.

Furthermore, a cross configuration has never been observed at the region of conjunction in this bivalent. This fact requires an emendation of the simple assumption that a single, subterminal chiasma is involved. Namely, it would have to be asserted that the portions of the homologs distal to the assumed chiasma remain paired over a sizeable length of chromosome at the very time (late diplotene through metaphase) the proximal three-fourths of the homologs evince no mutual attraction. Such an admission would in itself remove the theoretical requirement for the postulated chiasma. If the distal fourths of the homologs can remain conjoined through metaphase without chiasmata, then segregation is ensured. The disjunction figures actually found at anaphase are those to be expected in such an event. Accordingly, if the chiasmatype hypothesis is not to be modified by improbable subsidiary qualifications, then the bivalent must be interpreted as either conjoined by multiple chiasmata or by some mechanism other than chiasmata.

Assumption of an even number of randomly disposed chiasmata within the segment, or of an odd number of such multiple chiasmata, awakens the same sort of difficulty as the apparently invalid postulate of a single chiasma. The remaining chiasmatype interpretation is that conjunction in this bivalent is

brought about solely by reciprocal chiasmata as envisioned by DARLINGTON (1934a, 1934b, 1935, 1937, 1939b) for the sex chromosomes of male Drosophila. However, this assumption is not as simple as it appears. (Its validity as a hypothesis accounting for the conjunction of sex chromosomes in Drosophila will be examined in another section of this paper.) Unless the two-strand doubles involved in this hypothesis are further specified to have twists (that is, "chiasma direction") of the crossover chromatids reversed in direction and passing through approximately 180° at the two levels of exchange and unless sister strand relational coiling is absent between the loci of exchange, then chromatid locks and chromosome locks (terminology of SAX 1936) should occur. The reciprocal chiasmata would have to be entirely of the above restricted class because neither type of lock has been found, although both kinds would give easily recognizable configurations at anaphase. But the assumption that all the rod chromosome bivalents are of the requisite class implies the simultaneous existence of absolute and negative chromatid interference, localization of the chiasmata, and a restriction of relational coiling. That no line of separation corresponding with the rift between sister chromatids is laterally visible between the conjoined segments at metaphase affords but scant direct evidence that we are not dealing with the specified reciprocal exchange. But the fact that the paired segments do not form an open loop visible in polar view completes the vitiation of this already hopelessly complicated hypothesis, for further assumptions would have to be manufactured to account for the lack of such a loop.

Moreover, the pairing mechanism of the sex bivalent of Olfersia described above (page 546) and the pairing of the autosomes of Melophagus (COOPER 1941) make it obvious that conjunction of restricted chromosome segments may occur without benefit of chiasmata. The assumption of reciprocal chiasmata in the rod chromosome bivalent of Olfersia therefore falls of its own weight, and we may conclude that here, as in the cases mentioned above, we are dealing with what may be descriptively termed a "conjunctive segment" free of chiasmata. Comparison with the V chromosome bivalent adds further support to this conclusion.

Expecially striking in the case of the V chromosome bivalent is the uncomplicated separation of the proximal association in the loop of the long arm. The points of conjunction merely part from one another, there being no visible exchange of chromatids as would have to occur were a single chiasma the mode of conjunction at this locus. Accordingly we are reduced once again to a choice between postulating a reciprocal exchange of specified architecture, or granting the occurrence of conjunction without chiasmata. Furthermore five chiasmata, two pairs of reciprocal exchanges and a terminalized single exchange are required to account for the anaphasic disjunction configurations of the short arm association and long arm loop purely on the chiasmatype hypothesis. Since the arguments given above for the rod chromosome bivalent are all applicable to this case as well, the simplest conclusion is that meiotic conjunction is similar in mechanism in both of the large autosomes and in the sex chromosomes. It is therefore concluded that in the male Olfersia segregation is guar-

anteed for autosomes and sex chromosomes alike by a conjunctive mechanism which does not involve chiasma formation.

THE RECIPROCAL CHIASMATA HYPOTHESIS OF SEX CHROMOSOME CONJUNCTION

The metaphase pairing of the sex chromosomes in the Drosophila male presents a problem which is not only interesting in itself, but also bears directly on the meiotic mechanism in general. DARLINGTON (1931a, to date) has put forth the hypothesis that the X and Y chromosomes in Drosophila males are conjoined at meiosis through invariate, reciprocal chiasmata which are for the most part genetically undemonstrable. The argument of this hypothesis, to be treated in detail below, rests chiefly on the observation that the X and Y chromosomes form an intimate interstitial union for a short distance of their lengths. This fact loses much of its significance for DARLINGTON'S hypothesis in view of the findings reported above for Olfersia, as well as those for Melophagus (COOPER 1941). These new data are entirely consistent with the view that meiotic conjunction of homologous chromosomes in male Diptera may be brought about by intimate association of small regions devoid of chiasmata. Accordingly it is necessary to reexamine the premises and evidence on which the reciprocal chiasmata hypothesis is based.

