# THE ENTIRE COMPOUND AUTOSOMES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Three new unusual compound chromosomes have been synthesized in *Drosophila melanogaster*. They consist of two homologous autosomes joined together in the new order: right arm, left arm, centromere, left arm, right arm, for each of the two major autosomes, and one in which chromosomes *2* and *3* have been combined in the order: right arm of *2*, left arm of *2*, centromere, left arm of *3*, right arm of *3*. The attachments of the autosomal arms were accomplished by obtaining chromosome breaks at or very close to the ends of the left arms of the autosomes such that no essential chromosome material has been removed; the compounds derived from them are therefore referred to as entire compounds. These large chromosomes are recovered in progeny with frequencies lower than expectation partly because of zygote mortality associated with these chromosomes, and partly because of a failure of spermiogenesis.

IN 1916 ROBERTSON reported on the existence, in natural populations of grasshoppers and locusts, of rearrangements involving whole chromosomes; he called these V-shaped chromosomes formed by the combination of two independent rod-shaped chromosomes "compounds." He considered the transition of rod chromosomes to compounds, and *vice versa*, to be of general occurrence, and pointed out that the metaphase configurations of various species of Drosophila, as reported by METZ (1914), show different numbers of rods and V-shaped (*i.e.*, compound) chromosomes, although the total number of arms tended to remain constant from one species to the next. His prediction of the existence of naturally occurring strains of *D. melanogaster* with varying numbers of V's and rods has not been fulfilled, but the essence of his argument has been confirmed in other Drosophila species, for instance, in the *D. virilis* species group, where several different compound chromosomes are known (PATTERSON and STONE 1952).

The term "compound" was introduced into Drosophila nomenclature to describe experimentally produced combinations of two structurally similar arms (NOVITSKI 1954), as well as combinations of arms as structurally dissimilar as the *X* and *Y* chromosomes (LINDSLEY and NOVITSKI 1958). (Mammalian cytologists, on the other hand, have adopted the phrase "Robertsonian translocation" in place of ROBERTSON's own terminology, and use other nomenclature, such as centric fusions, isochromosomes or fusion chromosomes for compounds involving two homologous chromosome arms.)

Here we shall use the word compound in ROBERTSON's original sense, as any new chromosome made up of whole chromosome arms, and extend it to some new and unusual combinations that ROBERTSON could not have anticipated: those in which all the major autosomal arms of *Drosophila melanogaster* are combined into one large V-shaped compound.

Compound chromosomes have proved to be of unusual value, both from the genetic information obtained from their analyses, as well as from their use as tools in miscellaneous genetic experiments with Drosophila. The first compounds were made up of two *X* chromosomes, beginning with L. V. MORGAN's initial discovery of the attached-*X* chromosomes (1922) and followed by MULLER's accidental synthesis of a compound *X* in which the centromere was terminal and the two *X* chromosomes were attached in reverse order (1943). In a more extensive set of syntheses, NOVITSKI (1951) put together two *X* chromosomes in all possible combinations: in metacentric and acrocentric conformation, in reversed and tandem order, and as rings. Those syntheses that involved two *X* chromosomes attached end-to-end, and in which both chromosomes were present without any appreciable duplication or deficiency, were dependent upon the fortuitous appearance of an *X* chromosome, *In(1)EN*, which carried distal heterochromatin that could serve as an attachment point for adding all the essential genetic material (less the centromere) of another *X* chromosome.

Compound chromosomes of the autosomal arms (Figure 1B) have been widely used, with many cases of independent origin, since the pioneering work of RAS-

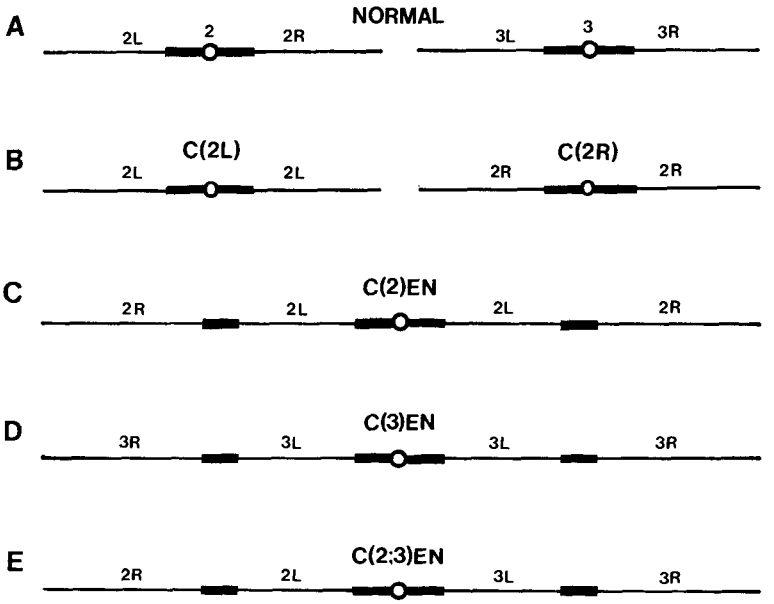


FIGURE 1.—A diagrammatic comparison of the structures of compound autosomes. (A) Normal chromosomes *2* and *3*. (B) Arm compounds of chromosome *2*, *C(2L)* and *C(2R)*. (C) The entire compound of chromosome *2*, *C(2)EN*. (D) The entire compound of chromosome *3*, *C(3)EN*. (E) The compound of chromosomes *2* and *3*, *C(2;3)EN*.

MUSSEN (1960; see also LEWIS 1967). The manufacture of compounds that are made up of two entire homologs, entire autosomes, without significant duplications or deficiencies of autosomal material, depends upon the availability of autosomal arms with heterochromatic segments attached beyond the locus of any essential terminal gene. Since such autosomal attachments have not appeared spontaneously, as they did for the *X* chromosome, it has been necessary to manufacture autosomes with such "terminal" attachment points. This has been done for both chromosomes *2* and *3* and, as a result, it is possible to synthesize chromosomes that consist of (a) two entire second chromosomes joined together, *C(2)EN* (Figure 1C), (b) two third chromosomes together, *C(3)EN* (Figure 1D), and (c) an entire second and an entire third together, *C(2;3)EN* (Figure 1E). This paper concerns the method of manufacture of these chromosomes, together with some of their more interesting genetic and cytological properties.

## PRODUCTION OF AUTOSOMES WITH "TERMINAL" ATTACHMENT POINTS

The attempt to put a heterochromatic region with a genetic marker onto the end of an autosome required a new set of experimental approaches. Such rearrangements, which place a genetic marker distal to all previously known loci and which involve a break so placed that no material can be detected as lost by the most sensitive genetic and cytological (polytene) tests available, will be referred to here as "terminal." We feel that in some of these cases, the break has probably occurred within the telomere region of the chromosome end (NOVITSKI *et al.* 1981). Clearly, the likelihood of finding such a terminal attachment on an autosome after the usual Drosophila radiation experiment, in which chromosomes in the sperm of males are randomly broken, seems fairly remote. In fact, the possibility of a terminal translocation, although reported as the first instance of an induced translocation in Drosophila by MULLER (1929), has now been discounted as an event occurring with any appreciable frequency in the usual experiments with irradiated sperm in Drosophila (ROBERTS 1975).

