GENETIC ANALYSIS OF THE MALE FERTILITY FACTORS ON THE Y CHROMOSOME OF DROSOPHILA MELANOGASTER[1]

GEORGE E. BROSSEAU, JR.[2]

Biology Division, Oak Ridge National Laboratory,[3] Oak Ridge, Tenn.

Received August 21, 1959

THE Y chromosome of Drosophila has been the subject of many genetic studies, but by and large its genetic properties and organization have remained a mystery. The intractibility of the Y to genetic study is caused by the lack of clear-cut genetic effects (mutants) associated with it and by its unsuitability for classical genetic study (e.g., lack of crossing over, frequent position effect). The Y is certainly much more than a pairing partner for the X; however, unusual genetic techniques are generally necessary to demonstrate many of its genetic properties.

The Y chromosome of *Drosophila melanogaster* is wholly heterochromatic (HEITZ 1933) and is very poor in classical gene content for its cytological length. From a cytogenetic investigation of the base of the X and the Y, MULLER and PAINTER (1932) concluded that these heterochromatic regions were genetically inert. This seems to be substantiated by the lack of any phenotypic effect associated with the presence or absence of a Y in the male and female fly (BRIDGES 1916), both with respect to viability and to sex-linked characters, although the normal-appearing X/0 males are completely sterile. STERN (1929) showed that each arm of the Y carries a complex of factors necessary for male fertility. The function of these fertility factors is still not well understood, but they are in some way related to sperm maturation and motility. Studies of spermatogenesis in X/0 males (SAFIR 1930; SHEN 1932) revealed that meiosis occurs regularly and that most stages of spermiogenesis appear normal. However, the sperm apparently degenerate before the completion of maturation since very few mature sperm are found in the vasa efferentia of X/0 males and these sperm are never motile.

In addition to the fertility genes, the Y carries a region that is homologous to part of the base of the X. STERN (1927, 1929) established that a wild type allele of bobbed (bb^+), a gene located in the proximal heterochromatin of the X, was represented on the Y. HEITZ (1934) found that a nucleolus-organizing region was present on both the X and the Y. The nucleolus organizer and probably bb^+ are on the short arm of the Y (Y^s) (see COOPER 1959 for discussion). Further evidence for homology between Y^s and the base of the X comes from studies of crossing over between the X and the Y (STERN 1929; KAUFMANN 1933; PHILIP 1935; NEUHAUS 1936, 1937; LINDSLEY 1955a,b). These exchanges occur very infrequently and may be mitotic in origin (gonial). These studies indicate that Y^s is

[1] Work performed while supported by an American Cancer Society Postdoctoral fellowship (No. F-276A).

[2] Present address: Department of Zoology, State University of Iowa, Iowa City, Iowa.

[3] Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

the arm most frequently involved in spontaneous exchange (see LINDSLEY 1955b for discussion). Finally, NEUHAUS (1938, 1939) gave evidence for a fertility factor common on the base of the X and the long arm of the Y (Y^L), but the existence of this factor and its possible location are not definitely established.

From a cytological study of pairing in meiosis in males, COOPER (1949, 1952) concluded that pairing between the X and the Y occurs at specific sites or collochores. There are two of these collochores on Y^S and perhaps two more on Y^L. Whether these collochores are regions of true homology is not certain, for in certain chromosomal rearrangements the base of the X seems to be able to pair with the fourth chromosome (SANDLER and NOVITSKI 1956) as can the Y (GRELL, 1959b; personal communication). LINDSLEY's analysis (1955b) of crossing over in XY compounds indicates that, if the collochores on the X and Y are regions of homology, these regions have no polarity and are equal to each other. The regular disjunction of the X and Y in normal males clearly indicates that these elements share a homologous region. Some of the detachment products produced by crossing over in the tandem metacentric and tandem acrocentric compound X chromosomes are lethal if no homolog is present in the meiotic nucleus. The presence of a Y greatly increases the frequency of single exchanges in the reversed acrocentric compound X (SANDLER 1954) but has no effect on crossing over in the reversed metacentric or on the autosomes. This effect is specific for the Y inasmuch as it is not duplicated by a free duplication for the base of the X.

Other genetic properties of the Y are even less clear-cut. The Y chromosome has a very strong modifying effect on V-type position effect (see LEWIS 1950 for review). The presence of an extra Y chromosome suppresses the variegation, making the phenotype more like the normal (GOWEN and GAY 1933, 1934; DUBININ and HEPTNER 1935; SCHULTZ 1936), whereas the absence of a Y enhances it (NOUJDIN 1936). The factors responsible have not been located but are probably present on both arms of the Y (NOUJDIN 1938). From work of BAKER and SPOFFORD (1958), it seems that discrete functional units may be responsible for the effect of the Y on variegation inasmuch as the ability of Y-chromosome fragments to suppress variegation is not related to their size.

It has been suggested that the Y chromosome carries a system of polygenes (BARRIGOZZI 1948, 1951; BARRIGOZZI and DI PASQUALE 1953; MATHER 1944; and others). These studies are based on minor differences between males and females in quantitative characters such as the number of sternopleural chaetae and the number of wing hairs. The evidence in favor of polygenes on the Y is by no means compelling; in fact in one case, where the comparison was made, there was no difference between the X/0 and X/Y males. COOPER (1945) pointed out the questionable nature of MATHER's (1944) results. NEUHAUS (1939) presented evidence for an effect of the Y on several metric characters, but again the data are only suggestive.