The chiasmatype hypothesis, first propounded by JANSSENS (1909) and further elaborated by WILSON and MORGAN (1920), held that the chiasmata are the direct consequences of genetic crossing over. It is DARLINGTON'S noteworthy distinction that he more than any other, through a series of brilliant observations and inductions (1930, 1931b) brought this hypothesis to its present status as one of the underlying theories of cytogenetics. Coincidentally with his development of the chiasmatype theory, DARLINGTON propounded (1929) a new hypothesis of metaphase pairing at meiosis. Since at diakinesis and first metaphase, in the forms he studied, chromatids are held together only in pairs, and chromosomes are held together only by means of exchanges of partners (that is, chiasmata) among these pairs of chromatids, DARLINGTON drew the conclusion that only chiasmata provide a mechanism for conjunction of homologs after pachytene. The well known fact that males of the genus Drosophila do not show genetic crossing over would seem to negate the generality of this subsidiary hypothesis. DARLINGTON (1931a), however, argued that since all forms satisfactory for chromosome study have their bivalents conjoined at first metaphase by chiasmata, the Drosophila male alike must have conjunction through chiasmata. Whereas the chiasmata (as inferred from crossing over data) in the female of Drosophila are distributed over the length of the chromosomes, those of the male, where interstitial, must be reciprocal and localized in genetically neutral segments of the chromosomes—namely, in the vicinity of the kinetochores. Thus autosomes and sex chromosomes alike were held by DARLINGTON to be conjoined by reciprocal chiasmata of such disposition that crossing over would be, for the most part, genetically undetectable. Cytological evidence in support of this interpretation was notably lacking at the time, but to certain of STEVEN'S (1908) figures of Drosoph-

ila DARLINGTON could give an interpretation compatible with his *ad hoc* hypothesis. It is true that METZ' (1926) figures of the autosomes of *Drosophila willistoni* and *D. pseudoobscura* (=*obscura* of METZ) were in obvious disagreement with DARLINGTON's hypothesis, but these observations DARLINGTON held less decisive than those of STEVENS.

Nevertheless METZ proved to be correct, a fact which both DARLINGTON (1934a) and DOBZHANSKY (1934) later affirmed. The autosomes of *Drosophila pseudoobscura* form bivalents which at metaphase have their kinetochores directed to opposite poles, but along their distal lengths the four chromatids are paired parallel to one another. A clear space customarily shows between the parallel limbs of the two chromosomes of each autosomal bivalent. On the other hand, the sex chromosomes possess a joint interstitial connection for a short segment of their lengths. The contrast between the two types or bivalents led DARLINGTON (1934a) to conclude that the autosomes are devoid of chiasmata and conjoined through specially exaggerated forces of somatic pairing (compare STEVENS 1908; METZ 1916; METZ and NONIDEZ 1921) whereas the sex chromosomes conjoin by reciprocal chiasmata as he had earlier postulated. The minimum specific assumptions of the reciprocal chiasmata hypothesis for conjunction of the sex chromosomes in male Drosophila appear to be the following:[5] (1) the chiasma hypothesis of metaphase pairing is valid for the sex chromosomes of the Drosophila male, despite the fact that it is not so for the autosomes; (2) chiasmata conjoining the inert homologous regions of the sex chromosomes are regularly present at meiosis in the male (see 3b *et seq.* below); (3) genotypic control guarantees in the male: (a) the complete suppression of all meiotic crossing over between autosomes, (b) the almost invariable production of two and only two chiasmata per sex bivalent, (c) an absolute negative chromatid interference in the homologous inert regions of the sex chromosomes so that if two chiasmata are formed, they are without exception reciprocal, (d) a reversal of chiasma direction at the second chiasma (see p. 553), (e) the suppression of sister chromatid coiling between chiasmata (see p. 553).

It is clear that such an elaborate hypothesis concerning the mechanism of sex chromosome conjunction is required only if assumptions (1) and (2) are valid. What appears to be the principal genetic and cytological evidence relevant to these two assumptions will now be examined.