The production of terminal attachments with a workable frequency depends upon the success of a different experimental approach. It has been shown that the chromosomes in the oocytes of Drosophila form a chromocentral type of configuration (DÄVRING and SUNNER 1973, 1976, 1977; NOKKOLA and PURO 1976; PURO and NOKKOLA 1977). MULLER and co-workers (MULLER and HERSKOWITZ 1954; ABRAHAMSON, HERSKOWITZ and MULLER 1954, 1956) have shown that after radiation of Drosophila females, whole-arm translocations or rearrangements, with breaks primarily (but not necessarily) in the heterochromatic regions, are not at all uncommon, the common example being the detachment of attached-*X* chromosomes after induced rearrangement (or pseudoexchange) between the heterochromatin of the *X* and the *Y* chromosomes. This basic observation has been repeated with many variations by others working not only with compound-*X* chromosomes, but with compounds of autosomal arms as well. There is also some reason to suspect that the ends of the chromosomes may bear some degree of genetic homology with heterochromatin in the chromocentral regions, since chromosome tips are often associated with the heterochromatic regions of the polytene chromosomes (KAUFMANN and GAY 1969).

The spontaneous occurrence of *X* chromosome rings on at least two different occasions (L. V. MORGAN 1933; R. D. BOCHE, unpublished; see SCHULTZ and CATCHESIDE 1937) suggests that, in germ cells, telomeres may at times be associated with centric heterochromatin and undergo exchange. It is not unreasonable to imagine that this occasional association of chromosome tips with heterochromatic regions might in itself lead either to a rearrangement between the two or to a chromosome configuration at interphase or prophase such that randomly occurring breaks would more likely lead to recovered rearrangements that involve these regions.

The genetic scheme to pick up such terminal attachments is based on the following consideration. If chromosome breaks are induced in two different chromosomes and a simple reciprocal translocation is produced, both members of the translocation must ordinarily be recovered

simultaneously in the progeny because the presence of only one of the two broken chromosomes will give rise to an inviable duplication-deficiency zygote. If, on the other hand, a terminal attachment occurs, that single chromosome can be recovered without its reciprocal, provided that the attached piece does not carry any essential euchromatic genes of such an extent as to cause inviability in the heterozygous state. For this purpose, the doubly marked *Y* chromosome ($y^+YB^s$) of BROSSEAU (1958) was put into females along with a compound *X* chromosome homozygous for *y*; these were irradiated with 3,000 r units from a Cobalt-60 source. The progeny were examined for cases where only one of the two markers $y^+$ and *B* was present in the progeny, indicating a breakage of the *Y* chromosome. These rare exceptions, which should include cases of simple rearrangement of the *Y* chromosome with itself, or some nonreciprocal translocation, can be tested for linkage with the major autosomes. In a sample of about a dozen such cases of *Y* chromosome fragmentation, three were found to show linkage with chromosome *2*. Further tests showed that in two cases the marker *B*, and in one case $y^+$, both derived from the *Y* chromosome, were attached to chromosome *2*.

The genetic position of the marker ($y^+$ in one case, *B* in the two others) was readily determined. In all three cases, it proved to be to the left of *al* (aristaless), suggesting a terminal location on the left arm of chromosome *2*. As a further test of the presumptive terminal attachment, these chromosomes were made homozygous on the assumption that if a break were not at the tip, but excluded some essential terminal loci, the homozygote would be inviable or otherwise affected in the homozygous condition. All three cases proved to be viable and fertile in homozygotes of both sexes, except for one that was male sterile.

In addition to these genetic tests, the chromosomes were examined cytologically. Photographs showing the apparent terminal appearance of these attachments in the polytenes have been presented elsewhere (NOVITSKI *et al.* 1981). Having a chromosome *2* with a terminal piece of heterochromatin at the left tip, along with an attached marker, made it possible to attach arms end to end in tandem fashion (Figure 1C). The precise procedure for making *C(2)EN* has been described in some detail earlier (NOVITSKI 1976). *C(2)EN* is illustrated in Figure 2, which shows the polytene configuration (A), as well as the appearance at anaphase (C) and metaphase (D) in ganglion neuroblasts. Note that in these well-stretched polytene examples (A and B), the attachment of the tip of *2L* and the base of *2R* appears to be threadlike, suggesting a failure of this region to polytenize.

## THE CONSTRUCTION OF *C(3)EN*

In order to manufacture a compound chromosome *3*, a combination of the two homologues, *C(3)EN*, it was necessary to obtain a chromosome *3* arm that had some terminal heterochromatin and an attached marker. Such a chromosome appeared in an experiment of BARRY LEIGH of Leiden University after irradiation of male spermatocytes that carried $y^+YB^s$. In this rearrangement, $y^+$ on the short arm was lost, but the *B* mutant on the long arm was retained. *B* was shown to be located to the left of *ru* (roughoid). The assumption that this marker was distal to all essential loci at the tip of the left arm of chromosome *3* is supported by the fact that homozygotes of both males and females are fully viable and the females are fertile, (although the males are sterile). The sterility of the homozygous male may be attributed to the segment of the *Y* translocated to the tip of *3L*, making such males triplicated for that segment. It has been shown that males with three *Y* chromosomes (COOPER 1956) and males with three segments from the long arm of that chromosome (WILLIAMSON and MEIDINGER 1979) are sterile. However, tests for fertility factors in the piece of the *Y* attached to the autosome have proved negative. Examination of the polytene chromosomes indicates that there is no obvious deficiency at the tip of *3L*, and we will assume that in this case, as in the preceding cases, the actual break occurred in the telomere region.