Other effects become evident when supernumerary Y's are present. Although the gross appearance of the flies and the sex balance are unaffected, flies with two Y's more than the normal complement have mottled eyes (COOPER 1956), and the percentage of flies with mottled eyes is related to the number of extra Y

arms. Males with three Y's are sterile (SCHULTZ in MORGAN, BRIDGES and SCHULTZ 1934; COOPER 1956), and four Y's are probably lethal (COOPER 1949). Female fertility is slightly reduced by one Y and strongly reduced by two (GRELL 1959a). The effect of Y hyperploidy on the fertility of the male and female is not dependent on the dose of the fertility factors but may depend on a factor or factors proximal to the fertility genes (COOPER 1956).

Work on the effect of a Y chromosome in the female on nucleic acid composition and content of certain cells has yielded new clues to a possible functional activity of the Y (see SCHULTZ 1956 for review). Females with a Y produce eggs that have a higher amount of ultraviolet-absorbing material (CASPERSSON and SCHULTZ 1938), which at first suggested that these eggs had an increased amount of nucleic acids. Later work demonstrated, however, that the total amount of ribonucleic acid (RNA) is essentially unaffected (CALLAN 1948; SCHULTZ 1956), but that its composition is altered. LEVENBOOK, TRAVAGLINI and SCHULTZ (1958) analyzed the base composition of the RNA in X/X and X/XY (the attached-XY chromosome) eggs and found that the X/XY eggs had significantly more adenine than X/X eggs. This altered RNA has a higher specific ultraviolet absorption than normal RNA, which accounts for the earlier results. Identification of the base composition of hydrolyzates of cold perchloric acid extracts of eggs of different genotypes reveals further differences. The purine content of X/XY eggs is more than double that of X/X eggs; principally owing to adenine-containing compounds, which are increased almost four times. The total amount of pyrimidine is about the same in the two genotypes; however, the X/XY eggs have only a trace of thymine and a large amount of cytosine, whereas the reverse is true for the X/X eggs (TRAVAGLINI, LEVENBOOK and SCHULTZ 1958). The XY chromosome is abnormal in its heterochromatic composition in that some of the heterochromatin from the proximal part of the X is present and it is likely that part of the Y heterochromatin is absent. Therefore, some of these studies were extended to include the effect of a normal Y. Comparisons were made between eggs from attached-X ($\overset{\wedge}{XX}$) females with and without a Y. The same general picture was obtained except that the increase in thymine in the $\overset{\wedge}{XX}$/Y eggs was only one sixth as great as the increase in the X/X eggs. The authors suggest that the effect of the Y is on the synthesis of nucleic acid precursors that accumulate in the unfertilized and to some extent in the fertilized X/XY egg. It is clear that disruption of the heterochromatin-euchromatin balance by addition of a Y chromosome affects nucleic acid metabolism but it is not yet clearly established that the Y normally has a significant role in the control of nucleic acid synthesis. An extra Y has no measurable effect on the amount of RNA in salivary gland cells (PATTERSON, LANG, DACKERMAN and SCHULTZ 1954) or in the adult fly (ALTORFER 1953). The Y does increase the amount of DNA in follicular and nurse cells (FREED and SCHULTZ 1956). This effect is independent of ploidy and is greater than can be accounted for by the DNA of the Y itself.

It is obvious from this brief survey that our present knowledge of the genetic properties of the Y chromosome are far from satisfactory. Of the known genetic

factors on the Y, the fertility genes provide the most promising approach to a further analysis of the Y chromosome. The first two questions to be answered about these factors are: how many genes are there in each complex and can they be linearly ordered? The results of these studies show that there are at least two fertility genes on Y^S and at least five on Y^L. These genes can be linearly ordered on the chromosome.

Terminology

An unambiguous symbolic representation for the various altered Y chromosomes described in this paper required a new terminology. A system for noting the constitution of XY compound chromosomes has already been presented by LINDSLEY and NOVITSKI (1959). STERN (1929) originally represented the fertility complexes by the symbol *K*, where K_1 is the fertility complex of Y^L, and K_2 that of Y^S. This system has serious limitations when the complexes are resolved into their components. The *K* can be retained and the Y^L complex as a whole symbolized by *KL* and the Y^S complex by *KS*. Lower case letters with a numeral added designate individual loci. The fertility genes of *KL* are arranged numerically starting at the centromere end of the complex, e.g., *kl-1*, *kl-2* . . . *kl n*, as are those of *KS*., *ks-1*, *ks-2* . . . *ks n*. If more loci are described in the future, the numbering system of the appropriate complex will be shifted so that the numerical order of the loci is maintained. In accord with the usual Drosophila notation, the normal allele is indicated by a "+" or specifically *kl-1*$^+$, *kl-2*$^+$, etc., and the mutant alleles are *kl-1*, *kl-2*, etc. A sterile Y is designated as Y^{KL-} or Y^{KS-}, depending on the location. The complete notation of an XY compound chromosome, such as the attached-XY, is $Y^SX{\cdot}Y^L$; *In(1)EN; KL* $y{\cdot}$*KS* y^+ (LINDSLEY and NOVITSKI 1959). The raised dot indicates the position of the centromere. In this paper this particular compound chromosome will be referred to simply as the attached—XY.

The symbols used in this paper are as follows: achaete (*ac*); Bar (*B*); bobbed (*bb*); bobbed-deficiency (*bb*-Df); brown (*bw*); Inversion (1) EN (*In(1)EN*); Inversion (1) delta-49 (*In(1)*dl-49); Inversions (2L + 2R) Curly (*Ins(2L + 2R)Cy*); Inversion (3LR) Dichaete (*In(3LR) DcxF*); miniature (*m*); vermilion (*v*); scute (*sc*); scute^8 (sc^8); scute^{S1} (sc^{S1}); shaven naked (sv^n); white (*w*); yellow (*y*); and Y chromosome long arm, closed (Y^{cl}).

