SOME CONSEQUENCES OF SPERMATOGONIAL EXCHANGE IN LONG INVERSIONS OF THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

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ABSTRACT

Among the progeny of males carrying the inverted X chromosome, In(1)sc^{8}, individuals are found which have lost the terminal euchromatic segment of the inversion. This has been interpreted in the past (Sidorov 1940, 1941) as the result of exchange between the distal heterochromatin of the inversion and the short arm of the Y chromosome, and, indeed, the reciprocal recombinant type may be recovered, i.e. a Y with its short arm replaced by the terminal euchromatin of In(1)sc^{8}. The fact that such derivatives occur in clusters and represent recombination which has occurred in males indicate that the events in question are mitotic rather than meiotic in origin.

An analysis of 44 such recombinant X chromosomes recovered from 273,721 offspring of males carrying the distal heterochromatin of In(1)sc^{8} reveals that previous notions represent an oversimplification of the actual case. When the chromosome carries large heterochromatic segments at its base, the distal heterochromatin may tend to pair with its own base in preference to pairing with the Y. Such pairing may occur in either direction, i.e. eucentrically or dyscentrically, as may pairing between the X and Y. Reversal of ordinary pairing relationships may result in the formation of dicentric bridges, and the evidence indicates that such bridges break at anaphase. It has been possible to demonstrate exchange between the distal heterochromatin of In(1)sc^{8} and all other sex-linked heterochromatic blocks except Y^{L}, and indications are that this event may not occur.

No evidence has been found favoring any sort of fusion cycle subsequent to bridge breakage, but the experimental arrangement favored detection of only a chromatid and not a chromosome type of cycle.

With respect to crossing over, Drosophila differs from other genetically studied organisms in two important respects. First there is no meiotic crossing over in males, and second somatic pairing of homologous chromosomes allows crossing over to occur in the course of mitotic divisions. Somatic crossing over does occur in males, however, and the occasional recombinant offspring of males are attributed to exchange during mitotic divisions of spermatogonial cells.

The heteromorphic sex chromosomes of Drosophila, the X and the Y, are homologous in part, judging from their pairing relationships and from the fact that occasional crossing over occurs between them. The region of homology is confined to the heterochromatin of the X chromosome, but, since the whole Y is heterochromatic, it is not nearly so well defined in the case of the Y chromosome. In the case of the male, and probably in females also, X-Y exchange seems to be limited to mitotic divisions.

Crossing over between the Y chromosome and the distal segment of heterochromatin of the sc^8 inversion was first recognized by Sidorov (1940, 1941). Among the progeny of sc^8 males mated with y sc^{13} w females he observed occasional y females and sc^{13} w males in addition to the regularly observed wild type females and y sc^{13} w males. The y females were shown to carry a sc^8 chromosome deficient for y and ac; the sc^{13} w males were sterile. Of seven Def sc^8 chromosomes tested by Sidorov, six were shown to carry the fertility factors of the short arm of the Y chromosome, while none carried Y^L fertility factors. These chromosomes, then, were interpreted as recombinants resulting from

exchange between the distal heterochromatin of $In(1)sc^{8}$ and the short arm of the Y (see Figure 1). To further test this assumption, $In(1)sc^{8}$ males were crossed to y v f females and v f females selected from the progeny. These females were shown to carry a Y chromosome bearing normal alleles of y and ac and lacking fertility factors of the short arm. Such a Y chromosome has also been reported by Crew and Lamey (1940) from a similar cross involving $In(1)sc^{S1}$.

Two interesting facts emerge from the above work: First there is an apparent specificity of pairing and subsequent crossing over between the X heterochromatin and Y^{S} to the exclusion of Y^{L}; secondly the direction of pairing is reversed compared with the direction of pairing between an X chromosome of normal sequence and the Y. With reference to the second point it should be pointed out that exchange between Y^{S} and an uninverted X does occur; Stern's (1929) XY' is almost certainly the result of such an event. It might be added that statements concerning X-Y homologies based on studies of detachment of attached X chromosomes should be viewed with caution, since, as pointed out by L. V. Morgan (1938), most attached X chromosomes probably have a Y centromere and a certain fraction of proximal Y heterochromatin at the base of each arm. Reverse pairing may also occur between the proximal heterochromatin of a normal X and the distal heterochromatin of an inverted X, a subsequent exchange resulting in a double X (Valencia, Muller, and Valencia, 1949).

Experiments involving $In(1)sc^{8L},EN^{R}$

The crosses used by Sidorov suffered from the disadvantage that

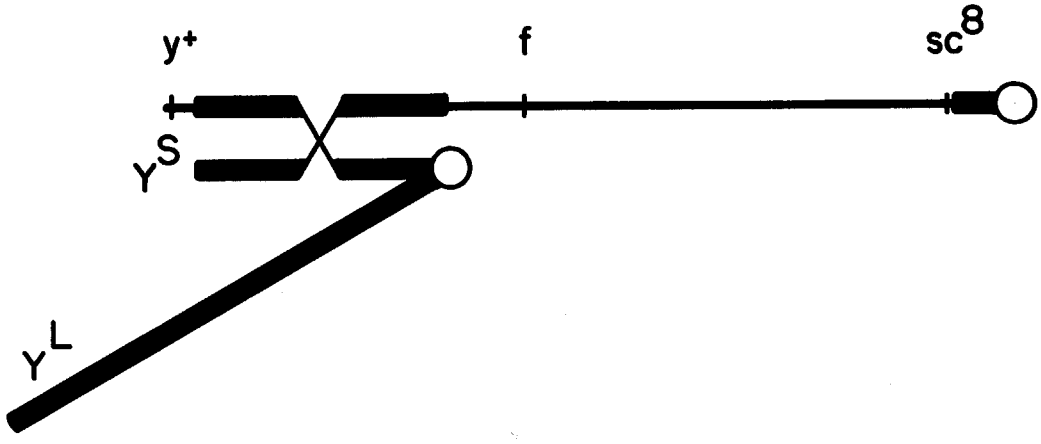

Figure 1. Exchange between the distal heterochromatic segment of $In(1)sc^8$ and the short arm of the Y chromosome, believed by Sidorov to be the event primarily responsible for the exceptional offspring of $In(1)sc^8$ males (heavy lines represent heterochromatin).

reciprocal crossover types could not be recovered, in usable form, from the same mating; a special chromosome has been employed to obviate this difficulty. Where Sidorov used In(1)sc^{8} males, males carrying a composite X chromosome with the left end of In(1)sc^{8} and the right end of In(1)EN have been used in the crosses to be presented below. The right end of In(1)EN differs essentially from that of In(1)sc^{8} in that it has all of the necessary genic material from the euchromatic left end of a normal sequence; it arose through the opening out of X^{c}, y (Novitski, 1949). The nature of the basal heterochromatin of In(1)EN is unknown except that it carries bb^{+}; there is also a small second arm of unknown origin which is probably heterochromatic in nature. In(1)sc^{8}, on the other hand, has no known heterochromatic markers at the base. The presence of the loci of y and ac at the base of In(1)EN allows the compound In(1)sc^{8L},EN^{R} X chromosome to survive in males when the distal euchromatic segment of In(1)sc^{8} has been removed. In(1)sc^{8L},EN^{R}, y^{+} f y males were crossed to y w females in single pairs and raised at 25° C; the pairs were kept for two five-day egg-laying periods and then discarded. In a second experiment the parent males were obtained from crosses of In(1)sc^{8L},EN^{R}, y^{+} f y males X y^{2}su-w^{a}w^{a} bb, M(2)S-7 females from which 24 hour egg-laying samples were placed for the next 48 hours at 34.5° C and then raised through to adulthood at 25° C; two types of males were recovered for subsequent crosses, In(1)sc^{8L},EN^{R} and In(1)sc^{8L},EN^{R}; M(2)S-7. Both the temperature treatment and M(2)S-7 markedly increase somatic exchange as detected by

observation of hypodermal spots. The above procedures were kindly suggested by Dr. William D. Kaplan (personal communication). The parental pairs in experiment 2 (i.e. the above males and their sisters) were transferred through five five-day egg-laying periods before being discarded. Results of the above two experiments are presented in Table 1.

Experiment number	Father	Progeny			
		y^+ males	y males	y females	y^+ females
1	$In(1)sc^{8L}{}_{EN}{}^{R}$, y^+ f y	33,527	19	31,723	5
2a	$In(1)sc^{8L}{}_{EN}{}^{R}$, y^+ f y	3,020	13	2,617	0
2b	$In(1)sc^{8L}{}_{EN}{}^{R}$, y^+ f y, M(2)S-7	2,008	1	1,819	0

Summarized results of experimental crosses involving $In(1)sc^{8L}$, EN^R males.

Table 1.

In experiment 1, five y males came from one parent male (F-7), four from another (D-6), and one from each of ten other males (B-1, C-4, D-8, I-7, L-7, O-7, P-7, P-8, Q-1, and U-5), while in experiment 2 eleven y males came from one male (C-1) and one from each of three others (B-7, C-6, C-13). Such clustering was not

observed by Sidorov. The y^+ females arose singly (L-3, U-7, U-8, V-0, V-4). Henceforth the y bearing chromosomes from the above experiments will be referred to as $In(1)sc^{8L},EN^{R}$ crossover X chromosomes, or abbreviated as $sc^{8}EN$ c.o. X, while the y^+ bearing chromosomes will be abbreviated as $sc^{8}EN$ c.o. Y. In view of the extreme rarity of the event in question it seems justified to consider clusters as arising, through cell division, from single mitotic events. Since there is no guarantee that sperm transferred during a given copulation represent a random sample of the total sperm population, it is not possible to derive an expression for the frequency with which the events in question occur.