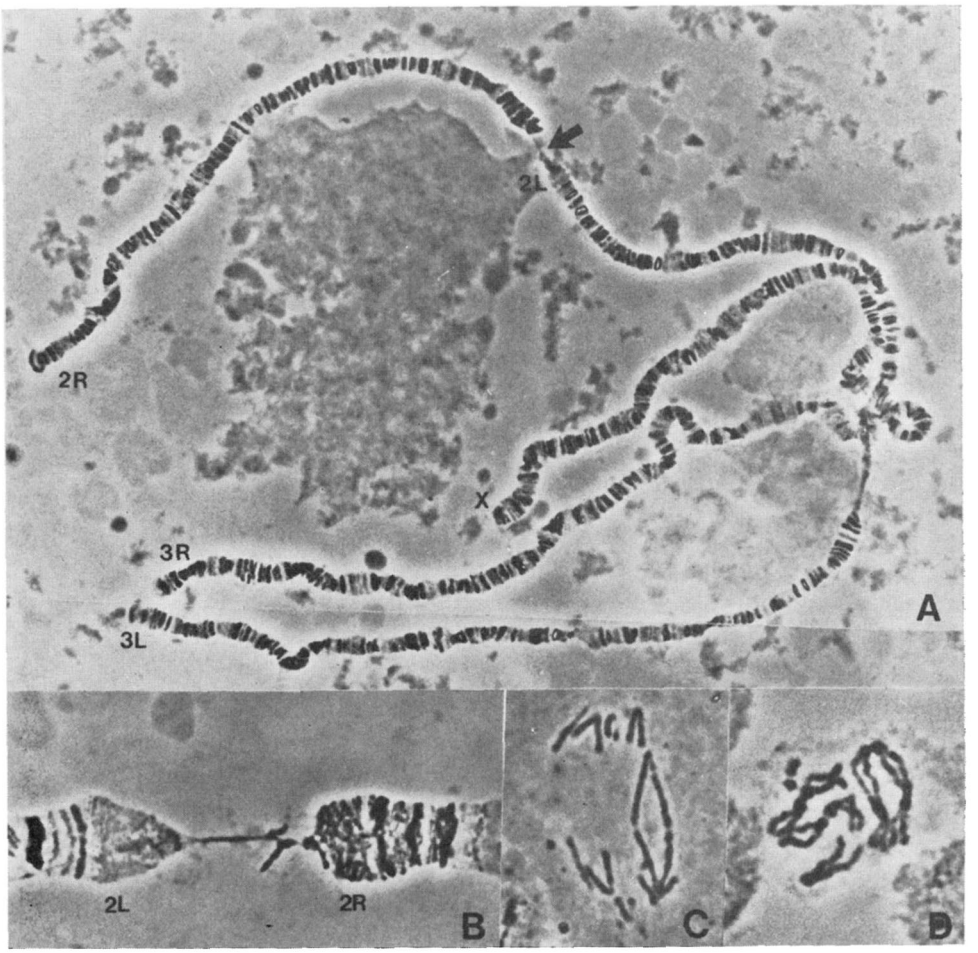


FIGURE 2.—Cytology of *C(2)EN*. (A) A polytene nucleus showing the appearance of the attachment of *2L* and *2R*. (B) An enlargement of the polytene region between the tip of *2L* and base of *2R*, showing the attenuation of the chromosome thread that ties the two chromosomes together. (C) A neuroblast ganglion anaphase with the long entire compound clearly evident. (D) A metaphase of an *XO* cell, with the compound at the right. At this stage the separated chromatids characteristically rejoin medially at the interstitial heterochromatic region.

In order to simplify the procedures for the synthesis of the entire compound third, a stock that consisted of a compound for the right arms of the third, *C(3R)*, with the two left arms free and unattached, *F(3L)*, was made up, using a *C(3L)*; *C(3R)*, *ca* stock (of unknown origin) to provide *C(3R)* and two translocation stocks, *T(3;4)Dubinin* and *T(Y;3)A95* to provide the free left arms. The *F(3L)*; *C(3R)*, *ca* line gives no progeny when mated to individuals with a normal chromosome constitution.

When females carrying the chromosome *3* with the distal attachment and a normal chromosome *3* were irradiated (3,000 r units, Cobalt source) and mated to *F(3L)*;*C(3R)* males, a few diploid progeny were produced. Among these ex-

ceptions were some that obtained, from the mother, a chromosome *3* to which an extra right arm of chromosome 3 had been attached (Figure 3) in place of the distal *B* marker. Such non-*B* exceptions survived because they received from their father a free left arm of chromosome *3*, restoring the normal diploid complement.

In these experiments, a total of eight non-*B* exceptions were recovered. Six carried the desired attachment; the other two proved to carry rings consisting of the entire left arm of chromosome *3* (Figure 4). These rings were recoverable because the male gamete contributed both a *F(3L)* arm and the compound *C(3R)*, thus completing the euploid complement. These rings have been designated *R(3L)EN*, the *EN* indicating that the rearrangement is unusual in that it involves the *EN*(tire) chromosome arm, unlike typical experimentally induced rearrangements that involve only interstitial chromosome breaks. A photograph of this autosomal ring has been presented elsewhere (NOVITSKI and PURO 1978).

It should be noted that the compound *C(3R3L·3R)* is highly unstable (Figure 3B). Crossing over between the *3L* segment of the compound and the free *3L* arm gives rise to a chromosome of normal structure that is favored by nonrandom disjunction. However, such a normal chromosome generated in the female would

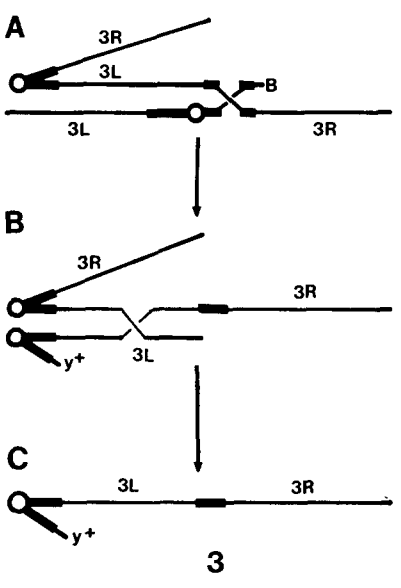


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FIGURE 3.—The manufacture of the chromosome type with *3L* and *3R* arranged in tandem. (A) The combination of a normal metacentric chromosome *3* with the terminal attachment of Bar (upper) along with a second normal *3* (lower). An exchange in the distal heterochromatic segment produces *3R3L·3R*. (B) The combination of *3R3·3R* (upper) with *y+·3L* (lower), which produces *y+·3L3R* (C) by crossing over.

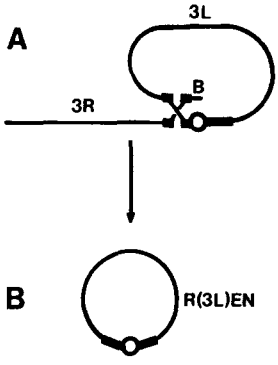


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FIGURE 4.—An alternative exceptional class from irradiated females with a terminally marked third chromosome. (A) A rearrangement between the tip and the proximal heterochromatin of the other arm of the same chromosome, producing a ring, as shown in (B).

yield inviable zygotes because of the absence of complementary gametes from the male to ensure its perpetuation. The complementary product of this normal metacentric after crossing over in the female is an acrocentric that is essentially *·3L3R*. These may be recovered by mating *C(3R3L·3R)/F(3L)* females to males with normal chromosomes. All such chromosomes produced in our experiments had the centromere region derived from *T(Y-3)A95* and, therefore, carried the $y^+$ allele of that translocation. It is more accurately designated as $y^+$*·3R3L* (Figure 3C). Although its recovery was impeded by nonrandom disjunction, it was easily detected because the *ru* allele at the *3R3L* juncture was $ru^+$ and all other *ru* loci carried the recessive allele. Six different *C(3R3L·3R)* chromosomes were used to derive over a hundred $y^+$*·3L3R* lines.