Recovery of the sterile Y chromosomes

Normally a Y chromosome with a mutation or deficiency in the fertility factor complex (a sterile Y) would be eliminated as soon as it arose owing to the inability of males receiving it to leave progeny. This difficulty can be circumvented by the use of the attached-XY chromosome, which carries attached to a single centromere all the sex-chromosome material necessary for male viability and fertility. Males carrying a normal X marked with yellow (*y*) and $sc^8{\cdot}$Y [a Y carrying as a duplication the tip of the X including the marker y^+ (MULLER 1948)] were irradiated with 3000r of X-rays and mated to homozygous XY, *y B* females. If any of the F_1 sons receives a sterile Y, he will still be fertile owing to the normal

fertility genes on the attached-XY. The F_1 males were pair-mated to *In(1)*dl-49, *y w m*/XY, *y B* females. The X part of the XY is in inverted order, thus in the heterozygote with *In(1)*dl-49, the two chromosomes differ by two inversions and crossing over is almost completely absent. After the females were allowed to ovaposit for six days, the parents were discarded. When these cultures were 15 days old, the progeny were shaken into a fresh culture vial and subsequently were discarded after six days. This progeny should contain males of two types: XY, *y B*/Y* and *In(1)*dl-49, *y w m*/Y* where Y* is the irradiated Y. Absence of a paternally derived dl-49 X chromosome in the next generation indicates the presence of a damaged Y, Y^{K-}. In practice this was recognized by the absence of *y w* non-*B* females among the progeny. These crosses are summarized in Table 1. The presence of *w*, non-*B* females can easily be detected by examination through the side of the vial, and a large number of cultures can be examined quickly. Those cultures in which no *w*, non-*B* females were found were retained for further testing since they were presumed to carry a sterile Y. The *In(1)*dl-49, *y w m*/Y* males were isolated and tested for fertility, and the XY, *y B*/Y* males were crossed to attached-X females to propagate the stock.

A total of 1650 sc^8·Y chromosomes were screened for fertility, and 57 putative sterile Y's were recovered. In addition to these, there were six sterile Y's recovered in the F_1 on the basis of loss of the y^+ markers. Actually a large number of y^+ losses were found, but unless they carried both bb^+ and one or the other fertility complexes, they were presumed to be deleted X's or whole Y-chromosome losses and the cultures were discarded. The sterile Y's were retested by crossing the XY, *y B*/Y^{K-} males to free X females and testing the F_1 males for fertility. In a number of the cultures, the marker y^+ did not segregate regularly from the XY chromosome. In these cases it was assumed that y^+ had been transferred to one of the autosomes, and these cultures were discarded. The possibility

TABLE 1

The crosses used to detect and recover sterile Y chromosomes

P	XY, *y B*/XY, *y B*	×	*y*/sc^8·Y*, y^+	
F_1	XY, *y B*/*In(1)*dl-49, *y w m*	×	XY, *y B*/sc^8·Y*, y^+	
F_2	Females		Males	
	XY, *y B*/XY, *y* B XY, *y B*/*In(1)*dl-49, *y w m*	×	XY, *y B*/sc^8·Y*, y^+ *In(1)*dl-49, *y w m*/sc^8·Y*, y^+	
F_3†	Female gametes		Male gametes	
	XY, *y B*		*In(1)*dl-49, *y w m* (when sc^8·Y* is fertile)	*In(1)*dl-49, *y w m* (when sc^8·Y* is sterile)
XY, *y B*	*y*, narrow-*B* female		*y*, wide-*B* female	None
*In(1)*dl-49, *y w m*	*y*, wide-*B* female		*y w m*, non-*B* female	None

• The males carrying this Y chromosome were irradiated with 3000r of X-rays. The irradiated Y is marked with an asterisk so that it may be followed through the crosses used.

† In the F_3 cross only the gametes giving rise to females are considered. Cultures that did not have *w*, non-*B* females were presumed to carry a sterile Y and were saved for retesting.

remains, however, that some of these cultures had Y chromosomes in which the pairing sites (collochores) had been deleted along with some of the fertility genes. In this event, deficiences for fertility genes closely linked to collochores would be selected against.

The sterile Y cultures that remained were tested to determine the affected arm. Attached-X females with the sterile Y's were crossed to X's with a single Y arm attached, X·Y^L or X·Y^S. If the sterile Y is fertile with X·Y^S but not with X·Y^L, then the sterility mutation is on Y^S; the converse is true for Y^L mutations. These sterile Y cultures were tested for autosomal translocation using *Ins(2L + 2R)Cy* for chromosome II, *In(3LR)DcxF* for the third, and sv^n for the fourth. All the translocations found were discarded lest the rearrangement cause a position effect and possibly yield results that would be difficult to interpret. All sterile Y's that survived these tests were presumed to be free of gross interchromosomal rearrangements and to carry mutations or deletions in the fertility regions. In this group there were 33 Y^{KL-}'s and 12 Y^{KS-}'s. When the y^+ losses were added we had 37 Y^{KL-}'s and 13 Y^{KS-}'s. The complementation tests described in the next section were carried out with 30 of the Y^{KL-}'s and 11 of the Y^{KS-}'s. The Y's that were excluded included the y^+ losses, and three Y^{KL-}'s that were lost before they could be tested, and one Y^{KS} that spontaneously reverted to fertility. This latter reversion was probably not a reverse mutation, but rather the result of an exchange with XY and is discussed in a later section.