The X chromosomes of the yellow males from experiments 1 and 2 were balanced by matings to y w females. The y^+ females were crossed to XYY, y males which were selected with the aid of Sb^{vg} (Lewis, unpublished); some of the sons of this cross received a Y from their father in addition to the sc^{8} c.o. Y chromosome they received from their mother. The non-yellow sons were inbred with their y w sisters and stock temporarily maintained by selection of non-yellow flies. Later these Y fragments were balanced against attached X breakdown products that carried the complementary Y arm.

The constitution, with respect to the Y chromosome, of the products of the above experiments was determined. The Y" chromosome described by Stern (1929) was used to test for the presence of Y^{L}; this chromosome contains two Y^{S} arms and lacks fertility factors of Y^{L}.

It is able to confer fertility to a male only in the presence of Y^L. Males carrying each of the sc^8EN c.o. X chromosomes recovered from the above experiments were crossed individually to y / Y" females; the progeny of these crosses were sc^8EN c.o. X / Y" and y / Y. The fertility of the F_1 males was tested in each case by inbreeding the F_1 and by individually backcrossing five F_1 males to y / Y" females. Similar crosses utilizing y v f / Y^{cl} females in the place of y / Y" were performed to test for the presence of Y^S. Y^{cl} is a ring shaped Y described by Muller (1948) in which the short arm and the tip of the long arm of sc^8·Y (Muller, 1948) have been removed, resulting in a chromosome which has lost Y^S fertility factors but none of those borne on Y^L. On the basis of the above tests it can be said that none of 34 sc^8EN c.o. X chromosomes tested carries Y^L, while 3 out of 34 (L-7, P-0, P-7) carry Y^S.

Attached X females carrying three of the sc^8EN c.o. Y chromosomes were crossed in pairs to X·Y^S, y w / Y^{cl} males and to X·Y^L, g^2 B / Y" males (the elevated dot indicates the position of the centromere). In all three cases X·Y^S, y w / sc^8EN c.o. Y F_1 males were found to be fertile, and the X·Y^L, g^2 B / sc^8EN c.o. Y males were sterile. The indications are clearly that when the Y chromosome is involved in a sc^8EN crossover, Y^S and never Y^L is the participating member.

If an exchange were to occur at such a position in the Y chromosome that some of the fertility factors of the arm involved were distal and others proximal to the point of exchange, the recombinant

chromosomes would be an X with the distal half of a Y arm attached to its tip and a Y with the distal half of one arm replaced by a piece of X. More than one arm of the Y and therefore probably more than Y^{cl} or Y" would be required to confer fertility upon a male carrying such a $sc^{8}EN$ c.o. X. A single Y arm would, however, be more than sufficient to compensate for the missing fertility factors in the reciprocal $sc^{8}EN$ c.o. Y. To check this possibility one must recombine reciprocal crossover types and recover fertile males. Most of the $sc^{8}EN$ c.o. X chromosomes from experiment 1 were crossed to attached X females carrying each of three $sc^{8}EN$ c.o. Y chromosomes and the fertility of the F_1 males tested. No additional fertile combinations were found.

From the work of Gershenson (1940) and from results obtained in these laboratories, some degree of homology between X heterochromatin and the base of chromosome 4 was suspected. Consequently each $sc^{8}EN$ c.o. X was tested for linkage with 4; no case of X-4 linkage was found.

The results reported above differ considerably from those reported by Sidorov in that the Y chromosome seems to be a rare participant in the formation of $sc^{8}EN$ c.o. X chromosomes. One might look to the essential difference in experimental design for an explanation of the discrepant results; that difference is the base of the EN inversion. Is it possible that, by doubling back to the chromocenter, the distal heterochromatin of the X chromosome can cross over with the short arm

of In(1)EN ? The demonstration of such an exchange, if it has occurred, is not possible since there is no way as yet to characterize the heterochromatin of the short arm. An alternative procedure is to eliminate the EN base at the outset and use the same chromosome that Sidorov employed; i.e., $In(1)sc^{8}$.

Experiments involving $In(1)sc^{8}$

Circumvention of the difficulties involved in recovering reciprocal recombinant types from the same mating in $In(1)sc^{8}$ crosses is more of a problem than in the case of $In(1)sc^{8L}{}_{,}EN^{R}$. To accomplish this $In(1)sc^{8}$, f v cv sc^{8} males were crossed to $Y^{S}X{\cdot}Y^{L}$ females carrying y. The regular types of offspring from such a cross are y^{+} females and y males, whereas the recombinant types are y females and y^{+} males, which carry a full Y chromosome from their mother irrespective of the contribution from their father. The $Y^{S}X{\cdot}Y^{L}$ chromosome has been fully described elsewhere (Lindsley and Novitski, 1950); essentially it is an In(1)EN chromosome with the tip of a $sc^{8}EN$ c.o. X chromosome which carries Y^{S}, and with the short arm replaced by Y^{L}. The resultant chromosome carries all of the necessary material of both the X and Y.

Experiments 1 and 2 have demonstrated the occurrence of the clusters of recombinants to be expected as the result of a mitotic event; it was deemed desirable to recover such clusters in as many cases as possible, especially in hopes of recovering reciprocal types from the same father. With this in mind, the two experiments

described below have been designed to encourage maximum yield of offspring from individual males. In experiment 3, 25 In(1)sc^8, f v cv sc^8 males were mated individually to five females of the constitution $Y^S X \cdot Y^L$, In(1)EN, B y. At the end of five days the males and females were separated; each male was placed in a new bottle with five new females of the same constitution, and the old females were put into new bottles for continued egg laying. After five more days the bottles containing females alone were transferred and the bottles containing one male and five females were subjected to the same procedure as described for the first series of bottles. At the end of the next five days the procedure was repeated except that the males were discarded; at this time there were theoretically nine bottles from each of 25 parent males. Experiment 4 was arranged in a similar manner except that only four females per male were used and subculturing was done every four days. Also it was discovered in experiment 3 that females, once removed from the males had sperm enough for only two additional subcultures; in experiment 4, therefore, females were discarded after the second subculture. In this case transfers were continued to the point where for each of 100 parent males there were maximally 14 bottles.

Careful examination of the cross used in experiment 3 reveals that the recombinant X chromosome (sc^8 c.o. X) is recovered as the heterozygote, $Y^S X \cdot Y^L$, In(1)EN, B y / sc^8 c.o. X. These two chromosomes have essentially the same sequence; consequently crossing over is expected to occur freely between them. Crossing over distal to

Bar results in exchange of tips which is undetectable in terms of the markers employed. Recovery of such a recombinant between $Y^{S}X \cdot Y^{L}$ and sc^{8} c.o. X in the process of establishing stock of a sc^{8} c.o. X chromosome would result in the loss of the critical distal region, which may or may not carry Y^{S}, and in its replacement by the Y^{S} tip of the $Y^{S}X \cdot Y^{L}$ chromosome. Actual measures of the rate of recombination between B and the tip in such a heterozygote yield a value of 21.9 %. To assure the recovery of the proper chromosome end in experiment 3, separate stocks were made and maintained for all further testing from three sons from each heterozygous female recovered. To eliminate the necessity for establishing more than one stock of each recombinant in experiment 4, the In(1)dl-49, car f v section of an $Ins(1)sc^{8}$,dl-49, car f v chromosome, kindly supplied by H. J. Muller, was transferred into the $Y^{S}X \cdot Y^{L}$ chromosome by a double crossover. It was anticipated that the presence of In(1)dl-49 would practically eliminate crossing over, but when test counts were made it was discovered that the rate of recombination between car and the tip was 9.3%, that between f and the tip being 20.9%. Speculation on possible mechanisms involved in this unexpected phenomenon are not pertinent to the present discussion. Since the presence of In(1)dl-49 has little or no effect on crossing over, it was also necessary in experiment 4 to establish several stocks of each recombinant X chromosome (four or five).

The results of experiments 3 and 4 are summarized in table 2.

In experiment 3 two of the y females and one of the y^+ males were

Experiment	y^+ females	y females	y males	y^+ males
3	11,381	3	8,085	3
4	93,675	6	85,818	0

Summarized results of experimental crosses involving In(1)sc^8 males.

Table 2.

among the offspring of one parent male (E_1), while the rest of the exceptional individuals arose separately (sc^8 c.o. X P_o, sc^8 c.o. Y Q_2, sc^8 c.o. Y T_o). In experiment 4 two y females came from one father (67h), and the remaining four came from separate fathers (89a, 25b, 91b, 99c).

The sc^8 c.o. X chromosomes are deficient for the loci of y and ac but not sc; the sc^8 c.o. X, f v cv sc^8 / Y^S X·Y^L, In(1)EN, B y females recovered from experiment 3 were subjected to a series of crosses such that balanced stocks containing sc^8 c.o. X, f v cv sc^8 / sc^8·Y males and y w / sc^8·Y females were established. Similar stocks were extracted from the sc^8 c.o. X, f v cv sc^8 / Y^SX·Y^L, Ins(1)EN, dl-49, car f v y females recovered in experiment 4. The sc^8·Y chromosome (Muller, 1948) is a complete Y chromosome with y^+ and ac^+ plus some of the distal heterochromatin of In(1)sc^8 attached to the tip of its long arm; the duplication of y and ac allows the sc^8 c.o. X chromosomes to survive as males. This chromosome, incidentally, seems to be an authentic case of exchange between Y^L and the distal hetero-

females recovered in experiment 4. The sc^8·Y chromosome (Muller, 1948) is a complete Y chromosome with y^+ and ac^+ plus some of the distal heterochromatin of $In(1)sc^8$ attached to the tip of its long arm; the duplication of y and ac allows the sc^8 c.o. X chromosomes to survive as males. This chromosome, incidentally, seems to be an authentic case of exchange between Y^L and the distal heterochromatin of $In(1)sc^8$.