Females homozygous for $y^+$*·3R3L* were irradiated and mated to *C(3L)*; *C(3R)* ♂♂. No viable progeny should be produced except for rare triploids, nondisjunctional progeny, or cases where the two acrocentrics have been joined to produce *C(3)EN*. From 450 cultures with 50 females and 25 males as parents, 191 exceptions were recovered. Two of the exceptions proved to carry the desired compound, *C(3)EN* (Figure 5).

It is interesting to note here that the male sterility, which had been characteristic of the homozygote for the terminal attachment with *B*, disappeared and the derived compound is fertile in the male, undoubtedly a consequence of the removal of the *B* locus along with some associated genetic material from the *Y* chromosome.

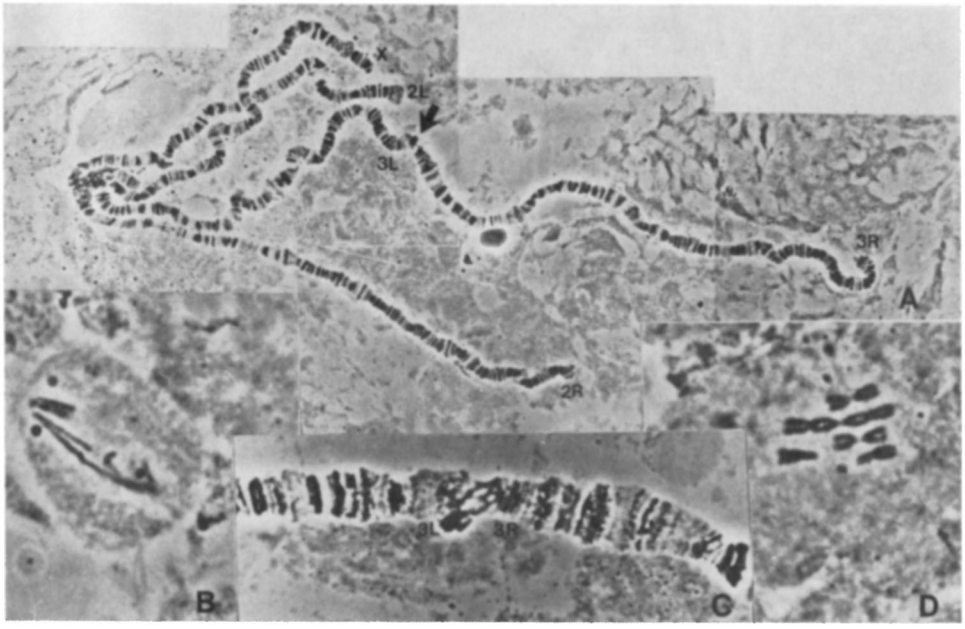


FIGURE 5.—The cytology of *C(3)EN*. (A) The polytene configuration of *C(3)EN*. (B) A neuroblast anaphase showing the appearance of the long compound. (C) A detail of the connection between the tip of *3L* and the base of *3R*. (D) A colchicinized metaphase; each arm of the compound shows the characteristic medial heterochromatic constriction.

## THE SYNTHESIS OF *C(2;3)EN*

With the availability of the entire compound autosomes, it is simple in principle to make up the compound of the composition *2R2·3L3R*; that is, one that has all of the haploid autosomal material hooked to the same centromere, except for the small fourth chromosome. One obvious method of attack would be to irradiate females of composition *C(2)EN;C(3)EN* and to mate them to ordinary diploid males. This cross would ordinarily produce no progeny (except for occasional triploids). However, if an exchange occurred between the heterochromatic regions of the two chromosomes, a new compound, *2R2L·3L3R* would be produced that would be found among the few viable offspring. In fact, this procedure was modified slightly with much the same result. A stock was made in which the *C(2)EN* chromosome was present with $y^+$·*3L3R*. Such females mated to ordinary males also will produce no progeny except for rare exceptional types. Some of the viable diploid progeny that appear in low frequency after irradiation can be shown to carry detachments of the *C(2)EN* compound with the acrocentric chromosome *3* to produce *C(2;3)EN* chromosomes. (Figure 6) More specificallly, females of the constitution *C(2)EN, ru* were irradiated and mated to *net ru* ♂♂ (80 bottles of 50 ♀♀ and 25 ♂♂). Of 179 exceptions, seven proved to be *C(2;3)EN*. The polytene configuration is shown in Figure 7.

## ZYGOTE MORTALITY WITH *C(2)EN*

Individuals with compounds consisting of two combined homologs, as *C(2)EN* or *C(3)EN*, will produce two kinds of gametes, those with the compound (and therefore with two homologs) and those without it (and therefore with no homolog). Such a stock will readily perpetuate itself because the combination of two gametes, one with the compound and the other without, will produce a diploid zygote. On the other hand, a compound-bearing individual, when mated to one with normal chromosomes, can produce no viable progeny because the zygotes will have either one or three second chromosomes, both lethal conditions. (Occasionally, viable exceptional triploid females are produced.)

After the entire compound second chromosome [*C(2)EN*] was synthesized, a few exceptional flies carrying it were found together in the same culture with a larger number of flies of composition *C(2L); C(2R)*, which had been part of the series of synthetic steps (NOVITSKI 1976). It was reasoned that since flies with the entire compound should in theory produce fewer aneuploid (and therefore inviable) progeny than should those with *C(2L)* and *C(2R)*, eventually *C(2)EN* should displace the arm compounds. After several generations of mass transferring, it became apparent that the entire compound was not competing successfully against the single arm compounds, although the former were theoretically only about half as fit as the former.

The reason for this unexpected behavior became apparent when the chromosome *2* mutants curved (*c*) and brown (*bw*), which had been used as markers in the synthesis of *C(2)EN*, appeared in homozygous condition in the compound. With two differently marked compounds, it was then possible to determine

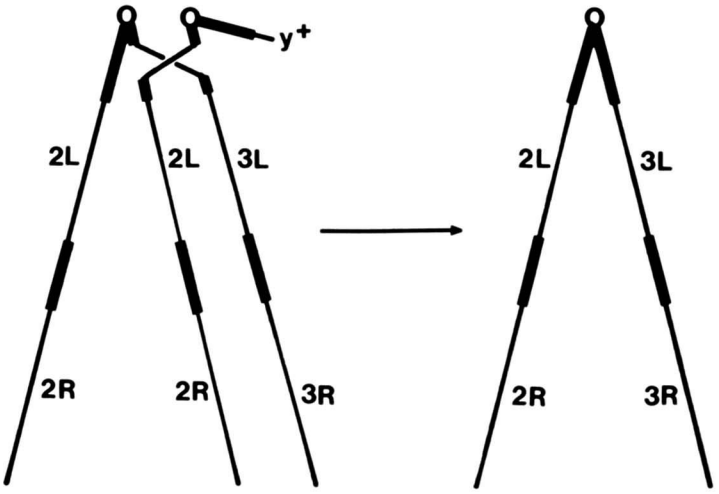


FIGURE 6.—The derivation of *C(2;3)EN*. The combination of *C(2)EN* and *y+·3L3R* producing, after heterochromatic interchange, the *C(2;3)EN* combination.