Complementation tests

Females that were *y v/ y v/* Y:bw^+; *bw* (a normal Y carrying as an insertion a duplication for a small part of the second chromosome including the locus bw^+) were crossed to XY, *y B*/Y^{K-}, y^+ males. The *yv*/Y:bw^+/Y^{K-}, y^+;*bw* males in the progeny were then crossed to $\overset{\triangle}{XX}$, *y*/Y^{K-}, y^+;*bw* females. Among the progeny of this cross are two kinds of y^+v; *bw* males in equal frequency, those with one Y^{K-}, y^+ and those with two. If the Y^{K-} from the female was different from the Y^{K-} from the male, resulting Y^{K-}/Y^{K-} males would be heterozygous for two different sterile Y's. Furthermore, this particular type of male could be made up in two ways depending on which parent contributed which Y^{K-}. Any two sterile Y's could be tested for complementation by testing the proper X/Y^{K-}/Y^{K-} males for fertility. In this manner all possible combinations of Y^{KL-} and Y^{KS-} were made up and tested. Each of the possible combinations were made reciprocally so that each test was replicated. In all cases where the results of the replicate tests were discordant a retest was made. The very few discordant tests found are discussed later.

The actual complementation test consisted of placing 40-50 *y v*/Y^{K-}/Y^{K-}; *bw* males in a bottle with 30 *y v/y v;bw* virgin females. The resulting cultures were classified as sterile or fertile on the basis of the presence or absence of larval tunnels on the food after seven days. The progeny of the fertile cultures were checked for evidence of nonvirginity of the test female and for possible inclusion of X/Y:bw^+/Y^{K-} males among the test males. As pointed out in the preceding

paragraph, the $X/Y^{K-}/Y^{K-}$ males were phenotypically identical to their X/Y^{K-} brothers; therefore 80–100 $y^+v;bw$ males were actually used in the test, ensuring the presence of an adequate number of the desired males. At a later time it was brought to my attention that males with two sc^s·Y, y^+ chromosomes could be distinguished by the presence of hairs in the second posterior cell of the wing. This phenotype was found to be reliable by progeny test of males with one or two normal sc^s·Y, y^+ chromosomes and was used to identify the proper males in all retests. Results of these tests and the few necessary retests are given in the following sections.

Tests with KS

Results of the complementation tests with the Y^{KS-}'s are given in Table 2, where it can be seen that there are three types. Sterile Y's 2, 4, 6, 7, 8, 10, 11, 12, and 13 are all sterile *inter se*, which indicates that they share a deficiency or mutation for the same locus. Since all members of this group are fertile with Y5, Y5 must be mutant at a different locus; therefore at least two fertility loci must be present on Y^S. Sterile Y 14 is sterile with either group and hence must lack both loci. This latter chromosome is the only sterile Y in the whole series (including the Y^{KL-}'s) that is also deficient for bb^+. Since there are only two genes in *KS*, no conclusions about the organization of the genes in this region can be drawn.

The relative frequency of breakage in the four regions defined by the centromere, bb^+, the two genes of *KS*, and the end of the chromosome gives an idea of the spatial separation of the fertility genes and their relative positions on the chromosome. This argument is based on the assumption that all the sterile Y's have deficiencies with two breakpoints (although these may be very close to each other). Alternative hypotheses such as differential mutability of the genes and differential sensitivity of the regions to X-rays cannot be ruled out. The gene deficiency represented by the group of nine Y^{KS-}'s has been designated *ks-1* and

TABLE 2

Results of complementation tests with the Y^{KS-}*'s*

Male	Number of Y^{KS-} introduced through the: Female										
	2	4	5	6	7	8	10	11	12	13	14
2	X†	—	F	—	—	—	—	—	—	—	—
4	—	X	F	—	—	—	—	—	—	—	—
5	F	F	X	F	F	F	F	F	F	F	—
6	—	—	F	X	—	—	—	—	—	—	—
7	—	—	F	—	X	—	—	—	—	—	—
8	—	—	F	—	—	X	—	—	—	—	—
10	—	—	F	—	—	—	X	—	—	—	—
11	—	—	F	—	—	—	—	X	—	—	—
12	—	—	F	—	—	—	—	—	X	—	—
13	—	—	F	—	—	—	—	—	—	X	—
14	—	—	—	—	—	—	—	—	—	—	X

† X, All homozygous combinations sterile; F, fertile combination; and —, sterile combination.

the other factor *ks-2*. Of a total of 22 breaks recovered, ten were between *ks-1* and *ks-2* whereas only two were distal to *ks-2* (assuming the most probable order of the genes to be centromere, bb^+, *ks-1*, *ks-2*). Nine breaks between *ks-1* and bb^+ were recovered but only one break proximal to bb^+ was found. This paucity of breaks proximal to *ks-1* and bb^+ may be caused by selection of Y^{K-}'s that disjoined normally from the XY since this region probably contains the collochores also. Thus these data, though only tentative, suggest that *ks-1* and *ks-2* are probably fairly well separated and that *ks-2* is near the end of Y^s.