The y^+ males from experiment 3, which were B in phenotype, were crossed to $\underline{y^2\ su\text{-}w^a\ w^a\ bb}$ / 0 (0 indicating no Y chromosome) females. In two cases (T_o and Q_2) the progeny of such crosses consisted of yB males and $su\text{-}w^a\ w^a$ bb females; the F_1 of each of these crosses was inbred and stocks containing $Y^S X \cdot Y^L$, In(1)EN, B y / y^+ males and $\underline{y^2\ su\text{-}w^a\ w^a\ bb}$ / y^+ females established. The nature of the y^+ bearing chromosomes has not been further investigated. In the third case (E_1) the mating of the B male with $\underline{y^2\ su\text{-}w^a\ w^a\ bb}$ / 0 females yielded only B males and $y^2\ su\text{-}w^a\ w^a$ females; further crosses involving this case are to be discussed later.

Tests carried out on the sc^8 c.o. X chromosomes for the presence of Y chromosome arms were the same as those employed in the case of sc^8EN c.o. X chromosomes except that the $\underline{y}$ / Y" and $\underline{y\ v\ f}$ / Y^{c1}-females also carried Dp (1;3) sc^{J-4} to insure survival of males carrying the incomplete Y chromosomes. Dp(1;3) sc^{J-4} carries the tip of the X chromosome, through the locus of sc, on the extreme left end of chromosome 3; it is part of T(1;3) sc^{J-4}. The three sc^8 c.o. X chromosomes from experiment 3 (E_1a, E_1b, P_o) were found to carry Y^S;

from experiment 4 three chromosomes were found to carry Y^S (89a, 91b, and 67h(2), but not 25b, 99c, or 67h(1)). In no case was the presence of Y^L demonstrable. Here then, the results are more nearly in agreement with Sidorov's assertion that Y^S is a common participant in sc^8 c.o. X formation.

Tests for X-4 linkage were negative for all sc^8 c.o. X chromosomes as they were for the sc^8EN c.o. X chromosomes.

Tests of chromosome sequence

Sidorov (1940) found that sc^8 c.o. X chromosomes can be recovered from the progeny of $In(1)sc^8$ / + females. In testing these chromosomes for sequence he discovered that some of them were reinversions; he does not record such observations for sc^8 c.o. X chromosomes derived from males. To extend Sidorov's observations to the case of spermatogonial events, the products of the above experiments were tested for sequence by measuring their recombination against both $In(1)sc^8$ and a normal sequence. In testing recombination with the $In(1)sc^8$ sequence the distance between cv and v was used in some cases and that between y and v in other cases as a measure. The values for the distance between cv and v were found to fall into two distinct groups with means 5.5 and 15.5. Those for the distance between y and v similarly fell into two groups with means 4.4 and 45.3. The higher values represent normal crossing over in the region defined, while the lower values represent crossover suppression. Recombination of sc^8EN c.o. X chromosomes with a normal sequence was measured in terms

of the sc-f distance in some cases and the y-v distance in others. In those cases in which the distance between sc and f was measured, the values fell into classes of means 6.1 and 45.6, and where the y to v region was studied, the mean was 8.1, whereas the same distance in a y^2 w^a / sc cv v f female was 31.1 map units. Here, as in the case of recombination with In(1)sc^8, the higher values represent normal crossing over.

With respect to the above measurements the sc^8EN c.o. X chromosomes fall into three distinct classes. First there are those which cross over normally with In(1)sc^8 but not with the normal sequence (B-1, C-4, D-6a, D-6b, D-6c, D-6d, D-8, F-7a, F-7b, F-7c, F-7d, J-3, K-1, L-7, O-7, P-0, P-7, Q-1, S-7, W-0, C-6); these chromosomes have retained their inverted sequence and represent a rather heterogeneous array of effects. Second, there are those which cross over normally with a normal sequence but not with In(1)sc^8 (F-7e, I-7); these are reinversions and have been interpreted as products of breakage of a mitotic bridge. Third, there are those chromosomes which cross over neither with a normal sequence nor with In(1)sc^8 (C-1a, C-1b, C-1c, C-1f, C-1g, C-1i, C-1j); these have been interpreted as ring chromosomes, and this interpretation has been confirmed by examination of larval neuroblast figures. The formation of the various types of chromosomes just mentioned will be discussed fully in the following sections.

Recombination values have been similarly measured for the sc^8 c.o.

X's recovered from experiments 3 and 4. All of these chromosomes (E_1-a, E_1-b, P_o, 89a, 25b, 91b, 99c, 67h(1), 67h(2)) prove to have retained their inverted sequence.

Interpretation of results with In(1)sc^{8L}, EN^{R}

The distal heterochromatin of the In(1)sc^{8L}, EN^{R} chromosome can be imagined to undergo somatic synapsis with either the Y chromosome or with the largely unknown heterochromatic regions of its own base. It will be profitable to treat each of these possibilities in some detail in an attempt to account for the chromosomes discussed in the preceding three sections.

Figure 2 shows the possible pairing configurations between the distal X heterochromatin and the Y chromosome. Y^S and Y^L have not been labelled as such; instead the terms synaptic arm (Y^{syn}) and asynaptic arm (Y^{as}) have been employed. Either Y^L or Y^S may be either synaptic or asynaptic. The synaptic arm may pair with the distal heterochromatin in two ways which may be described as eucentric and dyscentric pairing. These are terms used by Darlington (1937) to describe gene arrangements in structural heterozygotes. Eucentric refers to situations in which the changed and unchanged segments have the same linear order in relation to the centromere, whereas dyscentric refers to situations where the linear order of the changed segment is inverted with respect to the centromere. In the present context these terms are used to describe pairing relationships

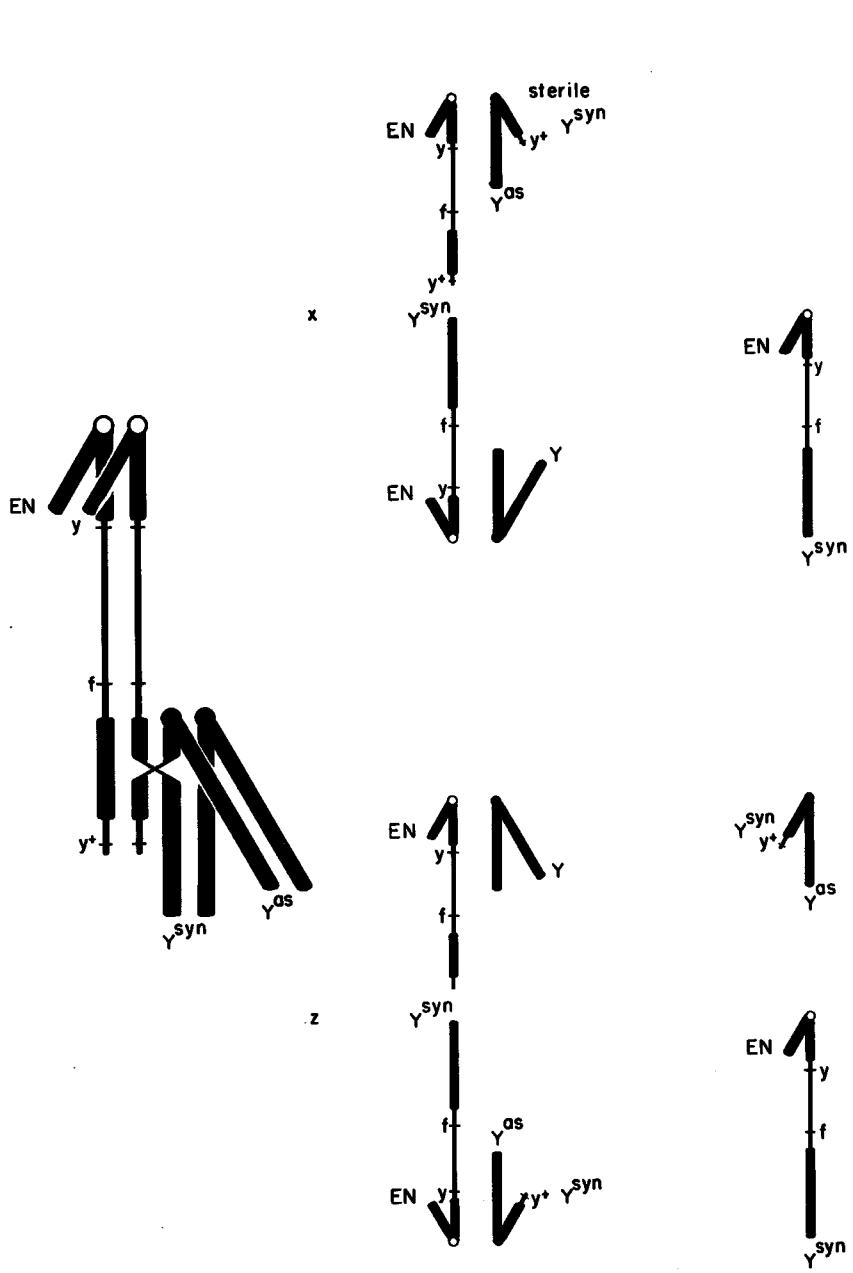

Figure 2. Genetic consequences of exchange following different pairing configurations between the distal heterochromatic segment of In(1)sc^{8L},EN^{R} and the Y chromosome (heavy lines represent heterochromatin).