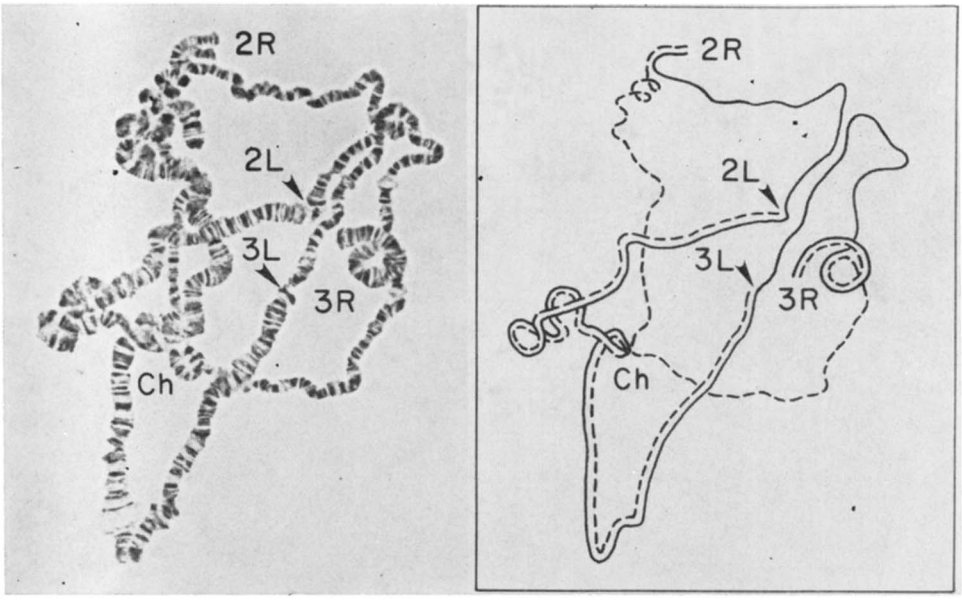


FIGURE 7.—The polytene configuration of a *C(2;3)EN* chromosome pairing with two normal homologs. In the line diagram, the chromocenter is indicated by Ch and the *X* chromosome is omitted for clarity. The arrows point to the juncture of the tips of the left arms with the bases of the right arms.

TABLE 1

*Progeny produced when males carrying* C(2)EN *or* C(2L);C(2R) *are mated to females with* C(2)EN *or* C(2L);C(2R)

| Parents | No. of eggs laid | Matroclinous progeny ♂ | Matroclinous progeny ♀ | Patroclinous progeny ♀ | Patroclinous progeny ♂ | Total progeny | Progeny/Eggs Obs. | Progeny/Eggs Exp. |
|---|---|---|---|---|---|---|---|---|
| 1. *C(2)EN*, + ♀♀ × *C(2)EN*, + ♂♂ | 228 | 13 | 30 | ? | ? | 43 | 0.19 | 0.50 |
| 2. *C(2)EN, c bw* ♀♀ × *C(2)EN, c bw* ♂♂ | 327 | 51 | 47 | ? | ? | 98 | 0.30 | 0.50 |
| 3. *C(2)EN*, + ♀♀ × *C(2)EN, c bw* ♂♂ | 582 | 72 | 49 | 1 | 4 | 126 | 0.22 | 0.50 |
| 4. *C(2)EN, c bw* ♀♀ × *C(2)EN*, + ♂♂ | 683 | 86 | 102 | 0 | 2 | 190 | 0.28 | 0.50 |
| 5. 3 + 4 | 1265 | 158 | 151 | 1 | 6 | 316 | 0.24 | 0.50 |
| 6. *C(2L), j;C(2R), px* ♀♀ × *C(2L), j;C(2R), px* ♂♂ | 251 | 30 | 31 | ? | ? | 61 | 0.24 | 0.25 |
| 7. *C(2)EN*, + ♀♀ × *C(2L), j;C(2R), px* ♂♂ | 485 | 19 | 12 | 16 | 11 | 58 | 0.12 | 0.25 |
| 8. *C(2)EN, c bw* ♀♀ × *C(2L), j;C(2R), px* ♂♂ | 420 | 27 | 25 | 2 | 10 | 64 | 0.15 | 0.25 |
| 9. 7 + 8 | 905 | 46 | 37 | 18 | 21 | 122 | 0.14 | 0.25 |

In experiments 1, 2 and 6, it is not possible to distinguish in the progeny the source of the parental compounds; thus, all progeny are classified as carrying maternal compounds.

whether these chromosomes were behaving according to simple expectation. At the same time, egg counts were made in order to obtain an estimate of the productivity in these crosses.

The results of these tests are shown in Table 1. From lines 1 and 2, it can be seen that instead of the ideal 50% egg hatch (assuming no residual egg inviability whatsoever), the hatch amounted to 19% and 30%, respectively, with an overall average in those two experiments of 25.4% (141/555), or about one-half of the expectation. Experiments 3 and 4 are added together on line 5. The frequency of adults with the *maternal* compounds among the total number of eggs laid is 24.7%, a close approximation to the expected 25% for this class. Line 6 gives a control set using *C(2L);C(2R)* males and females, which shows 23.9% hatchability in this stock (compared to the expected 25%).

As can be seen in Table 1, lines 3 and 4, there is a pronounced deficiency of progeny carrying the entire compound chromosome *2* from the parental male. This recovery of the parental male compound in the progeny is quite variable (see last column of Table 2). Such a result can come from either, or both, of two different causes: (1) The variability may originate in the proportion of viable zygotes that carry the compound from the male, or (2) in the proportion of zygotes with the female compound. Thus, what might appear as a deficiency of progeny with the paternal compound, could actually come about because there was, in fact, a variable number of eggs produced by the female with no compound. Lines 6 through 9 of Table 2 shed some light on this. Females with an entire compound *2* were mated to males with *C(2L)*; *C(2R)* from two different lines. From the last column of Table 2, it can be seen that the recovery of the compound from the female can be significantly different from 50% in both directions, and that the extent of the deviation may depend on the constitution of the male with which the female is mated.