Tests with KL

The results of the complementation tests with the Y^{KL-} chromosomes are given in Table 3. The chromosomes that gave identical results in the test crosses presumably have identical deficiencies and are grouped together in Table 3. The 30 sterile Y's fall into 12 unique groups. The first step in the analysis of this table is to define the genetic relation between these groups. We can assume that the group headed by 13 is deficient for a single locus that we can arbitrarily designate as position 1 and is normal or + for an as yet undetermined number of other loci. Then, since 37 complements all the members of the first group, 37 must be + at position 1 and deficient somewhere else, for instance position 2. Number 41 complements both the first two groups and hence is + for positions 1 and 2 but deficient somewhere else, say position 3. This procedure can be repeated for all groups. Results of this analysis are given in Table 4. The data can be completely

TABLE 3

The results of complementation tests with Y^{KL-}

Male	Number of Y^{KL-} introduced through the: Female											
	13, 25, 29, 33	37	11, 20, 27, 41	12, 23	3, 28, 34	36	38	7, 8, 14, 18, 19, 24, 39	32	10	1, 5, 9, 15	4
13, 25, 29, 33	X†	F	F	F	F	F	F	F	—	—	F	—
37	F	X	F	—	F	F	F	F	—	F	—	—
11, 20, 27, 41	F	F	X	—	F	F	—	—	—	—	—	—
12, 23	F	—	—	X	F	F	—	—	—	—	—	—
3, 28, 34	F	F	F	F	X	—	F	—	F	F	—	—
36	F	F	F	F	—	X	—	—	F	F	—	—
38	F	F	—	—	F	—	X	—	—	—	—	—
7, 8, 14, 18, 19, 24, 39	F	F	—	—	—	—	—	X	—	—	—	—
32	—	—	—	—	F	F	—	—	X	—	—	—
10	—	F	—	—	F	F	—	—	—	X	—	—
1, 5, 9, 15	F	—	—	—	—	—	—	—	—	—	X	—
4	—	—	—	—	—	—	—	—	—	—	—	X

† See footnote Table 2.

explained on the basis of five genes. Table 4 includes five additional sterile Y's that were not in the original complementation tests. The genotypes of these Y's were determined by testing against known deficiencies.

Eight of the 12 types of deficiencies are multiple gene deficiencies, several of them overlapping each other. All but one of these deficiencies are continuous, that is to say only adjacent genes are missing in any given deficiency. This observation is consistent with the hypothesis that the genes are linearly ordered on the chromosome. Number 10 is an apparent exception to this rule in that it seems to complement 37. The reason for this is not clear. The combination 10/37 does not restore full fertility to the male, but the combination consistently showed some complementation in three retests. It is not unreasonable that one multiple-break event should be recovered among 41 sterile Y's from an exposure of 3000r. Thus this case probably does not invalidate the rule. The organization of the fertility complex can be best pictured as a linear array of particulate units. In this respect this heterochromatic region is organized in a manner similar to the euchromatic regions of the chromosomes. The results of the analysis of *KL* are given in the form of a complementation map in Figure 1. It is obvious that there are fewer breaks between *kl-4* and *kl-5* and perhaps between *kl-3* and *kl-4* than between the other genes. This could mean that these genes are less widely separated spatially than are the others. However, again it is impossible to rule out differential radiation sensitivity.

In all the original experiments, the sterile Y's seemed to sterilize the male completely. But experiments undertaken for other purposes have revealed that some of the sterile Y's have a low level of fertility. The results of fertility tests with these very low fertility sterile Y's are given in Table 5. Two of these sterile Y's are multiple gene deficiencies. Although it may be imagined that loss of *kl-5*$^+$ or *kl-3*$^+$ alone might permit a low level of fertility, some other explanation is required for these multiple gene deficiencies. One hypothesis that might be

TABLE 4

Genotypes of the Y^{KL-}*'s*

	Sterile Y number											
Fertility factor no.	(30)† 13 25 29 33	37	11 20 27 41	12 23	3 28 34	36	38	(102) (103) 7 8 14 18 19 24 39	32	10	1 5 9 15	(101) (105) 4
kl-1	−	+	+	+	+	+	+	+	−	−	+	−
kl-2	+	−	+	−	+	+	+	+	−	+	−	−
kl-3	+	+	−	−	+	+	−	−	−	−	−	−
kl-4	+	+	+	+	+	−	−	−	+	+	−	−
kl-5	+	+	+	+	−	−	+	−	+	+	−	−

† Numbers in parentheses refer to additional Y^{KL-}'s not included in the experiments comprising Table 3.

TABLE 5

Fertility of the partially fertile Y^{KL-} chromosomes

Y^{KL-} number	No. males tested	No. males fertile	Percent fertile	Average no. of progeny per male	Factors deficient
L41	2948	14	0.47	10.93	*kl-3*
L19	2765	9	0.32	1.56	*kl-3–5*
L34	8881	68	0.76	11.31	*kl-5*
L3	5334	67	1.26	17.30	*kl-5*
L28	2802	20	0.71	5.60	*kl-5*
L36	7030	72	1.02	8.54	*kl-4–5*

tested further is that these chromosomes have localized damage with inactivation of the adjacent fertility genes by position effect. The consequences of this position-effect inactivation are the same as those for loss of the genes by deletion and do not alter the conclusions drawn from the complementation studies in any way. One consequence of this phenomenon was that some of these cultures that produced very few progeny were at first classified as fertile and yielded discordant results between reciprocal combinations of the same two sterile Y's. Retests showed that these were not cases of true complementation.

Other discordant results between reciprocal combinations of the same two sterile Y's turned out to have quite a different explanation. In these cases, an unmarked fertile Y was recovered from the progeny males. Since no unmarked fertile Y is used in any of the stocks or crosses associated with the fertility tests, and since the presence of other markers ruled out contamination, some other explanation for these Y's had to be sought. Further tests indicated that the unmarked Y's could have been the result of crossing over between the sterile Y and the Y^L attached to the centromere of the XY chromosome (BROSSEAU 1958). The frequency of this type of exchange is sufficient to explain the cases found in the fertility tests.