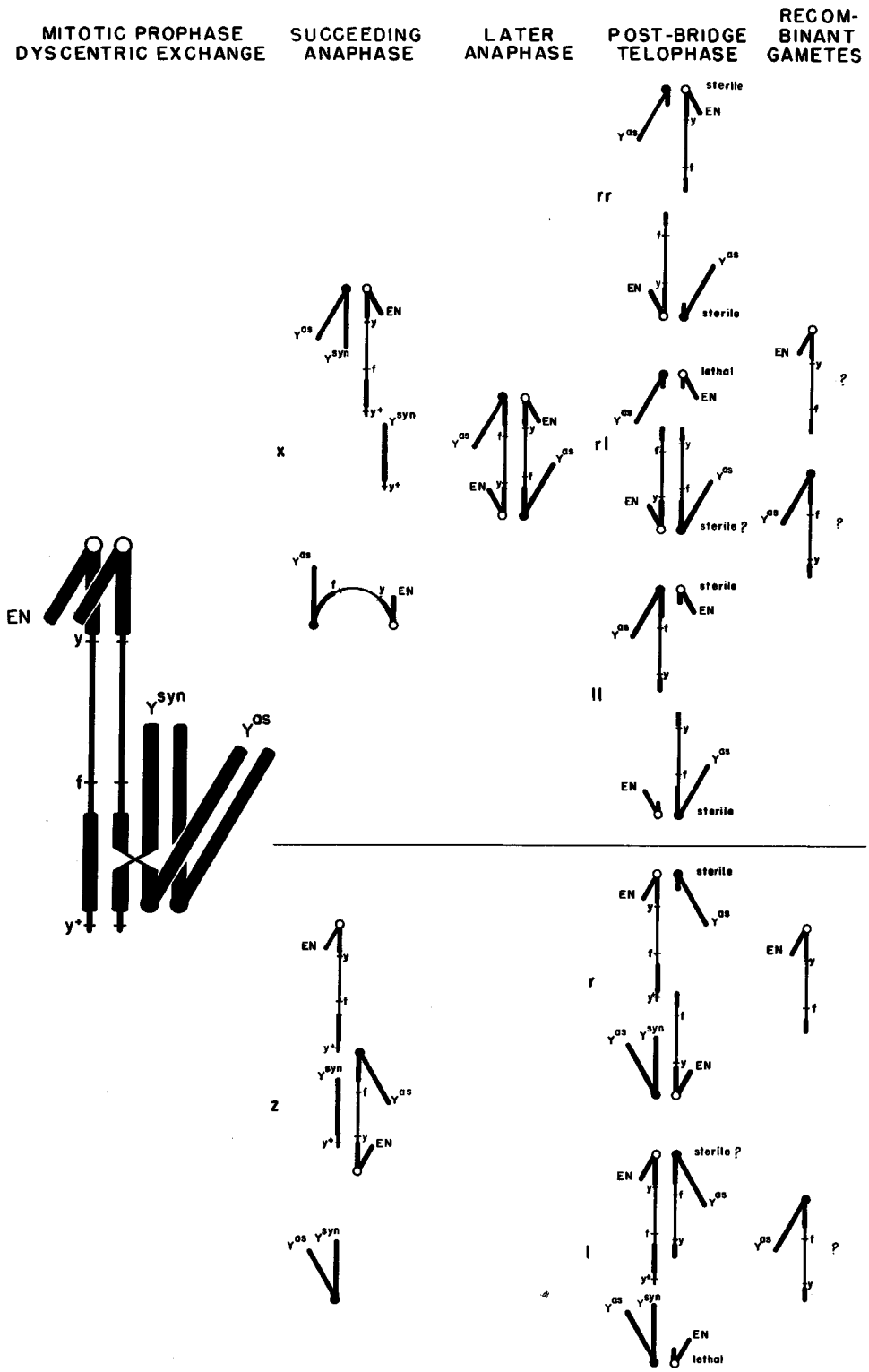

Figure 2 continued.

rather than gene order; in those cases in which the ends of the two heterochromatic segments nearest the centromere are paired and the ends most remote from the centromere are paired the pairing is eucentric. Where the basal end of one segment pairs with the more remote end of the other segment, the pairing is dyscentric. Eucentric and dyscentric exchange refer to exchange following these types of pairing.

Following a somatic exchange there are three possible types of two by two segregation from the tetrad. The chromatids may separate equationally with one recombinant and one non-recombinant proceeding to each pole or the two recombinants may proceed to one pole and the non-recombinants to the other. These two types of segregation have been termed the x and z types respectively by Stern (1936), and they will be so termed below. The third type of segregation is reductional (y), and according to Stern this does not occur. Henceforth only x and z segregation will be considered.

Considering first the case of eucentric pairing, it is seen that an exchange results in a $sc^{8}EN$ c.o. X, a $sc^{8}EN$ c.o. Y, and a non-recombinant X and Y. When x type of segregation occurs the $sc^{8}EN$ c.o. X and the normal Y go to the same pole and should give rise to a viable clone of cells; to the other pole proceed the normal X and the $sc^{8}EN$ c.o. Y. These also should give rise to a viable clone of cells, but since they lack the synaptic Y arm, whichever it may be, it is probable that they should be sterile. Stern and

Hadorn (1938) have established by transplantation techniques that male tissue deficient for Y^L, occurring contiguously with normal tissue in a mixed testis, is unable to produce viable sperm as detected by motility studies and progeny tests. From these results it can be said with fair certainty that if Y^L is the synaptic arm, the sc^8EN c.o. Y chromosomes resulting from eucentric exchange will not give rise to viable sperm if x type segregation ensues. There must be some point, however, in the lineage of a spermatozooan between the germinal primordia and the secondary spermatocyte where the Y becomes unnecessary for completion of formation of functional gametes. If this point is prior to the first meiotic division the possibility of obtaining Y deficient primary spermatocytes which will finish normal development still exists. It is probably reasonable to assume that the same facts outlined above with respect to Y^L apply equally well to Y^S, but experimental evidence is lacking.

Eucentric exchange and subsequent z segregation result in the passing of the two non-crossover chromatids to one pole and the recombinant chromatids to the other; the cell receiving the non-crossovers is of no further interest. The other cell still has a complete chromosomal complement and should therefore have no difficulty in giving rise to a clone of recombinants yielding equal numbers of sc^8EN c.o. X and sc^8EN c.o. Y gametes. Assuming x and z segregation to be equally frequent and Y-deficient cells to be invariably sterile, a ratio of 2 sc^8EN c.o. X : 1 sc^8EN c.o. Y is expected from eucentric

exchange between the distal heterochromatin of In(1)sc^{8L}, EN^{R} and the Y chromosome. In the above scheme if Y^{L} is the synaptic arm the $sc^{8}EN$ c.o. X will contain all or part of Y^{L}, and the $sc^{8}EN$ c.o. Y will lack all or part of Y^{L}; similarly, where Y^{S} is the synaptic arm the $sc^{8}EN$ c.o. X will carry all or part of Y^{S}, and the $sc^{8}EN$ c.o. Y will lack all or part of Y^{S}. As pointed out in the treatment of the In(1)sc^{8L}, EN^{R} experiments it has been possible to demonstrate the presence of Y^{S} on only 3 out of 34 $sc^{8}EN$ c.o. X chromosomes studied (L-7, P-0, P-7), whereas no indication of the presence of Y^{L} or of only part of either Y^{S} or Y^{L} has been obtained. The only $sc^{8}EN$ c.o. Y's investigated (B-2, T-0, U-8) have been shown to lack part of Y^{S}. Thus evidence exists for eucentric pairing of Y^{S} but not Y^{L} with the distal In(1)sc^{8} heterochromatin.

Turning now to dyscentric pairing, it is seen that exchange results in the formation of a dicentric and an acentric, with the y^{+} allele being lost to the acentric. Segregation of type x sends non-recombinants to one pole and the intact dicentric to the other. The non-recombinant cell need not be further considered, but the fate of the dicentric is of interest. During the ensuing mitosis the dicentric reduplicates and sister centromeres separate, those at one end of the dicentric separating independently of those at the other. This being the case two bridges may be formed as shown in figure 2 or one dicentric may proceed to each pole. Each dicentric that survives a given mitotic division intact is again subjected to the

possibility of bridge formation in the succeeding division.

As mentioned previously, reinversions have been interpreted as products of breakage of dicentrics. Precedent for such an interpretation is found in the work of Stern (1936) who has classified hypodermal spots observed in ring and inversion heterozygotes as carrying products of fragmentation of dicentric bridges formed through somatic exchange. Stern's evidence suggests that the bridge may break at any point between the two centromeres. Breakage at almost any point in the euchromatin results in passage of a grossly deficient chromatid to each pole; since in a certain proportion of cases occurring among females a normal chromatid will also pass to each pole, some of the cells containing deficient chromosomes should survive. In the present experiments, however, since males were employed, any deficient chromosome thus formed would exist in the hemizygous condition in the daughter cell; this would almost invariably be cell lethal. For this reason it is practical to assume that only the breaks occurring in the heterochromatin result in viable cells. Therefore the dicentrics considered in figure 2 may break to the left (in a normal sequence) of y resulting in a reinverted $sc^{8}EN$ c.o. X with a Y centromere bearing, in addition to the X, the asynaptic arm of the Y; or it may break to the right of f resulting in a $sc^{8}EN$ c.o. X carrying the base of In(1)EN.