It is, unfortunately, not possible to make a decisive test for recovery of the entire compound in the male by mating to *C(2L)*; *C(2R)* females. Such matings are almost completely sterile, because the most prevalent type of segregation in

TABLE 2

*The recovery of* C(2)EN *chromosomes in the progeny of various matings with marked compound chromosomes*

| Constitution of parents | | Progeny | | | | |
|---|---|---|---|---|---|---|
| | | Matroclinous | | Patroclinous | | |
| Female | Male | Female | Male | Female | Male | Pat./Mat. |
| 1. *C(2)EN S28,+* | *X/B$^{s}$Y+;C(2)EN, b bw* | 3659 | 2255 | 995 | 954 | 0.33 |
| 2. *C(2)EN S28,+* | *X/B$^{s}$Y+;C(2)EN, c bw* | 2224 | 1468 | 119 | 105 | 0.06 |
| 3. *C(2)EN, b bw* | *X/Y;C(2)EN S28,+* | 792 | 1386 | 121 | 119 | 0.11 |
| 4. *C(2)EN, bw sp* | *X/Y;C(2)EN S28,+* | 412 | 738 | 106 | 64 | 0.15 |
| 5. *C(2)EN, sp* | *X/Y;C(2)EN S28,+* | 347 | 631 | 64 | 38 | 0.10 |
| 6. *C(2)EN S28,+* | *C(2L)b;C(2R), px* | 373 | 235 | 383 | 466 | 1.40 |
| 7. *C(2)EN, b bw* | *C(2L)b;C(2R), px* | 505 | 338 | 352 | 361 | 0.85 |
| 8. *C(2)EN S28,+* | *In(1)w$^{m4}$/B$^{s}$Y;C(2L)j;C(2R), px* | 280 | 227 | 159 | 104 | 0.52 |
| 9. *C(2)EN, b bw* | *In(1)w$^{m4}$/B$^{s}$Y;C(2L)j;C(2R), px* | 318 | 186 | 116 | 98 | 0.43 |

TABLE 3

*The recovery of* C(3)EN *from matings of parents carrying marked compounds*

| Composition of parents | Compound in progeny | | Pat./Mat. |
|---|---|---|---|
| | Matroclinous | Patroclinous | |
| *C(3)EN Z170,st cu ca* × *C(3)EN W96,cu ca* | 454 | 1182 | 2.60 |
| *C(3)EN W96,cu ca* × *C(3)EN Z170, st cu ca* | 5402 | 1724 | 0.32 |
| *C(3)EN W96,th st ca* × *C(3)EN W96,st cu ca* | 2587 | 2475 | 0.96 |

such females is of the two arm compounds from each other, leading to inviable zygotes because of the gross duplications and deficiencies, for the chromosome arms. The rarer eggs with both, or neither, of the *C(2L)* and *C(2R)* chromosomes might possibly be used for this determination, but serious questions can be raised about the validity of any assumptions regarding their relative frequencies.

The variable recoveries of the other two entire compounds, *C(3)EN* and *C(2;3)EN*, are given in Tables 3 and 4, respectively. The latter chromosome is more amenable to analysis because it can be used in crosses, in both directions, involving chromosomes of normal structure. It is also not subject to homozygosis for alleles conferring poor viability, which could be responsible for some lowering of the maternal class in *C(2)EN* and *C(3)EN*.

In the first part of Table 4 are given the recoveries of *C(2;3)EN* from males in six independently derived lines; all are below the expected 50%. In the second part of the table, the reciprocal cross is given; these also show a depression in recovery, but one note of caution is necessary in the interpretation of this table. When two normal chromosomes pair completely with *C(2;3)EN*, the left arms of

TABLE 4

*The recovery of* C(2;3)EN *from males (1) and females (2 and 3) heterozygous for the compound and normal second and third chromosomes carrying* net *and* ru, *respectively, (1 and 2) or carrying* Ins(2)Cy *and* In(3)Cx,D (3)

| | Strain | Progeny with | | Percent recovery* |
|---|---|---|---|---|
| | | *C(2;3)EN* | Normal 2;3 | |
| (1) | A6 | 88 | 209 | 42.1 |
| | A131 | 106 | 689 | 15.4 |
| | A106 | 170 | 865 | 19.7 |
| | A100 | 91 | 651 | 14.0 |
| | A160 | 263 | 710 | 37.0 |
| | A162 | 308 | 1003 | 30.7 |
| (2) | A85 | 39 | 172 | 22.7 |
| | A160 | 76 | 228 | 33.3 |
| | A100 | 175 | 396 | 44.2 |
| | A162 | 151 | 342 | 44.2 |
| (3) | A100 | 316 | 449 | 70.4 |
| | A101 | 1512 | 1709 | 88.5 |
| | A131 | 1453 | 1381 | 105.2 |

These heterozygotes were backcrossed to *net;ru* (1 and 2) and to Oregon-R (3).

* The percent recovery is calculated as the number of compounds/the number of normal chromosomes × 100.

the normal autosomes are paired with the interstitial left arms of the compound (Figure 7) such that single crossovers (and half the double crossovers) between the homologous left arms will give rise to asymmetric dyads in which the *2L2R* or *3L3R* chromatid of the compound is replaced by a *2L* or *3L* chromatid. Such an asymmetric dyad will be preferentially included in the egg, but it will be inviable.

To check on the extent to which nonrandom disjunction might be responsible for the poor recovery of *C(2;3)EN* from the female, crosses were made up in which the two free autosomes carried the *Cy* and *DCX* inversions, respectively, to reduce crossing over. The results of this cross are shown in part 3 of Table 3. The greatly increased recovery of the compound when crossing over is suppressed (part 3 of Table 4), compared to the recovery when crossing over occurs more freely (part 2 of Table 4), is strong evidence that transmission of the compound in the female is greatly reduced by nonrandom disjunction.

In order to determine whether the large chromosome size was inherently a factor involved in the low recovery, *C(2)EN* was broken into single arms by translocation with the *Y* chromosome. This kind of single chromosome is easily constructed because it is found in the predominant diploid survivor in an otherwise sterile cross of irradiated *XXY;C(2)EN* females by normal males. These long chromosomes, which have their essential elements in the order *2R2L·*, with or without an additional smaller arm from the *Y* chromosome beyond the centromere, appear cytologically as acrocentrics and are commonly referred to as such. This terminology, however, is uninformative with respect to the order of the loci with respect to the centromere, the most important consideration in predicting genetic consequences, and may even be misleading in that it suggests that the basic change is a shift in the position of the centromere from a median to a terminal (or subterminal) position. We suggest the term "*t*erminal *p*ericentric inversion" (*In(2L)tp*) for this kind of chromosome, indicating its essential equivalence to one in which an inversion occurred with breaks at the end of one arm and just adjacent to the centromere in the other.