Results of crosses with attached–X detachments

It is well known that both induced and spontaneous detachment of attached–X chromosomes frequently involves one arm of the Y. The common procedure in characterizing these detachments is to test them against one or the other arm of the Y to determine whether the detachment carries one of the fertility complexes. In the preceding section, however, it was shown that a deficiency of one of the genes in each of the complexes is generally as effective in sterilizing the male as loss of the whole complex; thus deficiency of any one of the genes will look like loss of its entire complex. With the sterile Y's, it is possible to demonstrate the presence of part of one of the complexes on some of the detachment products. The usefulness of such a demonstration is evident from the following considerations. (Details of this discussion can be followed in Figure 2.) The exchange pictured involves Y^L; however, exactly the same argument applies to exchanges with Y^S. The detachment can be visualized as a heterochromatic

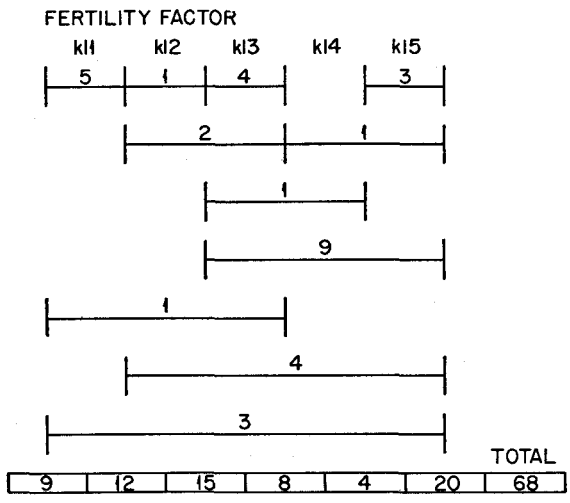

FIGURE 1.—Complementation map for the fertility region of the long arm of the Y chromosome. Figures above the lines, representing the genetic length of each deficiency, indicate the frequency with which that deficiency was observed. The numbers at the bottom of the figure give the number of breaks that occurred between each fertility gene.

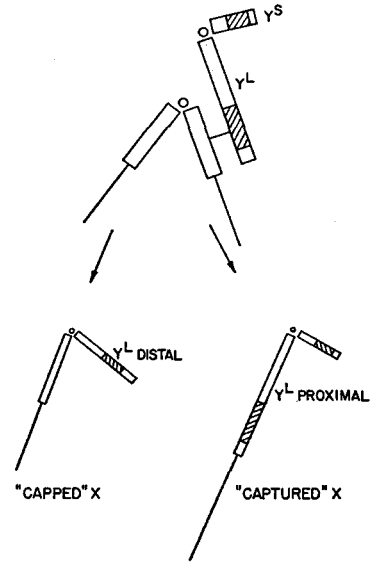

FIGURE 2.—The origin of the detachments of an attached-X chromosome where the detachment involved an exchange between the base of one of the X's and Y^L. The crosshatched areas represent the region of the fertility complexes.

exchange between the arm of the Y and the proximal heterochromatin of one of the X's. By the terminology of ABRAHAMSON, HERSKOWITZ and MULLER (1956), the detachment product that carries a Y centromere with the X arm attached is a "captured" X and the reciprocal product is a "capped" X. If the location of the exchange in the Y arm is within the limits of the fertility complex, the resulting captured X's will carry the proximal portions of the complex and the capped X's will sample the distal parts of it. The detachments can be combined with known deficient Y's and the males tested for fertility to determine which of the genes are proximal and which are distal.

The detachments used in these studies were kindly supplied by DR. D. R. PARKER (PARKER and HAMMOND 1958), who induced them by X-rays. Five of 13 captured X's with an exchange located in Y^L carried a portion of the fertility complex. The results of the tests with these detachments are given in Table 6. These data show that *kl-1*$^+$ is the proximal-most gene. Further confirmation of this is found among the Y^{KL-}'s that had also lost the marker y^+, which is located at the end of the long arm. Sterile Y's 102 and 103 (see Table 4) simultaneously lose *kl-3–5* and y^+, and thus are located distally to *kl-1*$^+$ and *kl-2*$^+$. Only one of the 15 tested capped X's carried any portion of the fertility complex, but this portion was from the middle of the complex (*kl-2*$^+$ and *kl-3*$^+$) indicating that

TABLE 6

Results of tests for Y^L fertility factors on captured-X's from detachment of attached-X's

Detachment no.	Fertility factor deficiency†				
	kl-1	*kl-2*	*kl-3*	*kl-4*	*kl-5*
108–7	+	+	+	+	—
108–43	+	+	—	—	—
110–10	+	+	+	+	—
129–30	+	—	—	—	—
129–34	+	+	—	—	—

† +,Covers deficiency and —, does not cover deficiency.

the detachment involved a complex event. These results place *kl-1*$^+$ at the proximal end of the fertility region and to a certain extent confirm the order of the genes found in the complementation studies.

A similar analysis was carried out with detachments involving Y^s. Only two captured X's of this type were available; however, one of these carried *ks-1*$^+$ indicating that this factor is proximal to *ks-2*$^+$. In the capped X's where Y^s is the cap and the exchange is located within or distal to the fertility region, it is not possible to state positively that the Y is involved in the detachment. The other chromosomes can be ruled out by means of genetic tests with the possible exception of the very tips of the autosomes; it is therefore likely that this sample of "unidentifiable" detachments consists mostly of Y^s caps. Ten out of 37 of these detachments gave evidence of Y^s involvement. Two of these ten carried *ks-2*$^+$ alone, confirming its distal position. The remaining eight detachments are fertile with single gene deficiencies for *ks-1*$^+$ and *ks-2*$^+$ but are sterile with sc^{s1}-Y^L and with a double deficiency for *ks-1*$^+$ and *ks-2*$^+$. These detachments were also fertile with Y^{c1}. These results suggest that a third factor is present, probably on the short arm, and is not represented as a single gene deficiency in the sample of sterile Y's. If a third factor exists then it must be located between *bb*$^+$ and the centromere since Y^{c1} lacks *bb*$^+$ but may contain a short proximal portion of Y^s. BAKER's results (1955) indicated that *KS* is located distally to *bb*$^+$. The failure to recover this possible third factor by itself may be attributable to the selection exercised against the sterile Y's that did not disjoin normally from the X because the pairing sites are probably also located in this region. There is also a possibility that this is the factor shared by the X and the Y, the existence of which was suggested by NEUHAUS (1939). It cannot be stated definitively that this factor is on the Y^s and not the Y^L side of the centromere, although it is most likely very close to the centromere.