Consideration of the validity of the chiasma hypothesis of metaphase pairing for Drosophila.—GOWEN (1928, 1933) has shown that there is a high rate of non-disjunction correlated with the almost complete suppression of crossing over in female *Drosophila melanogaster* homozygous for *c3G*. At first thought this might appear as strong support for DARLINGTON's (1929) chiasma hypothesis of metaphase pairing, but in fact there are two conditional points which must be stressed and which allow of alternative interpretation. First, the time of action of *c3G* appears to be at meiosis (GOWEN), but not necessarily at the time of crossing over (chiasma formation). For example, *c3G* may act by sup-

[5] Relevant discussions or explicit treatment of most of these assumptions will be found in DARLINGTON 1931a, 1934a, 1934b, 1935, 1937, 1939b.

pressing a necessary antecedent condition to crossing over. If *c3G* shortens the time available for leptotene pairing so that meiotic synapsis is rarely normal in the female, then the consequent non-disjunction would at most indicate only a need for synapsis in the female rather than an absolute requirement of chiasmata for bivalent conjunction and subsequent regular disjunction. Second, while crossing over (hence chiasma formation) is virtually completely suppressed and there is attendant non-disjunction, maturation nevertheless favors the production of normal gametes. There is a considerably greater number of normal haploid eggs (about 18 per cent more) formed than can be accounted for by random distribution of the chromosomes alone. Hence, since homozygous *c3G* does not cause non-disjunction in the male of either sex chromosomes or autosomes (GOWEN 1928, 1933; DARLINGTON 1934a), it seems reasonable to suppose there is some meiotic factor common to male and female meioses which tends to guarantee some measure of pairing and normal segregation in spite of suppression of crossing over. That disjunction of the sex chromosomes in the male is totally unaffected by homozygous *c3G* strongly suggests that X and Y do not require chiasmata for conjunction and hence segregation. DARLINGTON (1931a, 1934a, 1937), however, prefers to suppose that *c3G* suppresses both pairing and crossing over, but that in the male genotypic control (assumption 3 above) inhibits the anticipated effects of *c3G* on crossing over in the sex chromosomes as well as on the simple, non-chiasma pairing of the autosomes of the male.

Be that as it may, it is now certain that crossing over between sex chromosomes in the female is not always required for their normal segregation. STURTEVANT and BEADLE (1936) found that in In(1)dl-49/+ females about half of the sex chromosome tetrads underwent no exchange. Despite this gross failure of detectable crossing over, no matroclinous female exceptions appeared among 3,238 daughters. Although BROWN's (1940) observations show crossing over to be very infrequent in the inert region, it might nevertheless be contended that undetectable exchanges in the inert regions had been responsible for the resulting normal disjunction. However, STURTEVANT and BEADLE showed this to be implausible, for heterozygous inversions upsetting homologies within the inert regions themselves (In(1)Df(*bb*), In(1)*sc-8*, In(1)Df(*sc-8*)) result in considerable numbers of non-crossover tetrads and yet fail to increase the matroclinous female exceptions above the normal rate. Crossing over is therefore not essential for normal disjunction of the sex chromosomes in the female of *Drosophila melanogaster*.[6] Since crossing over (hence chiasmata) is not a prerequisite to regular disjunction of the sex chromosomes in female Drosophila, or of the autosomes in Drosophila males, it is not likely that crossing over (hence chiasmata) is necessary for metaphase pairing of X and Y in the male.

Accordingly assumption (1) that the chiasmata hypothesis of metaphase pairing is valid for the sex chromosomes in male Drosophila may be concluded to be unsupported and not required by genetic data.

[6] Experiments by the author, shortly to be published, demonstrate that exchanges in the small right arm of the X cannot be held accountable for normal segregation in these experiments.