The six acrocentric elements so derived showed recovery rates of 30% to 40% of the normal homologue. A crossover between one of these and a whole-arm *2–3* translocation (translocation F46-H of PURO) yielded a chromosome of composition *2R2L·3R*; this chromosome was recovered only 64% as often as the homologs. Subsequently, the method of derivation of the compound *C(3)EN* involved a stage at which pericentric third chromosomes ($y^+ \cdot 3L3R$) were produced as a step in their syntheses. These chromosomes can be readily tested by mating directly to marked diploid females; although they show a somewhat reduced transmission (Table 5), the depression is not so great as that of the corresponding second chromosomes. The low recovery of these modified chromosomes indicates that neither the size of the chromosome in itself nor the nature of the centromere or the centromere region is responsible for the poor recovery.

## EXPERIMENTAL DIFFICULTIES WITH ENTIRE COMPOUNDS

In addition to the zygote mortality inherent in the crosses involving these compounds, unexpected difficulty in setting up experiments has been encountered

TABLE 5

*The recovery from the male of the single-armed acrocentric* In(3L)tp, y+ cu *with* ru st Ki *(1) and with* Dl *(2) when mated to* rucuca *(1) and to Coos Bay (2) females*

| Strain | Progeny with | | Percent recovery |
|---|---|---|---|
| | *In(3L)tp* | Normal 3 | |
| (1) Z33 | 356 | 902 | 39.5 |
| Z62 | 296 | 462 | 64.1 |
| Z109 | 293 | 413 | 70.9 |
| (2) Z33 | 1485 | 1661 | 89.4 |
| Z62 | 522 | 601 | 86.9 |
| Z109 (run 1) | 1023 | 1001 | 102.2 |
| Z109 (run 2) | 1514 | 1327 | 114.1 |

because of the low viability and fertility of some combinations of these compounds with ordinary chromosomes. Thus, a stock with the combination of an *X* chromosome carrying *Basc* (Muller-5) and either *C(2)EN* or *C(3)EN* has been difficult to make and perpetuate. This is also the case for lines with compound-*X* chromosomes together with either of those two compound autosomes. Although a stock homozygous for an *X-Y* chromosome and *C(2)EN* has been reported by R. H. MADDERN of CSIRO, Canberra, Australia (personal communication), we have not yet been able to duplicate his result (although we do have a line that carries an *X-Y* chromosome heterozygous in the female).

In general, the productivity in crosses involving these chromosomes has been lower than would be expected if the only factor disturbing complete productivity was the lethality of zygotic types carrying the chromosomes of the male. Further, it might be anticipated that as the recovery of the compound from the male improved, the total number of progeny from a mating would increase. In a set of 18 matings in which males of various origin were pair-mated to females from a single line and allowed to lay eggs for a period of three days, there was no apparent relation between the percent recovery of the paternal compound and the number of progeny produced. In fact, a simple correlation analysis suggested a slight negative correlation. Although such experiments do not exclude some unusual meiotic behavior of the compounds, they do suggest that developmental irregularities predominate in these lines. Other experiments in which the genetic background is more closely controlled will be discussed elsewhere.

Further information on the nature of the sterility of males carrying these compounds has been provided by J. MOLÉ-BAJER, who undertook an EM examination of spermatid development. The early work of KIEFER (1966, 1969, 1970), TATES (1971) and the later series of papers by TOKUYASU and co-workers (for references, see TOKUYASU, PEACOCK and HARDY 1977), provide the details of spermiogenesis in males with both normal and abnormal genetic makeups. In Figure 8 are shown the crossections of the flagellae of a normal male (A), a male with *C(2)EN* (B) and a male with both *C(2)EN* and *C(3)EN* (C). The similarity of (B) with figures of KIEFER (1969), showing the degeneration in males lacking a *Y* chromosome fertility factor, are striking. The axial fibers become disorganized in orien-

tation; the helmet-shaped nebenkern loses its characteristic shape and breaks away from its axial fiber. In (C), the degeneration is extreme, with a complete breakdown of the orientation, loss or abnormal development of the nebenkern and extensive vacuolization. There is no suggestion of any pattern of normal and abnormal spermatids of the sort that might result from the influence of spermatids of one genetic constitution *versus* the complementary constitution (*i.e.*, with or without the compound), which is seen in preparations of segregation distorter heterozygotes (TOKUYASU, PEACOCK and HARDY 1977).

For these reasons, no effort has been made to analyze the behavior of the compounds during the meiotic divisions. However, PECK (1980), working in the laboratory of P. ROBERTS, has undertaken such a study without finding any unusual behavior of the compound chromosome *C(2)EN* during spermatogenesis.

From the above considerations, it appears that the primary effects in our experiments can be attributed to a developmental influence of these long chromosomes, possibly by presenting some unusual configuration in the interphase nucleus disruptive to normal synthetic reactions. A more incisive analysis of the behavior of these compounds may depend on the study of lines less heterogeneous than those described here.

## DISCUSSION

The first step in the construction of the three large autosomal compounds described here depended upon obtaining attachments of nonessential material from the *Y* chromsome to the tips of two of the four autosomal arms at the same time that no loci necessary for viability or fertility was lost from the autosomal arm. That no essential genetic material was lost is supported by the following considerations: first, the absence of any deleterious effects in the homozygote that would be deficient for any genetic material distal to the point of attachment;

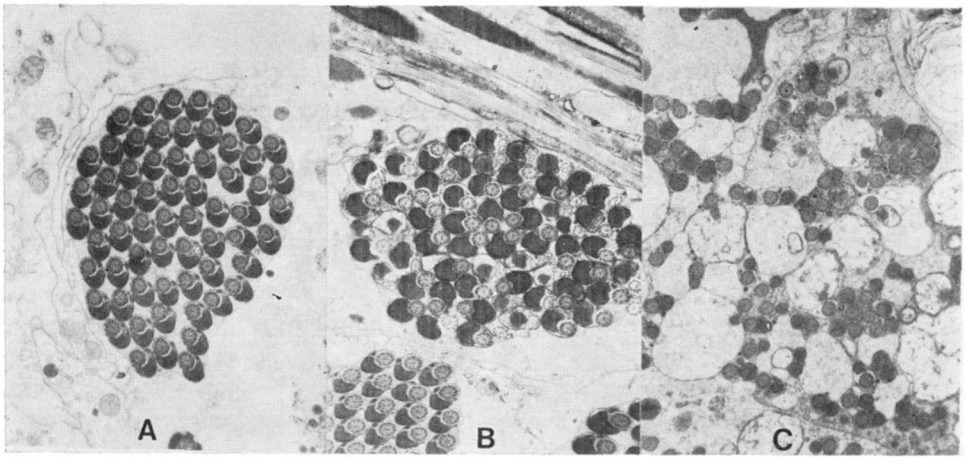


FIGURE 8.—Electron microscope photographs of cross sections through the axial filaments of sperm of males carrying (A) normal chromosomes, (B) *C(2)EN* and (C) the combination of *C(2)EN* and *C(3)EN*.

second, the failure to find any terminal bands missing in the polytene chromosomes and, third, the apparently unpolytenized thread seen in the region of the attachment. Although we have suggested for these reasons that these breaks may, in fact, have occurred within the telomere (NOVITSKI *et al.* 1981), the ultimate decision must rest on tests more definitive than those presented here.