Spermiogenesis

Aceto-orcein squash preparations of the sterile males were made for each of the sterile Y's to determine if the various genes could be distinguished from one another on a phenotypic basis. The testes from five males for each genotype, including several X/0 males, were examined. In no case was any phenotypic distinction

possible with the possible exception that the sterile Y's produced a slightly more normal picture than the X/0 males. The X/0 males do not seem to proceed quite so far in sperm maturation as the males with a sterile Y, but this difference is of doubtful significance. In all cases meiosis seems to be completely normal as do the early stages of spermiogenesis. The breakdown apparently occurs at the time of sperm elongation and maturation. Few fully elongated sperm were found, and then these were never motile. The vasa efferentia were usually empty (the presence of a very few sperm would not have been detected in this examination). Thus it has not been possible to distinguish between the fertility genes phenotypically.

DISCUSSION

The fertility region of the Y chromosome was studied by NEUHAUS (1938, 1939), who induced a series of Y-4 translocations that produced only sterile males in the absence of another free Y. His method was to produce males that carried the proximal portion of one translocation and the distal portion of another. He tested these males for fertility. This test is based on the assumption that the translocation carries a mutant fertility gene at the site of the translocation. If the breakpoint of the fragment is located proximal to the breakpoint of the translocation from which the proximal piece is drawn, then the mutation will be covered by the normal genes and the male will be fertile. The opposite is true if the mutation is distal to the breakpoint. A low probability nondisjunctional event is necessary to produce the proper male for testing. Because this male is phenotypically indistinguishable from his nonfragment-bearing brothers it was necessary to test large numbers of males.

NEUHAUS concluded that there were at least four fertility genes on the long arm of the Y and five on the short. These results are at variance with mine. The difference for Y^L is slight (four *vs.* five) and is probably attributable to the small number of Y^L-4 translocations available to NEUHAUS. The difference for Y^S is not so easily explained (five *vs.* two) but is probably the consequence of the different methods used. NEUHAUS could not be sure that he had a male of the right genotype in his test sample, which could have resulted in misclassification. Other errors are also possible. Careful examination of Table 3 of the NEUHAUS paper (1939) reveals several inconsistencies in the results of the fertility tests, which tends to rob the work of any real meaning.

The nature and function of the fertility genes is far from clear at present. All the genes seem to be producing the same effect as evidenced by the similarity of their effects on spermiogenesis, yet each must differ in function as loss of one gene is phenotypically equivalent to loss of the whole set. Superficially, it seems as though each complex is one functional unit; however, the complete complementarity of the mutants indicates that actually multiple-functional sites are involved. Another possibility is that the genes perform successive or related steps in some synthetic pathway. But in the complete absence of any information about the function of the fertility genes in a chemical or physiological sense, it is

not possible to say anything further about this idea—an idea that might have some implications of one possible origin of these genes. The fertility genes could represent the end product of genetic duplication and subsequent divergence of function such as that postulated by SEREBROVSKY (1938) for *ac* and *sc*; there is no experimental evidence for or against this idea.

Several hypotheses have been suggested for the origin of the Y chromosome in Drosophila (MULLER 1918, 1932; HALDANE 1933; NEUHAUS 1939). These hypotheses all have in common the idea that the Y is some kind of degenerate homolog for the X that has resulted from enforced heterozygosis and lack of crossing over. It is beyond the scope of this paper to discuss the genetic inertness or activity of heterochromatin except to point out that the failure to associate a discrete function with a portion of the genome cannot be considered as definitive evidence that this material has no function. The conclusion that the heterochromatin is inert is the result of asking whether it is essential to the individual organism. Often the answer is no. However, the error may be that this is the wrong question. Perhaps we should ask whether heterochromatin is important to the species or population. To this question, we have few answers. The further the "functionless", inert materials of the Y are investigated, the more functions become apparent.

The Y has no genetic regions homologous to most of the X and the view may also be taken that the X has no homolog for most of the genes or known genetic activities of the Y. This view is diametrically opposed to the notion that the Y is a degenerate X. From this new view the Y is not protected from natural selection by the X, but is constantly haploid and therefore constantly exposed to the forces of selection.

If the Y is a degenerate X, then some residual homology between the X and the Y should be found and, as pointed out earlier, this is the case. A portion of Y^S is clearly homologous to a portion of the basal part of the X. However, Y^S has an additional region, the fertility complex, which is not represented on the X. If an additional fertility factor that is shared by the X and the Y exists, the genetic data place it in the Y^S region of X homology not in the fertility complex. There is no good evidence that any of the Y^L has any counterpart on the X. The location of bb^+ has some bearing on this question, for, if Y^L contains a mutant allele of *bb* (NEUHAUS 1939) or if bb^+ is really on the long arm (the evidence against this latter possibility is rather strong but not conclusive, see COOPER 1959), then Y^L does have some homology with the X. The fact that all of the Y^L deficiencies are bb^+ and that the only sterile Y lacking bb^+ is also deficient for the Y^S fertility genes, argues that bb^+ is on Y^S. The *bb*-Df sterile Y also does not cover a lethal *bb*-Df, which indicates that Y^L (presumed to be completely normal in this chromosome) does not carry even a mutant *bb*. It seems likely that the only region of the Y that is homologous to any of the X is the proximal portion of Y^S.