Consideration of genetic evidence relative to the regular occurrence of crossing over at meiosis between the sex chromosomes of the male Drosophila.—There is no longer any doubt that X and Y can crossover in the male, for this has been shown by the work of STERN (1929, 1936), PHILIP (1934, 1935), STERN and DOAN (1936) and NEUHAUS (1937). Excepting PHILIP's data for the moment, and STERN's (1936) instances of crossing over in somatic cells, the remaining cases show detectable exchanges between X and Y to occur with a frequency of roughly $2\text{–}8\times10^{-4}$ in the male. Furthermore, crossovers tend to occur in small clusters in the progeny of individual males suggesting, as STERN and DOAN point out, that the exchanges occur not at meiosis but probably during gonial divisions.[7] Discovery has been made of similar spontaneous but exceedingly rare crossing over between autosomes in males of *Drosophila melanogaster* (MULLER 1916; BRIDGES and MORGAN 1919; PATTERSON and SUCHE 1934), *D. simulans* (STURTEVANT 1929), *D. virilis* (KIKKAWA 1933) and *D. ananassae* (MORIWAKI 1937). MULLER's case requires the original crossover to have occurred in an embryonic cell[8]. As BRIDGES and MORGAN point out, if the 43 apparent crossovers between purple and vestigial in a total of 573 offspring which they obtained from a single male are truly crossovers, then, as with MULLER's case, their occurrence was probably due to an exchange which occurred in a cell far antecedent to normal meiosis. Likewise crossovers between homologous autosomes which have been induced, by heat or X-rays, in Drosophila males tend to occur rarely, in clusters from individual males, and with unequal reciprocal classes (review in WHITTINGHILL 1937). Thus autosomal as well as X-Y crossing over occurs in the male, but both types of crossing over probably occur not at meiosis but in gonial or gonial-precursor cells—that is, they are due to mitotic crossing over (WHITTINGHILL 1938). Since the autosomes of those Drosophila males which have been studied do not show any evidence of chiasmata at meiosis,[9] the occurrence of rare crossing over between the sex chromosomes in males cannot be taken as implying the occurrence of chiasmata in the sex chromosomes at meiosis of spermatogenesis. Expressed otherwise, the capacity for regular crossing over at spermatogenesis is not prerequisite to gonial or somatic crossing over in the male. Nevertheless the structural changes resulting from the X-Y crossovers mentioned above have been presented by DARLINGTON (1937) as support for his assumptions. It is clear from what has just been said that they furnish no such support.

[7] WHITTINGHILL's (1937) suggestion that spermatid multiplication could also account for clustering of rare crossovers is improbable, as he himself appears to feel. Such an explanation is not applicable to the female for which only embryonic or oogonial crossing over may be invoked to account for similar data.

[8] STURTEVANT (personal communication) believes MULLER's case is more likely a consequence of mutation than of crossing over.

[9] See figures of *D. funebris* (METZ 1926); *D. melanogaster* (STEVENS 1908; METZ 1926; GUYÉNOT and NAVILLE 1929; ZUITIN 1929; HUETTNER 1930; WOSKRESSENSKY and SCHEREMETJEVA 1930); *D. miranda* (DOBZHANSKY 1935, 1937; KOLLER 1939); *D. pseudoobscura* (METZ 1926; KOLLER and TOWNSON 1933; DARLINGTON 1934a; DOBZHANSKY 1934; STURTEVANT and DOBZHANSKY 1936); *D. virilis* (METZ 1926); *D. willistoni* (METZ 1926).

PHILIP (1934, 1935) maintains that her cytogenetic experiments demonstrate reciprocal exchanges to occur between X and Y chromosomes in male *Drosophila melanogaster* with a frequency of approximately $3\text{–}7\times10^{-4}$ (that is, of the same order of magnitude as apparent single exchanges in the male). Since her work was done before NEUHAUS' (1936b, 1939) findings to the contrary, PHILIP assumed the normal allele of bobbed to be located in the long arm of the Y chromosome (Y^L), that crossing over between Y^L and X may take place to the left of bobbed, and lastly that Y crosses over with X for the most part, if not exclusively, through exchanges in Y^L. The latter assumption was also made by DARLINGTON (1931a) who, to account for the formation of STERN's (1929) $\widehat{XY}'$ ($=X\widehat{Y^L}$) chromosome in the male, held that it arose through an "inverted chiasma" between X and Y^L. KAUFMANN (1933) quite properly pointed out that such an aberrant exchange would nevertheless fail to give $\widehat{XY}'$. DARLINGTON's escape from the dilemma was not to accept the obvious alternative suggested by KAUFMANN—that is, that an ordinary exchange between Y^s and X may give rise directly to $\widehat{XY}'$—but to shelter his initial assumption under an additional one. He now postulated (1934b) crossing over to occur within and between the "attachment chromomeres" (=kinetochores) of X and Y which were further assumed to have paired in an inverted way! PHILIP (1935) avoided such a complicated explanation by assuming that the small arm of X to the right of the kinetochore (KAUFMAN, 1934; PROKOFIEWA 1935) is homologous with part of Y^L and inverted with respect to Y^L. If this were so, a simple crossover between X^R and Y^L would give $\widehat{XY}'$ (PHILIP terms this an "inverted crossover"). While PHILIP's suggestion merits consideration, NEUHAUS (1937) has given good reason to believe that in the male effectively single exchanges are far more frequent between Y^s and X than Y^L and X.