The recovery of the entire compounds is, in some lines, rather low, particularly for some of the *C(2)EN* compounds from the parental male. A number of possible causes have been ruled out: the larger mass of the double-armed entire compound, the inadequacy of the centromere region, the length of the chromosome with two autosomal arms in tandem and loci affecting the recovery carried on the compound itself. The data on egg hatch strongly suggest that the poor recovery is primarily a matter of zygote inviability, rather than any unusual meiotic segregation phenomenon or sperm dysfunction. At the present stage in these investigations, we feel that these differences in recovery result primarily from zygote inviability, which depends on the chromosome (and genetic) composition of each individual zygotic type, including a substantial role of the paternal *Y* chromosome. For this reason, the use of these chromosomes in experiments that depend on some knowledge of the gametic frequencies should be limited to those combinations of stocks that show good recovery. Why the effect on zygote mortality appears to be so much greater when the compound is transmitted through the male than when it is transmitted through the female remains one of the more intriguing puzzles posed by these entire compound autosomes.

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## LITERATURE CITED

ABRAHAMSON, S., I. H. HERSKOWITZ and H. J. MULLER, 1954 Genetic proof for half-translocations derived from irradiated oocytes of *Drosophila melanogaster* (Abstr.). Rec. Genet. Soc. Amer. **23**: 28; and Genetics **39**: 955. ——, 1956 Identification of half-translocations produced by X-rays in detaching attached-*X* chromosomes of *Drosophila melanogaster*. Genetics **41**: 410–419.

BROSSEAU, G., 1958 Crossing over between Y chromosomes in the male *Drosophila*. Drosophila Inform. Serv. **32**: 115–116.

COOPER, K. W., 1956 Phenotypic effects of *Y* chromosome hyperploidy in *Drosophila melanogaster*, and their relation to variegation Genetics **41**: 242–264.

DÄVRING, L. and M. SUNNER, 1973 Female meiosis and embryonic mitosis in *Drosophila melanogaster*. I. Meiosis and fertilization. Hereditas **73**: 51–64. ——, 1976 Early prophase in female meiosis of Drosophila melanogaster. Further studies. Hereditas **82**: 129–131. ——, 1977 Late prophase and first metaphase in female meiosis of Drosophila melanogaster. Hereditas **85**: 25–32.

KAUFMANN, B. P. and H. GAY, 1969 The capacity of the fourth chromosome of *Drosophila melanogaster* to establish end-to-end contacts with the other chromosomes in salivary-gland cells. Chromosoma **26**: 395–409.

KIEFER, B. I., 1966 Ultrastructural abnormalities in developing sperm of X/0 *Drosophila melanogaster*. Genetics **54**: 1441–1452. ——, 1969 Phenotypic effects of *Y* chromosome mutations in *Drosophila melanogaster*. I. Spermiogenesis and sterility in *kl-1* males. Genetics **61**: 157–166. ——, 1970 Development, organization and degeneration of the Drosophila sperm flagellum. J. Cell Sci. **6**: 177–194.

LEWIS, E. B., 1967 Genes and gene complexes. pp. 17–47. In: *Heritage from Mendel.* Edited by R. A. BRINK. University of Wisconsin Press, Madison, Wisc.

LINDSLEY, D. L. and E. NOVITSKI, 1958 Localization of the genetic factors responsible for the kinetic activity of X chromosomes of *Drosophila melanogaster.* Genetics **43:** 790–798.

METZ, C. W., 1914 Chromosome studies on the Diptera. I. A preliminary survey of five different types of chromosome groups in the genus Drosophila. J. Exp. Zool. **17:** 45–59.

MORGAN, L. V., 1922 Non-criss-cross inheritance in *Drosophila melanogaster.* Biol. Bull. Woods Hole **42:** 267–274. ——, 1933 A closed *X* chromosome in *Drosophila melanogaster.* Genetics **18:** 250–283.

MULLER, H. J., 1929 The first cytological demonstration of a translocation in Drosophila. Am. Naturalist **63:** 481–486. ——, 1943 A stable double X chromosome. Drosophila Inform. Serv. **17:** 61–62.

MULLER, H. J. and I. H. HERSKOWITZ, 1954 Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster.* Am. Naturalist (Suppl.) **88:** 177–208.

NOKKALA, S. and J. PURO, 1976 Cytological evidence for chromocenter in *Drosophila melanogaster* oocytes. Hereditas **83:** 265–268.

NOVITSKI, E., 1951 The compound X chromosomes. Drosophila Inform. Serv. **25:** 121–122. ——, 1954 The compound X chromosomes in Drosophila. Genetics **39:** 127–140. ——, 1976 The construction of an entire compound two chromosome. pp. 562–568. In: *The Genetics and Biology of Drosophila.* Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

NOVITSKI, E., D. GRACE, C. STROMMEN and J. PURO, 1981 Terminal chromosome attachments. Am. J. Human Genet. **33:** 55–60.

NOVITSKI, E. and J. PURO, 1978 The genetics and cytology of some new chromosome types. Drosophila Inform. Ser. **53:** 205.

PATTERSON, J. T. and W. S. STONE, 1952 *Evolution in the Genus Drosophila.* The Macmillan Co., New York.

PURO, J. and S. NOKKALA, 1977 Meiotic segregation of chromosomes in *Drosophila melanogaster* oocytes. A cytological approach. Chromosoma **63:** 273–286.

PECK, M., 1980 An analysis of transmission of the compound (2)-Entire in anaphase II spermatocytes from *Drosophila melanogaster.* M.S. Thesis, Oregon State University.

RASMUSSEN, E. E., 1960 Reports on new mutants. Drosophila Inform. Serv. **34:** 53.

ROBERTS, P., 1975 In support of the telomere concept. Genetics **80:** 135–142.

ROBERTSON, W. R. B., 1916 Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae: V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae: Chromosomes and variation. J. Morph. **27:** 179–331.

SCHULTZ, J. and D. G. CATCHESIDE, 1937 The nature of closed X-chromosomes in Drosophila melanogaster. J. Genetics **35:** 315–320.

TATES, A. D., 1971 Cytodifferentiation during spermatogenesis in *Drosophila melanogaster.* Ph.D. Thesis. Leiden University.

TOKUYASU, K. T., W. J. PEACOCK and R. W. HARDY, 1977 Dynamics of spermiogenesis in *Drosophila melanogaster.* VII. Effects of segregation distorter (SD) chromosome. J. Ultrastruct. Res. **58:** 96–107.

WILLIAMSON, J. H. and E. MEIDINGER, 1979 Y chromsome hyperploidy and male fertility in *Drosophila melanogaster.* Can. J. Genet. Cytol. **21:** 21–24.

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