As pointed out above, segregation of type x following dyscentric exchange results in the formation of two dicentrics in one or more

of the succeeding mitoses; these may both break to the left of y (ll) or to the right of f (rr), or one may break to the right of f while the other breaks to the left of y (rl). Examination of figure 2 reveals that rr or ll breakage arrangements result only in cells with incomplete Y's, and probably, therefore, never yield functional gametes. Bridge breakage of the rl type also results in a cell lacking a complete Y chromosome, but this cell is of the constitution 2X:2A. Two questions may be asked about a cell of this nature: Do patches of genetically female cells arising in a testis complete spermatogenesis? If so, do such cells require all or part of a Y chromosome for fertility? Neuhaus (1936) has reported clusters of attached X females among the progeny of $X \cdot Y^S$ males; these attached X's are probably products of eucentric exchange between the Y^S arm of one chromatid and the basal heterochromatin of its sister. Disjunction should occur such that an attached X passes to one pole, and a Y"-like chromosome passes to the other; the free Y chromosome should undergo normal mitotic division sending a chromatid to each pole. In some of his experiments Neuhaus used $X \cdot Y^S$ / Y males and in others he used $X \cdot Y^S$ / Y^L males, but he fails to specify whether clusters of attached X's were recovered from one or both types. This point is important with respect to the second question asked above; if any such clusters were recorded from $X \cdot Y^S$ / Y^L males, the unimportance of Y^S in conferring fertility upon 2X:2A spermatogonial cells would be indicated. If the genetically female cells arising from rl breakage

of the double dicentric diagrammed in figure 2 do give rise to functional gametes, there will result clusters containing both of the derivatives expected from bridge breakage. Examination of table 3 will reveal that the cluster F-7 contains both inverted and reinverted $sc^{8}EN$ c.o. X chromosomes; if this cluster had arisen from the event described above, however, the reinverted X would contain the complete asynaptic Y arm as a second arm; no member of the cluster carries demonstrable Y material. Indeed no case of a $sc^{8}EN$ c.o. X was found carrying a basal Y arm.

Before terminating the discussion of the fate of double bridges it should be mentioned that, theoretically, breaks in positions other than in the heterochromatin are recoverable from this type of configuration. Orientation of the bridges is such that the y end of one chromatid and the f end of the other proceed to each pole. A series of breaks is conceivable in which the portion lost by the breakage of one bridge is complemented by that gained by breakage of the second. If both bridges were to break at v, for example, each pole would receive one fragment carrying the region from y to v and one with the region from bb to v. If the breaks were not at exactly the same place in the two chromatids, one daughter cell would be deficient for the region between the breaks, while the other would have this region duplicated. Where the duplicate-deficient region is very small both cells may be able to continue spermatogenesis, for slightly larger regions, only the cell carrying the duplication should

survive. As the region included between the break points increases in length the viability of the resultant duplicate daughter cell should decrease until complete inviability is reached. When the region between the two break points approaches the length of the euchromatic region of the bridge, the duplicate cell becomes in essence a deficient female cell and should again be viable. Of the gametes derived from cells containing the complements just discussed, relatively few could give rise to a viable zygote in a given cross. In all of these cases the X chromosome complement is made up of one fragment carrying the left end and one carrying the right end of the chromosome, and these two fragments should segregate from one another at reduction division. The possibility of recovering one of these fragments depends upon its length. Very short pieces may give rise to duplication patroclinous males lacking a Y in crosses to regular females and to duplication females in attached X crosses. Fragments of intermediate length may on occasion be recovered as duplication females from attached X crosses; long fragments are probably recoverable only as deficiency females from unattached X crosses. No evidence of such breakage is evident from the data, but it must be admitted that markers were not chosen from this point of view and most or all such fragments would have gone undetected or been discarded as the results of non-virginity.

Segregation of type z following a dyscentric exchange between the distal X heterochromatin and the Y results in the immediate

formation of a bridge and the passage of the non-recombinant X and Y to opposite poles. Fragmentation of the bridge to the right of f yields to one pole a $sc^{8}EN$ c.o. X which still carries the inversion. Breakage to the left of y results in the inclusion of a reinverted $sc^{8}EN$ c.o. X, carrying the asynaptic arm of the Y at its base, in a 2X:2A cell. Subterminal breaks at the y end of the bridge should give deficient 2X:2A cells.

The only derivative of pairing between distal X heterochromatin and the Y which has been unequivocally demonstrated is the result of eucentric exchange when Y^{S} is the synaptic arm, while it seems fairly certain that eucentric exchange with Y^{L} as the synaptic arm does not occur. Eucentric exchange with Y^{S} is the same event claimed by Sidorov to result in sc^{8} c.c. X and sc^{8} c.o. Y chromosomes. The case for dyscentric synapsis is not at all convincing. If genetically female spermatogonial cells lacking complete Y chromosomes give rise to functional gametes, for every $sc^{8}EN$ c.o. X lacking Y material resulting from the breakage of bridges formed by dyscentric exchange, one expects to find a reinverted sc^{8} c.o. carrying a Y arm basally. Neither of the reinverted $sc^{8}EN$ c.o. X chromosomes recovered, however, bears a Y arm. If, on the other hand, such 2X:2A cells are sterile no reinversions are expected and the only expected derivative is that arising from breakage to the right of y of the bridge formed by z segregation of a tetrad in which dyscentric exchange has taken place. Nothing can be said as to the identity of the synaptic and asynaptic

arms if such is the case. The above event may account for some of the $sc^{8}EN$ c.o. X's which lack a Y arm, but other explanations must be sought for the rings and reinversions and probably for the bulk of the Y-deficient $sc^{8}EN$ c.o. X chromosomes.

In addition to the possible relationships between the distal heterochromatin of the X and the Y just discussed, new pairing possibilities are presented by the base of In(1)EN. These possibilities are diagramed in figure 3; in the first column it will be noticed that each configuration is defined with respect to three properties. The first item tells with which of the two possible basal heterochromatic segments the distal segment pairs, i.e., the short arm or the proximal heterochromatic segment of the long arm of In(1)EN. The terms eucentric and dyscentric have been defined previously. The third item in the definition refers to the strands involved in an exchange. The terms employed are borrowed from attached X terminology, an exchange being reciprocal when the segments involved are attached to the same centromere, and non-reciprocal or diagonal when they are attached to sister centromeres.

It should be noticed that the configurations in figure 3 are dyads and not tetrads, as in figure 2. Since sister centromeres are known to separate following somatic exchange there is only one type of segregation possible from these dyads; the Y chromosomes in the same cells undergo normal mitotic separation; all daughter cells, therefore, receive a full Y complement.

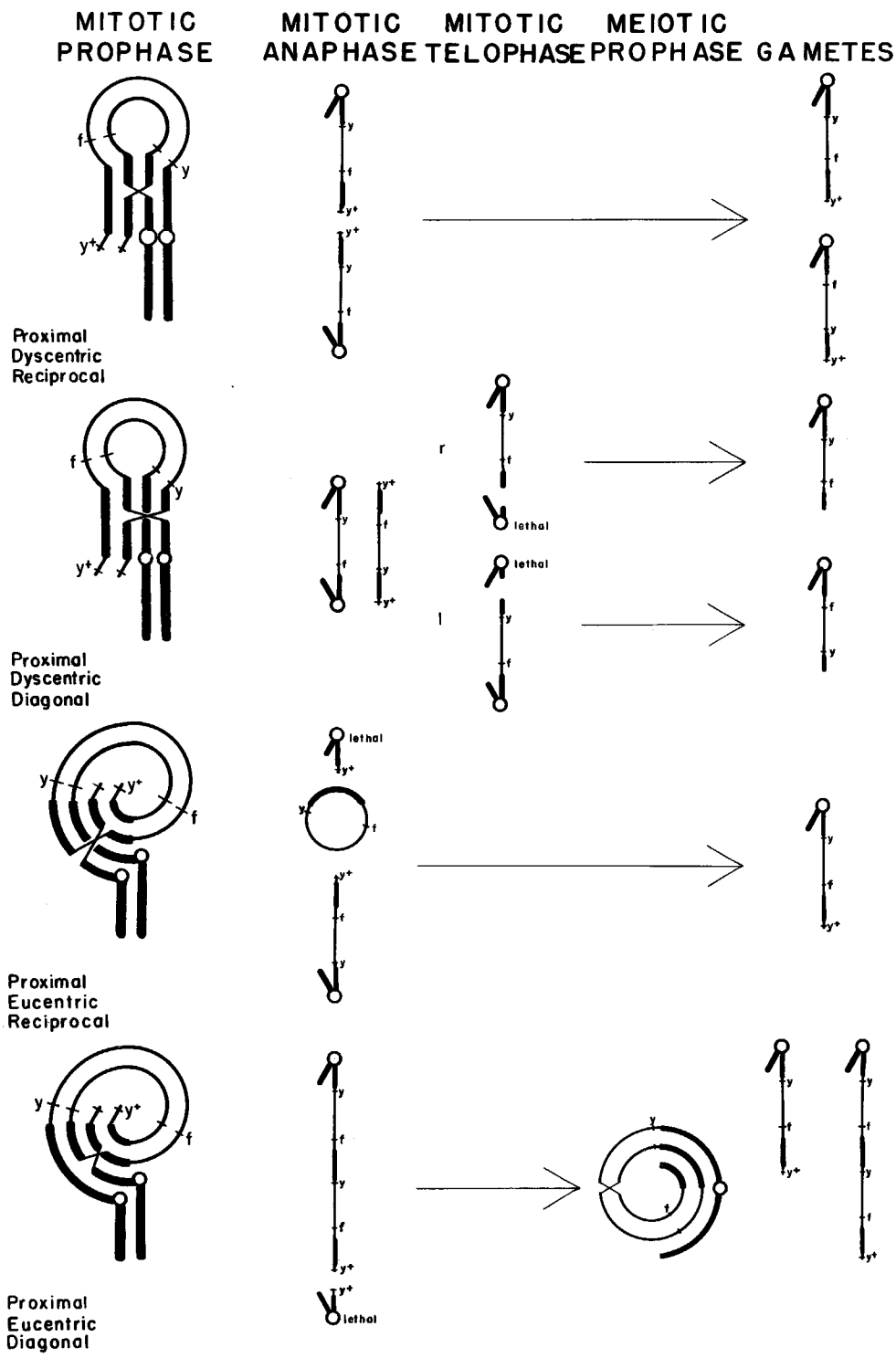

Figure 3. Genetic consequences of exchange following different pairing configurations between the distal heterochromatic segment and the base of $In(1)sc^{8L},EN^{R}$ (heavy lines represent heterochromatin).