With regard to PHILIP's demonstration of double crossing over between X and Y, some hesitance must be felt in accepting these data for it is not clear from her unfortunately ambiguous statements that the cytological and genetic tests were sufficiently rigorous to justify the conclusions she has drawn. But if it is assumed that she has indeed discovered double reciprocal exchanges between X and Y in the male, it is by no means shown that these followed from meiotic crossing over. There is no mention of whether or not the rare exceptions tended to occur in small clusters, but as BAUER (1937) points out, the numerical inequalities between her experiments suggest that PHILIP's crossovers are the results of gonial exchanges. That double "crossing over" may occur mitotically has already been made probable by BRIDGES and MORGAN's (1919) "doubles" between vermilion and sable which appeared in every offspring of a single female, as well as STERN's (1936) analysis of somatic crossing over. Although DARLINGTON (1935, 1937, 1939b) and PHILIP both consider her data as strong support for the reciprocal chiasmata hypothesis, such detection of rare crossovers between X and Y in the male can not be brought forward as proof of chiasmata at meiosis, as BAUER (1939) has already pointed out.

One additional genetic problem concerns the regular disjunction of Y from $\widehat{XX}$ in females. It is DARLINGTON's (1937) assumption that the genotypic determination of reciprocal chiasmata between X and Y does not occur in the female. In this event, if crossing over is necessary for regular disjunction, as DARLINGTON maintains, the rate of detachments of $\widehat{XX}$ should be very high with resultant formation of $\widehat{XY}^L$ and $\widehat{XY}^s$ in large and approximately equal numbers. Such detachments of $\widehat{XX}$ owing to crossing over of $\widehat{XX}$ with Y are rare and occur about as frequently as does gonial crossing over between X Y in the male, having a frequency of approximately 6.6×10^{-4} (KAUFMANN 1933; NEUHAUS 1936b; etc.) Thus DARLINGTON is faced with either granting that regular disjunction may occur without benefit of chiasmata, as has been shown probable on other grounds, or supposing that the efficient genotypic mechanism resides within the Y chromosome itself and does cause reciprocal chiasmata formation between Y and X in the female. Briefly, the Y must be conceived as almost invariably eliciting reciprocal exchanges with the X at meiosis whether in male or female. This seems very unlikely.

It may be concluded, therefore, that no genetic data are available which require assumption (2) that chiasmata are regularly formed between the homologous inert regions of X and Y in the male. Indeed there is no satisfactory genetic evidence that chiasmata ever occur between the sex chromosomes of the male at meiosis.

Consideration of the validity of the cytological evidence for reciprocal chiasmata in the Drosophila male.—Although, as has been shown above, the genetic data neither require nor support the primary assumptions of the reciprocal chiasmata hypothesis, it remains possible that cytological phenomena make this hypothesis necessary. Consideration of the cytological literature does not show this to be the case.

Shortly after the publication of DARLINGTON's (1931a) initial hypothesis of reciprocal chiasmata in both autosomes and sex chromosomes of the male Drosophila, KOLLER and TOWNSON (1933) recorded the occurrence of possible chiasmata in both the autosomes and sex chromosomes of *Drosophila pseudoobscura* males. The later work of DARLINGTON (1934a) and DOBZHANSKY (1934) showed the autosomes to be devoid of chiasmata, and the evidence for chiasmata in the sex chromosomes to be purely indirect. KOLLER's (1939) recent account of reciprocal chiasmata between X^1 and Y in *Drosophila miranda* males must also be discounted, for the author states that the X^1 X^2 Y chromosomes "are too small to allow a critical study." Indeed, KOLLER could not even decide whether a sex chromosome trivalent was formed in the interracial hybrids he studied. Accordingly the observations on the sex chromosome bivalent of male *Drosophila pseudoobscura*, which have been assembled by DARLINGTON (1934a), constitute the primary cytological data upon which the reciprocal chiasmata hypothesis rests. The actually visible mechanical relations and behavior of the sex chromosome bivalents described by DARLINGTON have been corroborated by DOBZHANSKY (1934) and STURTEVANT and DOBZHANSKY (1936) for *Drosophila pseudoobscura* itself, and seemingly iden-

tical properties have been discovered in the autosomes of Melophagus (COOPER 1941) and autosomes and sex chromosomes of Olfersia (present paper). The merits of DOBZHANSKY's claim (*vide* COOPER 1941) that he has cytological proof of reciprocal chiasmata between X and Y in *Drosophila duncani* can be assessed only after publication of the data.

The cytological evidence for reciprocal chiasmata in the sex chromosomes of *Drosophila pseudoobscura* are catalogued by DARLINGTON (1934a, 1937, 1939b) much as follows, and the quotations are from his work. Each item of this list is accompanied by my judgment of its significance. All the points he gives involve directly or indirectly a comparison of sex chromosomes with autosomes. It is by no means clear that his contrast of the sex chromosome bivalent with the autosomes may legitimately be employed to argue the existence of chiasmata in the former, for DARLINGTON (1937, p. 372) points out that "*the autosome pairs . . . would be incapable of showing chiasma formation, even if they had crossed over or not*, because their four chromatids are equally attracted to one another, lying equally parallel at diakinesis" (italics mine). However, there is no difficulty in judging whether the evidence presented justifies the conclusion that chiasmata are present.

(a) *At diakinesis the autosomes are associated at their kinetochores, whereas the sex chromosomes are associated not at the kinetochores, but at interstitial loci near their kinetochores.* There is nothing here that suggests or denies the existence of chiasmata. DARLINGTON (1934b) has already committed himself to both the belief that (a) the sex chromosomes can pair at their kinetochores and (b) that crossing over may occur within the "attachment chromomere" itself. Furthermore, DARLINGTON (1934a, p. 97) states that the proximal and distal ends of the autosomal bivalents are not distinguishable at this stage.

(b) *The X and Y are only in contact over a short portion of their length and this is evidently not the whole of the homologous segment.* The cases of Olfersia (*vide ut supra*) and Melophagus (COOPER 1941) show such localized conjunction to be possible between homologous chromosomes, both interstitially and terminally, without involving chiasmata. By itself, this feature of sex chromosome conjunction is not *a priori* evidence for chiasmata.

(c) *In the restricted region of conjunction (b above) the sex chromosomes "come into closer contact than the autosomes, sometimes, and at other times rather less close—no visible connection joining them."* This observation concerning some sex chromosome bivalents which are less closely associated than the autosomes awakens the suspicion that conjunction of the sex chromosomes in *Drosophila pseudoobscura* may occur much as described in Olfersia above.[10] STURTEVANT and DOBZHANSKY (1936), in considering the action of "sex ratio" in *Drosophila pseudoobscura*, point out that the failure of X and Y to form a bivalent may be owing to persistence of an initially separate state of the two chromosomes. DARLINGTON (1934a, pp. 98, 100, 109) states that at

[10] KOLLER and TOWNSON (1933, p. 134) remark that X and Y are seen to pair during condensation in some spermatocytes of *D. pseudoobscura*. Their figures are too poor to give weight to this observation.

diakinesis there are some sex chromosome pairs between the two chromosomes of which *no* connection is visible. This he interprets (p. 109) as a "lateral chiasma" which is not to be expected to give a visible connection between X and Y. Such a chiasma could arise only by terminalization, but DARLINGTON has shown that there is no repulsion between sex chromosome kinetochores in diakinesis. The required mechanism of terminalization is therefore in abeyance at this stage. Perhaps the observed loose association of X and Y may be accounted for by a chiasma interpretation. Nevertheless it cannot be marshalled as evidence demanding the occurrence of chiasma in the sex bivalent.

(d) *The connection (locus of conjunction) may be on either side of the kinetochore, but is never on both sides. "It therefore shows the variation and the interference characteristics of chiasma formation."* If homologous loci exist in both arms of Y, but in only one arm of X, this observation should follow whether chiasmata are the mechanism of conjunction or not. This observation cannot be considered as proof, or indeed evidence, for chiasmata between the sex chromosomes. Similar observations are described above for the sex chromosomes of Olfersia where chiasmata are clearly not involved.

(e) *"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."* This observation affords no proof of chiasmata. Tension develops between the spindle attachments of the autosomes and their paired regions as well (see figures in DARLINGTON 1934a, DOBZHANSKY 1934, STURTEVANT and DOBZHANSKY 1936, etc.), as DOBZHANSKY (1934) has stated. Furthermore the supposed suddenness of separation may be questioned. It should be noted that the sex bivalent has only a very short length to disjoin relative to that of the autosomes, and perhaps only for this reason appears to disjoin suddenly. Lastly, these observations are paralleled by those on the autosomes of Melophagus and all large chromosomes of Olfersia (this paper) where an assumption of chiasmata is unwarranted.

(f) *The X and Y are unchanged when they separate at anaphase (that is, they preserve their respective identities).* This is likewise a characteristic of the autosomal bivalent of male Drosophila between the respective chromosomes of which no chiasmata need be inferred. In fact any mode of conjunction not involving crossing over will give this result, whereas only one type of chiasma production, namely reciprocal pairs of chiasmata, can account for these results. While explicable on the latter hypothesis, it cannot be considered as evidence which requires this hypothesis.