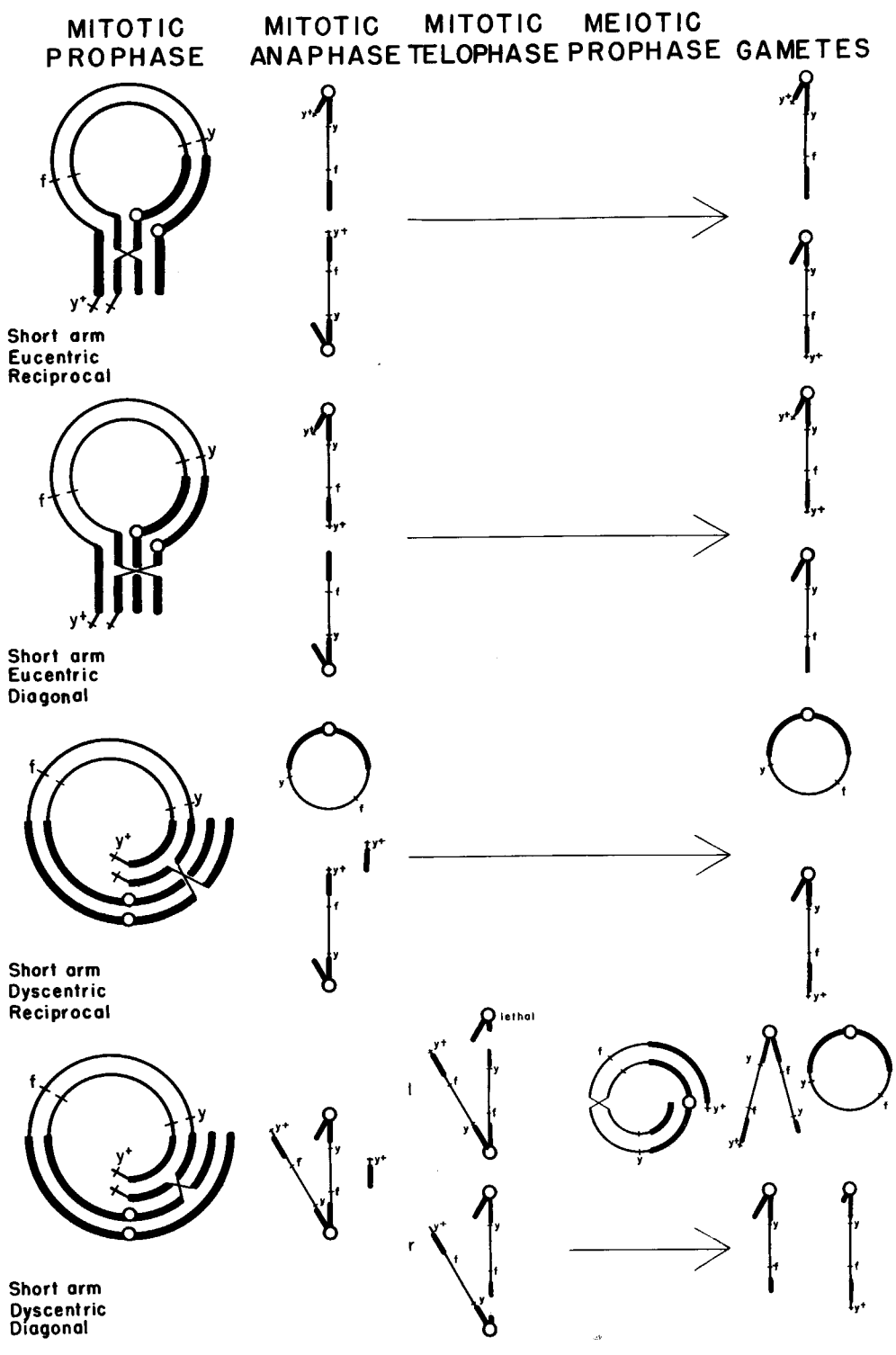

Figure 3 continued.

It will now be profitable to examine figure 3 in considerable detail. Dyscentric pairing of the distal heterochromatic segment with the proximal heterochromatic segment followed by a reciprocal exchange results in a reinversion of the interstitial euchromatic region of one member of the dyad. No change of the markers occurs, however, and such an event is impossible to detect. A diagonal crossover in place of the above reciprocal exchange, however, results in the formation of a dicentric chromatid. This dicentric may break at either end, yielding in one case a typical $sc^{8}EN$ c.o. X and in the other case a reinverted $sc^{8}EN$ c.o. X, a Y arm being absent in both cases. No other configuration in figure 3 results in both of these types of chromosomes, and it can be seen from the diagram that even this configuration should yield either one chromosome or the other, but never both as a result of a single event. Looking back to cluster F-7 previously discussed (page 24), it will be remembered that both of these chromosome types were recovered from a single cluster, presumably the result of a single event. Either of two possibilities can explain this cluster: Sister centromeres may have gone to the same pole following the exchange giving rise to two dicentrics in the subsequent division; or anaphase chromatids may already be split into half-chromatids for the succeeding division. There seems to be no clear way of resolving this question.

Eucentric pairing between the distal and proximal heterochromatin followed by reciprocal exchange results in no viable recombinants, but

with diagonal exchange a tandem double X (terminology of Novitski 1951) is produced, and it passes, together with a complete Y chromosome, to the daughter nucleus. Viability and the ability to produce sperm of genetically female cells arising in the testes has been discussed previously, but in the present situation a new question becomes important. Does the mere fact that such cells are 2X:2A allow their chromosomes to undergo meiotic crossing over? It can be seen in figure 3 that a reciprocal meiotic exchange would result in an apparent non-recombinant ^{X} and an acentric ring. A diagonal crossover would have no observable consequence (not shown because four strand stage not depicted). From the event described above tandem double X chromosomes should result. No evidence of this possibility has been obtained.

Reciprocal exchange following eucentric pairing between the short arm and the distal heterochromatin of $In(1)sc^{8L}$, EN^{R} results in the transposition of y^{+} from the distal heterochromatic segment to the short arm in one chromatid and no recombination in the other; this event is undetectable. Such pairing followed by diagonal crossing over, on the other hand, results in transposition of y^{+} from one chromatid to the short arm of its sister. The separating chromatids, then, are an In(1)EN chromosome with the right end of $In(1)sc^{8}$ located on the distal end of each arm (undetectable), on one hand, and a $sc^{8}EN$ c.o. X on the other. Such a $sc^{8}EN$ c.o. X carries the short arm of In(1)EN in place of the $In(1)sc^{8}$, y^{+} tip.

It seems likely that many of the chromosomes recovered from experiments 1 and 2 which carry no Y fragment and are still inverted are of this nature.

Dyscentric pairing between the distal heterochromatin and the short arm followed by reciprocal exchange yields a non-recombinant and a ring from which y^+ has been lost as an acentric fragment. The large cluster of 11 sc^8EN c.o. X's (C-1) probably arose through such an event. The final possibility is dyscentric pairing between the distal heterochromatin and the short arm with a subsequent diagonal exchange. The dicentric thus formed can fragment in several ways. Breakage to the right of f results in one product which cannot be distinguished from a non-recombinant and one sc^8EN c.o. X chromosome with no Y arm. Breakage to the left of y results in one inviable daughter cell and one carrying a tandem attached X; assuming the occurrence of meiotic crossing over in 2X:2A primary spermatocytes, ring X bearing gametes should be recovered in addition to the attached X gametes. It will be noticed that fragmentation somewhere in the euchromatic portion of the bridge will result in the passage to one pole of a complete X chromosome plus a portion of the normal right end of the X, the size of which depends upon the point of breakage. No change in markers would result, however, if the break were near the f end of the bridge, and such flies would have to be recognized by symptoms of duplication in males; if such males were detected they would most likely be sterile. If the bridge were to break near the

y end an attached X deficient for the tip of one arm would result; it would be subject to the same meiotic crossing over possibilities as in the case of breakage to the left of yellow. Breaks in the middle of the bridge should yield inviable combinations to each pole.

Examination of figure 3 reveals that, barring the doubtful occurrence of meiotic crossing over in 2X:2A primary spermatocytes, no case of segregation following exchange in a dyad gives rise to more than one recognizable recombinant. One might conclude therefore that clusters arising from such events can yield no more information than a single recombinant offspring. The occurrence of cluster F-7, however, seems to invalidate such a conclusion, and it is probable that all future experiments should be designed with maximal cluster production in mind.

The sc^{8}EN c.o. X chromosomes, which carry no Y arm, are of three types; two of these types may originate from more than one of the configurations diagramed in figure 3. The most frequent type recovered is that in which the distal euchromatic segment has merely been removed and no recognizable material put in its place; in these cases the EN inversion is still present. Such a chromosome may be a product of breakage of the bridge arising from a diagonal exchange following dyscentric pairing between distal and short arm heterochromatin. Only a break to the right of f can result in this type of chromosome. There is no a priori reason for expecting one end of the bridge to break preferentially; consequently for every

inverted $sc^{8}EN$ c.o. X there should be a reinverted $sc^{8}EN$ c.o. X. Since, at most, two reinverted $sc^{8}EN$ c.o. X chromosomes were recovered from the $In(1)sc^{8L}$, EN^{R} experiments the breakage of such bridges cannot be a major contributor to the inverted $sc^{8}EN$ c.o. X class of effects. A second source of inverted $sc^{8}EN$ c.o. X chromosomes is diagonal crossing over when the distal heterochromatin pairs eucentrically with the short arm; this results in replacement of the distal $In(1)sc^{8}$ euchromatin with the short arm. This event may very well be important in the production of most of the $sc^{8}EN$ c.o. X chromosomes. A third configuration which can give rise to $sc^{8}EN$ c.o. X's is dyscentric pairing between the distal heterochromatin and the short arm with subsequent diagonal crossing over. The bridge thus formed must then break to the right of f; a break to the left of y which should be equally frequent should give rise to tandem attached X's, which have not been recovered. A second argument against the last configuration as a significant source of $sc^{8}EN$ c.o. X's is that for any configuration, reciprocal and diagonal exchange should be equally frequent. A reciprocal exchange in place of the above postulated diagonal one would result in ring formation, and only one case of ring formation has been recorded.

Removal of the left end of $In(1)sc^{8}$ and reinversion of the In(1)EN sequence results in the second class of Y independent $sc^{8}EN$ c.o. X chromosomes. This type can only arise through breakage, to the left of y, of the bridge formed by diagonal crossing over

between dyscentrically paired distal and proximal heterochromatin of $In(1)sc^{8L}$, EN^{R}.

Rings form the third class of Y independent recombinants recovered. They can come about through exchange following dyscentric pairing between the distal heterochromatin and the short arm. A reciprocal crossover immediately gives rise to a ring; this seems to be the most likely method of ring production. Diagonal crossing over may result in the tandem attached X which may form a ring by meiotic crossing over. The first of these two schemes is the only one which can account for the one case of ring formation recorded. Cluster C-1 contained 11 $sc^{8}EN$ c.o. X chromosomes, all of which were presumably rings (7 tested); any such large cluster resulting from the second scheme outlined above would be a mixture of rings and tandem attached X's.

Interpretation of results with $In(1)sc^{8}$

As shown by Muller, Raffel, Gershenson and Prokofyeva-Belgovskaya (1936) the distal heterochromatin of $In(1)sc^{8}$ contains the locus of bb, Block A and Block B; Kaufmann (1944) has demonstrated the presence of the nucleolus organizer in this region. This accounts for a major portion of the mitotic heterochromatin, although the inversion may not include the most proximal synaptic blocks of the X reported by Gershenson (1940). With respect to salivary gland chromosomes, however, Muller et. al. report that a considerable portion of the chromocentral region of X lies to the right of the right break of

In(1)sc^{8}. If heterochromatic regions can indeed exhibit heterologus pairing, the possibility of pairing between distal and proximal heterochromatic segments exists, but in absence of evidence bearing on this point it will not be further considered. By far the most important type of heterochromatic association is almost certainly that between the distal heterochromatin of In(1)sc^{8} and the Y. Figure 2 can be used to discuss this relationship and its consequences, but it must be recalled that the base of the X chromosome in this case carries no heterochromatin to speak of and no alleles of y and ac. Recombinant X chromosomes therefore are deficient for the y ac region, and cells hemizygous for this deficiency result from many of the events depicted in figure 2. Demerec and Hoover (1936) have shown, by obtaining homozygous deficient hypodermal spots, through somatic segregation, that y ac deficient tissue is not cell lethal in the female. Ephrussi (1934) has shown in males that somatic elimination of a small covering fragment gives viable patches of y ac deficient tissue. It is reasonable to assume, therefore, that spermatogonial cells carrying deficient recombinant X chromosomes should be able to proliferate. Such cells, however, may be rather inefficient in competition with normal cells, or a critical mass of deficient tissue may limit the size of clusters obtainable.

The theoretical results of eucentric exchange are similar to those postulated for In(1)sc^{8L}, EN^{R} in figure 2. The 2 sc^{8} c.o. X: 1 sc^{8} c.o. Y ratio may be slightly altered toward 1:1 due to the

possible selective disadvantage of the deficient sc^8 c.o. cells resulting from segregation of type x. The analysis of the sc^8 c.o. X's is in agreement with that of the sc^8EN c.o. X chromosomes in that six out of nine cases show Y^S to have been the synaptic arm, where Y^L can be implicated in none of the nine (E_1-a, E_1-b, P_0, 89a, 91b, 67h(2)).

The three sc^8 c.o. X chromosomes carrying no Y arm (25b, 99c, 67h(1)) may be explained on the basis of dyscentric exchange. Since none of the sc^8 c.o. X's is a reinversion, and therefore fertility of female cells deficient for a Y arm is not indicated, breakage, to the right of f, of the bridge formed by z segregation following dyscentric exchange is sufficient to account for all three Y deficient sc^8 c.o. X's. Which arm of the Y was synaptic in such cases cannot be determined.

Cluster 67h is of interest in that it fails to conform to any of the schemes so far postulated; although its members can be explained individually, as they have been above, they cannot be explained as the result of a single event. Both retain an inverted sequence, yet one carries Y^S (67h (2)) while the other carries no Y arm (67h (1)). Explanation of this pair of chromosomes in terms of one event, however improbable, should be attempted before resorting to two independent events with frequencies ofthe order of those being discussed. Since Y^S is carried on one of the members, the event must have involved pairing between the distal heterochromatin of $In(1)sc^8$ and the Y. Slight modifications may be imposed upon one

of the chains of events shown in figure 2 to account for cluster 67h. Imagine first dyscentric pairing with Y^S as the synaptic arm; exchange should now be possible at such a point on Y^S as to include all but the extreme tip of this arm within the consequent dicentric. Segregation of type x results in the passage of this dicentric intact to one pole; the following division must also result in the passage of an intact dicentric to each pole. Once there are two cells containing dicentrics, bridge formation may commence. It should be noticed that the dicentrics thus formed contain a complete Y complement so that the usual sterility complications resulting from dyscentric exchange need not exist. Imagine now double bridge formation in two of the descendents of the cell containing the original dicentric; rr breakage in one case such that both chromatids fragment between f and Y^S fertility factors results in passage to each pole of a Y deficient sc^8 c.o. X and a complete Y (67h (1)); rr breakage in the other case such that one or both chromatids breaks between Y^S fertility factors and the Y centromere results in passage to one or both poles of Y^L and a sc^8 c.o. X with Y^S carried terminally (67h (2)). The fact that 67h (2) carries Y^S terminally, in so far as it is not present as a second arm, has been confirmed in larval neuroblast figures.

If the above explanation is a correct one, serious questions are raised as to the general occurrence of such a phenomenon. It

is possible that other sc^8 c.o. X and sc^8EN c.o. X chromosomes, both with and without Y^S, arose through inclusion of Y^S within the bridge. A terminal Y^S segment originating through dyscentric exchange should be inverted with respect to one originating through eucentric exchange, but the demonstration of such a difference in sequence does not seem possible.

The other cluster obtained from experiments involving $In(1)sc^8$ contains one member that is difficult to explain. It will be remembered from the discussion of experiments involving $In(1)sc^8$ that cluster E_1 contained two sc^8 c.o. X chromosomes, each of which carried Y^S; it also seemed to carry a sc^8 c.o. Y, but the results of test crosses were completely contrary to expectations. The B male originally recovered (assumed to be $Y^SX \cdot Y^L$, In(1)En, B y / sc^8 c.o. Y) when crossed to y w females yielded only B males and y w females. The y^+ allele which was presumed to have originated from the paternal $In(1)sc^8$ chromosome appeared to be attached to the maternal $Y^SX \cdot Y^L$ chromosome. The possibility of reverse mutation of y in $Y^SX \cdot Y^L$ was considered, but further analysis of the B chromosome has ruled out this possibility. The results of this analysis show that this chromosome is the $Y^SX \cdot Y^L$, In(1)EN, B y chromosome, but with the terminal Y^S replaced by the y^+ from $In(1)sc^8$. For want of a better explanation the following events have been postulated to account for this unusual combination of maternal and paternal chromosome elements: The original zygote received a sc^8 c.o. Y from its father and a $Y^SX \cdot Y^L$,

In(1)EN, B y chromosome from its mother; in one of the early cleavage divisions eucentric exchange between the Y^S arm of the sc^8 c.o. Y and the distal Y^S of the $Y^SX \cdot Y^L$ chromosome took place. Subsequent segregation of type z resulted in daughter cells of the composition $X \cdot Y^L$, $In(1)sc^{8L}$, EN^R, y^+ B y / Y and $Y^SX \cdot Y^L$, In(1)EN, B y / sc^8 c.o. Y. Only former type gave rise to germinal primordia, whereas both types could have participated in hypodermis formation, since they both are y^+ B in phenotype.

Reversals of forked

Among the sc^8EN c.o. X chromosomes from experiment 1, after they had been kept in stock for over a year, three stocks were noticed to be f^+ (F-7e, I-7, P-7). It cannot be said with certainty whether the loss of the f phenotype occurred at the time of the original exchange or whether it happened in a subsequent generation and replaced the f allele in the course of successive generations of stock transfers. It will be noticed that two of these three cases are the only two reinversions (F-7e, I-7). Two possible explanations of the existence of f^+ in the reinverted sc^8 c.o. X chromosome immediately come to mind. All stocks were balanced against y w, and the possibility exists that the f^+ allele from the attached X has been transferred to the sc^8 c.o. X; this could occur much more easily in the case of the reinversions than in that of the sc^8 c.o. X's which have retained their inverted sequence, since the former have the same sequence as the attached X arms. Although there are others, one of the simplest

mechanisms which might bring about such an interchange of f alleles is the formation of a female heterozygous for a breakdown product of the attached X and the $sc^{8}EN$ c.o. X; subsequent exchange of bases distal to f would give rise to sc^{8} c.o. X's carrying f. This mechanism would have two predictable consequences: First there would be attached X breakdown products in addition to the regularly expected classes of individuals in stock, and second, the f^{+} chromosomes should carry a Y arm at their base. Neither of these conditions was fulfilled. The second possibility is contamination; to check this the tips and the bases of these two chromosomes were studied more carefully. No residual heterochromatin could be found at the tip of either reinversion; recombinants carrying the tip of the reinversion and the base of a chromosome carrying bb were bb in phenotype in both cases, and the tips of the salivary X chromosomes showed no tendency to pair with the chromocenter. Examination of the centric region in larval neuroblast metaphase plates reveals that F-7e still carries the base of In(1)EN (at least it carries a short arm of the proper length), whereas I-7 appears to be a rod. It seems probable that P-7 is not due to contamination since it still carries the inversion and that F-7e is not contamination since it still carries the base of In(1)EN as it should. In the case of I-7, however, it has not as yet been possible to rule out contamination because of the lack of diagnostic characteristics in this chromosome.