(g) *"The lack of time coordination between spindle, autosomes, and sex chromosomes* (that is, the sex chromosomes may precede or lag—K.W.C.) *found also in D. melanogaster, is an indication that two independent processes of development are at work in the same nucleus."* This certainly cannot be taken as evidence for the occurrence of reciprocal chiasmata in the sex chromosomes. Were the argument pursued to its logical end, each chromosome would have to be considered conjoined by a unique mechanism, for all the bivalents appear to vary in their timing of anaphasic disjunction.

(h) "*The sex chromosomes fail to pair in a proportion of the cases, although the autosomes are regularly paired.*" Failure of any conjunctive mechanism should give rise to non-disjunction. Hence this is not evidence for the occurrence of chiasmata between X and Y. Furthermore the figures (DARLINGTON 1934a, 28 *et seq.*) in support of this statement may be given alternative interpretations. For example, none of these figures shows both sex chromosomes to have gone to the same pole, and figure 29 of supposed non-disjunction compares very favorably with figure 22 purporting to show precocity of the sex chromosomes.

There are additional cytological considerations (see pp. 552–554) which make the reciprocal chiasmata hypothesis difficult to maintain and require assumptions (3c–e) above. Furthermore, the characteristic loop of the reciprocal chiasmata and the clear rift between sister chromatids (DARLINGTON 1937, fig. 115, C3; fig. 36B) have never been found in the case of fly sex chromosome bivalents. It may be argued that these criteria are below the limits of resolution, in which event the hypothesis cannot be put to direct proof.

Thus it may be concluded that there is no genetic or cytological evidence known which requires the assumption of reciprocal chiasmata between X and Y in male Drosophila or the male of any other fly. Indeed some of the evidence available is directly opposed to such a hypothesis.

DISCUSSION

Comparison of meiotic conjunction of the chromosomes of Olfersia, the autosomes of Melophagus, and the sex chromosomes of Drosophila suggests a common conjunctive mechanism which does not directly involve any elements of either chiasmatype theory or the chiasma hypothesis of metaphase pairing. But until further data are accumulated, little more than a descriptive analysis can be given.

The chromosomes under discussion behave as though they are provided with one or more relatively short "conjunctive segments" which are responsible for cohesion of homologous chromosomes in bivalents and hence necessary for segregation at meiosis. These segments may also be responsible for the initial coming together of homologs which inaugurates bivalent formation at meiosis, but this does not necessarily follow from what is known. In Melophagus and Olfersia the conjunctive segments appear to act as loci from which the factor(s) customarily described as "forces of somatic pairing" emanate and operate during ordinary mitotic divisions. It is not known whether such strict localization of somatic pairing forces occurs also in the chromosomes of Drosophila,[11] but it is clear that two interstitial regions in the Y and at least one in the X of *D. pseudoobscura* males behave as though they alone possess a capacity for cohering at meiosis. On the other hand the autosomes of Drosophila appear to succeed in conjoining as bivalents at meiosis without such an apparently intimate contact as that provided by the localized conjunctive segment in the cases under discussion. Whatever the mechanism may be which holds these

[11] See Addendum, page 568.

autosomes together, the simplest interpretation seems to be that the same mechanism operates in all the cases considered, but that the means of actual cohesion is restricted and localized by some regional specialization of those chromosomes which are endowed with a conjunctive locus. Now if this is so, the relatively small segment must be capable of producing a cohesion of homologs fully as effective as that provided by the entire length of chromosome which participates in the conjunctive effort of an autosome of the Drosophila male. Such a consideration perhaps accounts for the fact that homologs appear to be actually locally united when they are paired by means of conjunctive segments.

Such restricted loci which do not cross over but which do bind homologs together as bivalents in the male nicely account for such genetical and cytological properties of the sex chromosomes of Drosophila as have been discussed in the preceding section. Furthermore such a mechanism is in harmony with additional data that militate against any hypothesis involving regular reciprocal crossing over between the inert regions of X and Y. "In(1)Df*bb*" is an X chromosome of *Drosophila melanogaster* which has lost about one-third its length, and this from the inert region. In spite of the extensive loss of inert material, only about three percent non-disjunction of X^{bb-df} and Y occurs in the male (SIVERTZEV-DOBZHANSKY and DOBZHANSKY 1933). Presumably loss of most of the inert region has not resulted in considerable loss of efficiency of the conjunctive segment, although the defect in the X chromosome is of such magnitude that it should prohibit reciprocal chiasmata formation. GERSHENSON (1933) has described another bobbed-deficient X which, while giving high non-disjunction of X and Y in the male of *D. melanogaster*, does not result in purely random segregation of X and Y, there being a pronounced tendency toward regular disjunction. Here it may be supposed that the loss from the inert region includes a relatively large one from the hypothetical conjunctive segment, but such that enough remains to preclude absolutely random segregation. On the reciprocal chiasmata hypothesis disjunction should remain normal, or produce many single exchanges between X and Y, or become purely random.