An attempt was made to demonstrate the presence of a supressor

of f in both F-7e and I-7; the progeny of $sc^{8}EN$ c.o. X, y / ec ct s car females were examined for f, which could arise through the separation of f and a suppressor by crossing over. No f flies were found among 437 progeny of females carrying F-7e or among 440 progeny of females carrying I-7. All but quite closely linked suppressors, then, are ruled out.

In experiments 2, 3, and 4 each fly was examined for reversal of f, and no case of f reversal in the paternal chromosome was found. In examining stocks from time to time, cultures containing high proportions of f^{+} males have been observed; tests for the presence of a Y arm resulting from exchange with attached X breakdown products have been negative in the two cases tested. Indications are that loss of the f phenotype occurs spontaneously at an appreciable rate in these stocks, and that the chromosomes in which this change has occurred tend to become established. Whether this change is true reverse mutation is a question that is unanswered, but it seems that there is an unusual phenomenon here that merits further investigation.

P_1 male	Experiment	Designation	Fertility over Y^{cl}	Fertility over Y"	Fertility over T(Y-2)	Normal recombination with +	Normal recombination with $In(1)sc^8$	Reversal of f	Phenotype over nucleolus-less	Phenotype of XO male	X-4 linkage
$In(1)sc^{8L},EN^R$	1	B-1	-	-		-	+	-	+	lethal	-
		C-4	-	-	+	-	+	-	+	lethal	-
		D-6a	-	-	+	-	+	-		lethal	-
		D-6b	-	-		-	+	-		lethal	-
		D-6c	-	-		-	+	-			-
		D-6d	-	-	+	-	+	-		lethal	-
		D-8	-	-	+	-	+	-	+	lethal	-
		F-7a	-	-	+	-	+	-	+	+	-
		F-7b	-	-	+	-	+	-		lethal	-
		F-7c	-	-	+	-	+	-	+	lethal	-
		F-7d	-	-		-	+	-		lethal	-
		F-7e	-	-		+	-	+			-
		I-7	-	-		+	-	+			-
		L-7	+	-	+	-	+	-	+	+	-
		O-7	-	-		-	+	-			-
		P-7	+	-	+	-	+	+	+	+	-
		Q-1	-	-		-	+	-			-
	2	B-7	-	-		-		-			-
		C-1a	-	-		-	-	-			-
		C-1b	-	-		-	-	-			
		C-1c	-	-		-	-	-			-
		C-1d	-	-			-	-			-
		C-1e	-	-		-		-			-
		C-1f	-	-		-	-	-			-
		C-1g	-	-		-	-	-			-

Table 3. Summary of pertinent observations made on derivatives from experiments 1 to 4. For the sake of completeness, observations have been included in this table which have not been discussed in the text due to their, as yet, fragmentary nature.

P_1 male	Experiment	Designation	Fertility over Y^{cl}	Fertility over Y"	Fertility over T(Y-2)	Normal recombination with +	Normal recombination with $In(1)sc^8$	Reversal of f	Phenotype over nucleolus-less	Phenotype of XO male	X-4 linkage
$In(1)sc^{8L},EN^R$	2	C-1i	-	-		-	-	-			-
		C-1j	-	-		-	-	-			-
		C-6	-	-		-	+	-			
		C-13	-	-		-		-			-
	Miscel-laneous	J-3	-	-		-	+	-			-
		K-1	-	-		-	+	-		lethal	-
		P-0	+	-	+	-	+	-	+	+	-
		S-7	-	-		-	+	-			-
		W-0	lethal	-	+	-	+	-	+	lethal	-
$In(1)sc^8$	3	E_1-a	+	-	+	-	+	-	+	+	-
		E_1-b	+	-	+	-	+	-	+	+	-
		P_o	+	-	+	-	+	-	+	+	-
	4	89a	+	-	+	-	+	-	+	+	-
		25b	-	-	+	-	+	-	bb	bb	-
		91b	+	-	+	-	+	-	+	+	-
		99c	-	-	+	-	+	-	+	+	-
		67h(1)	-	-	+	-	+	-	+	+	-
		67h(2)	+	-	+	-	+	-	+	+	-
	Miscel-laneous	XY^S B-1	+	-		-		-			-

Table 3 (cont'd)

Discussion

Non-homologous pairing has generally been associated with duplications of specific euchromatic material or with heterochromatin; non-homologous exchange, however, has been associated only with duplication, more specifically with repeats. It seems possible to interpret the data presented above in terms of the pairing configurations possible within a heterochromatic reverse repeat system.

If it is assumed that strict pairing relationships are maintained within the heterochromatic elements of the chromosome complement and further that within the sex chromosomes all heterochromatic segments have at least one block of pairing sites in common, it can be seen that one definite direction of pairing between any two segments will be enforced. If one segment, however, carries a reverse repeat for this common region, it may pair eucentrically or dyscentrically with any of the other heterochromatic segments. A reverse repeat in the distal In(1)sc^{8} heterochromatin will allow all configurations diagramed in figures 2 and 3 to occur; certain further conditions must be imposed to account for certain aspects of the observed results. If Y^{L} contains a region homologous with that carried in the reverse repeat postulated for X, evidence of eucentric exchange between these elements should exist. Gershenson (1940) points out that although Y^{S} and Y^{L} both contain regions homologous to X, one arm (probably Y^{S}) is synaptically more active than the other. It may be that Y^{L} is potentially able to undergo exchange with X heterochromatin, but is less able to realize

this potentiality in competition with Y^S.

The presence of a reverse repeat in the X heterochromatin is by no means proved, but postulation of its existence eliminates the necessity of thinking in terms of exchange between non-homologous regions. It may be possible in the future, through genetic studies of the heterochromatic changes that have taken place in the derivatives described in this work, to obtain some evidence on the presence or absence of a heterochromatic repeat.

The postulated rupture of an anaphase bridge results in the inclusion of a freshly broken chromosome end in both daughter nuclei, whereas breakage following double bridge formation produces daughter nuclei containing two freshly broken ends. These facts immediately raise questions concerning the possibilities of fusion of sister chromatid ends in the first case and of homologous chromosome ends in the second; McClintock's work (1938, 1941, 1942, 1944) on maize has served to emphasize the importance of such a possibility. In short, her findings are as follows: If a dicentric is formed during meiosis, it forms a bridge which fragments at anaphase I sending a broken chromosome end to each daughter nucleus; the daughter chromatids of each broken chromosome fuse with one another at the point of breakage giving rise to new dicentrics which initiate new bridge-breakage-fusion cycles. This chromatid cycle is continued throughout the gametophytic generation, so that fertilization results in the transfer of a chromosome with a freshly broken end to both embryo and endosperm

nuclei. In the embryo the broken end almost invariably heals, but in the endosperm the cycle is continued and results in variegation. Under certain genetic conditions, the chromatid cycle may occur in the sporophyte generation, but there seems to be a tendency for broken ends to heal rather than continue the breakage-fusion cycle indefinitely in this tissue. If two newly broken chromosomes are introduced into the embryo, one via the maternal and the other via the paternal nucleus, the chromosome ends undergo fusion and initiate a chromosome cycle as opposed to the chromatid cycle discussed above. In the case of the chromosome cycle healing rather than fusion may occur in certain nuclei; if these nuclei contain a reasonably complete chromosome complement they will proliferate rapidly at the expense of the nuclei still showing the fusion cycle since the latter nuclei are constantly producing highly unbalanced daughters.

Examination of figures 2 and 3 reveals that with the exception of dyscentric exchange between X and Y followed by x segregation, there is never more than one broken chromosome end distributed to a pole. The chromatid cycle might be expected to occur in such a situation (dyscentric z in figure 2 and proximal, dyscentric, diagonal and short arm, dyscentric diagonal in figure 3); the freshly broken chromosome which gives rise to a viable cell in these cases is generally a complete X; fusion of sister chromatids would result in a tandem attached X with a centromere at each end and with a large heterochromatic segment between the two X's. If McClintock's observations on maize hold for

Drosophila, non-random breakage favoring the site of the fusion is expected at bridge formation during the succeeding anaphase. Cases of breakage at positions other than that of previous fusion are also expected, however; certain euchromatic breaks should survive as duplications for one end or the other of the X, while heterochromatic breaks at either centromere should result in a unicentric tandem double X. These derivatives, of course, may participate in further cycles; the fact that bridge breakage products are recovered, however, indicates that such broken ends do heal in a certain proportion of nuclei. Such being the case, were a chromatid cycle in operation, indications of this fact might be expected among the derivatives recovered from experiments 1 to 4, but no such indications have been obtained.

Although one cannot generalize from the sporophytic generation in maize to the spermatogonia of Drosophila, McClintock's observations have indicated that a given type of cell is potentially capable of exhibiting a chromatid cycle or a chromosome cycle, but generally not both. It may be that in Drosophila spermatogonia, if conditions are right, the chromosome cycle may occur, but never a chromatid cycle. Figure 2 shows that all of the cells in which double bridges are formed yield daughter cells which are probably sterile. Such being the case products of a chromosome cycle, even though it had occurred, may not have been possible to recover from the experimental arrangement employed. On the basis of the data, fusion of broken chromosome ends cannot be definitely ruled out. It should be pointed out that studies of irradiated sperm (Pontecorvo and Muller 1941, Muller 1950) indicate

that dicentrics formed by fusion of broken ends are inviable to a much greater extent than is possible to suppose due to imbalance following loss of the accompanying acentric fragment. It has been claimed by these authors that inclusion of a dicentric in the zygote is almost invariably lethal *per se*. This apparently is not the case when smaller patches of tissue are involved.

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