As DARLINGTON (1937, p. 392) points out, the origin of the homologous inert segments remains unexplained on the basis of the reciprocal chiasmata hypothesis. If, however, the dipteran ancestral types did have their meiotic segregation conditioned by chiasmata in both male and female, then with reduction of crossing over in the male there must have been selection for efficient mechanisms capable of supplanting crossing over in the male, or for rigid localization of simple crossing over. Both appear to have evolved in the Diptera, but a non-chiasmate mechanism seems to be the dominant if not the only mode of conjunction in male muscoids and Pupipara. Such a mechanism results in maintaining the Y in a continually heterozygous state. This, as MULLER (1914, 1918; MULLER and PAINTER 1932) first pointed out, means that Y is largely insulated from the action of natural selection and may accumulate degenerative mutations to inertness.

If, as is suspected, the "conjunctive segment" proves to be a non-genic

organelle of the chromosome, like the kinetochore complex, matrix, nucleolus organizer, etc., then the name "collochore" is suggested for it. No implication as to the mechanism of action is to be considered implied by the literal meaning of the Greek compound. As appears to be the case for both the kinetochore and nucleolus organizer, the collochore seems to be able to function after it has been fractured.

SUMMARY AND CONCLUSIONS

In the male of the fly *Olfersia bisulcata* the diploid number of chromosomes is eight. The haploid chromosome set comprises a dot-like, a rod-like, and a V-shaped autosome, as well as either a large submedian X′ or a smaller nearly median Y′.

At the spermatogonial mid-prophase somatic pairing is restricted to a distal region in the rod-shaped autosomes, and to one terminal and two interstitial regions in the V-shaped autosomes; at points other than these "conjunctive segments" the homologs are either indifferent to or repel one another. A remarkable feature of the somatic pairing of the V-shaped autosomes is the fact that they twist about one another only at their interstitial pairing loci.

Although the sex chromosomes show no tendency for somatic pairing at gonial prophase, they as regularly lie adjacent to one another at metaphase as do homologous autosomes (that is, nearly always).

At diplotene and early diakinesis the bivalent configurations simulate their corresponding somatically paired homologs of gonial prophase. Thus the rod-shaped autosomes are conjoined distally only, and the V-shaped autosomes are conjoined at one terminal and two interstitial loci. The sex chromosomes are totally separate from each other.

At diplotene the homologs of the V-shaped autosome bivalent are twisted about one another at the interstitial pairing segments. Accordingly this bivalent superficially appears to possess at least three chiasmata. With approach to metaphase I, however, the twists are largely undone and the disjunction figures—coupled with antecedent phenomena—show this bivalent as well as the others to be devoid of chiasmata.

The sex chromosomes, which may be a nuclear diameter apart at diplotene, come together and ankylose in a short interstitial region before the close of diakinesis. Although the resulting bivalent behaves and appears thereafter identical in structure with the sex chromosome bivalent of the male *Drosophila pseudoobscura*, clearly it cannot be provided with chiasmata unless, as is very improbable, condensed chromosomes can undergo crossing over.

The autosomal and sex chromosomal bivalents thus owe their paired condition at first metaphase not to the formation of chiasmata, but to the possession of short regions of their lengths ("conjunctive segments") to which appear restricted the means of cohesion as bivalents. Whether these regions are truly anatomical specializations of the chromosomes is undecided.

The phenomena described above throw a wholly new light on the observations of the sex chromosome bivalent of male *Drosophila pseudoobscura* where

reciprocal chiasmata are said by DARLINGTON to be the sole mode of conjunction at first meiotic metaphase. The reciprocal chiasmata hypothesis is accordingly precisely formulated and analysed. Detailed consideration of the cytological and genetical evidence available for Drosophila leads to the conclusion that the reciprocal chiasmata hypothesis is unnecessarily involved and neither required nor supported by the relevant data.

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ADDENDUM

Since this went to press, Philip *et al.* (Nature **154**: 260–262, 1944) state that, unlike the case of the autosomes, somatic pairing of X and Y occurs only at the proximal ends in *Drosophila subobscura*.