It is obvious that the genetic properties of the Y would have the following characteristics. The genes should be sex limited in their usefulness, as the male fertility factors are. The Y genes might even be expected to be detrimental to

the female, and, indeed, some of them are as evidenced by the loss of fertility in females with extra Y's. The detrimental effects of Y-chromosome hyperploidy could account for the relative stability of the Y in karyotype evolution in the genus Drosophila. Very few of the many chromosome interchanges that have occurred during the evolution of this genus have involved the Y (PATTERSON and STONE 1952), but if the Y is truly inert, many interchanges would have been expected. Only four species of Drosophila have dispensed with the Y altogether and are X/0 in the male. In these cases the essential material has probably been attached to some other chromosome (WHARTON 1944; PATTERSON and STONE 1952), but it is not clear how the potentially detrimental effects have been circumvented. In all the other species in the genus, the Y chromosome has been maintained by natural selection although in the laboratory it is not possible to demonstrate any phenotypic effect of the Y except on fertility. The existence of X/0 species demonstrates that the male fertility factors can be put onto the autosomes; thus these factors are probably not sufficient to maintain the Y as a separate entity. One possible exception is *D. busckii* where the X/0 male is lethal (KRIVSHENKO 1952). The lethality of these males is caused by the absence of the euchromatic short arm of the Y. This arm is derived from a fusion of the microchromosome (corresponding to the fourth chromosome in *D. melanogaster*) and the base of the X and the Y (KRIVSHENKO 1955).

Perhaps the best way to look on the Y chromosome is not as a degenerate chromosomal relic but rather as a unique, specialized element subject to and maintained as an entity by natural selection. The challenge is to uncover the genetic activities of this chromosome. The genetic definition of the fertility factors and the new genetic tools that they provide are a step in this direction.

SUMMARY

The number and genetic organization of the male fertility regions of the heterochromatic Y chromosome were investigated by complementation studies with sterile Y chromosomes. The results indicate that there are at least two fertility genes on Y^S and at least five on Y^L. The data for Y^L are compatible with a linear order of the genes. In this respect this heterochromatic region is organized in a manner similar to euchromatin. Tests with detachments of attached—X's were the detachment has involved an exchange with Y have established the proximal-distal orientation of the fertility genes and confirmed the order of the genes found in the complementation studies. These results also suggest the presence of an additional fertility factor common to the X and the Y. Studies of spermiogenesis uncovered no obvious phenotypic differences in the action of the various fertility genes. It is hypothesized that the Y chromosome is a unique, specialized structure rather than an inert chromosomal relic.

LITERATURE CITED

ABRAHAMSON, S., I. H. HERSKOWITZ, and H. J. MULLER, 1956 Identification of half-translocations produced by X-rays in detaching attached-X chromosomes of *Drosophila melanogaster*. Genetics **41**: 410–419.

ALTORFER, N., 1953 Teneur en acide ribonucléique de différents génotypes chez *Drosophila melanogaster*. Experientia **9**: 463–465.

BAKER, W. K., 1955 On the structure of the sc^8-Y: bw^+ chromosome of *D. melanogaster*. Drosophila Inform. Serv. **29**: 101–103.

BAKER, W. K., and JANICE B. SPOFFORD, 1958 Heterochromatic control of variegation in *D. melanogaster*. (Abstr.) Proc. 10th Intern. Congr. Genet. Vol. **2**: 11–12.

BARIGOZZI, C., 1948 Role of the Y chromosome in the determination of cell size in *Drosophila melanogaster*. Nature **162**: 30–31.

1951 The influence of the Y chromosome on quantitative characters in *D. melanogaster*. Heredity **5**: 415–432.

BARIGOZZI, C., and A. DIPASQUALE, 1953 Heterochromatic and euchromatic genes acting on quantitative characters in *Drosophila melanogaster*. Heredity **7**: 389–399.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1–52, 107–163.

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

CALLAN, H. G., 1948 Ribose nucleic acid in the Drosophila egg. Nature **161**: 440.

CASPERSSON, T. E., and J. SCHULTZ, 1938 Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Nature **142**: 294–295.

COOPER, K. W., 1945 Normal segregation without chiasmata in female *Drosophila melanogaster*. Genetics **30**: 472–484.

1949 The cytogenetics of meiosis in Drosophila; mitotic and meiotic autosomal chiasmata without crossing over in the male. J. Morphol. **84**: 81–122.

1952 Studies on spermatogenesis in Drosophila. Am. Phil. Soc. Ybk. 1951 pp. 146–147.

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

1959 Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin". Chromosoma **10**: 535–588.

DUBININ, N. P., and M. A. HEPTNER, 1935 A new phenotypic effect of the Y chromosome in *Drosophila melanogaster*. J. Genet. **30**: 423–446.

FREED, J. J., and J. SCHULTZ, 1956 Effect of the Y chromosome on the DNA content of ovarian nuclei in *Drosophila melanogaster* females. J. Histochem. Cytochem. **4**: 441–442.

GOWEN, J. W., and E. H. GAY, 1933 Eversporting as a function of the Y chromosome in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **19**: 122–126.

1934 Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. Genetics **19**: 189–208.

GRELL, R. F., 1959a The Dubinin effect and the Y chromosome. Genetics **44**: 911–922.

1959b Non random assortment of non-homologous chromosomes in *Drosophila melanogaster*. Genetics **44**: 421–435.

HALDANE, J. B. S., 1933 The part played by recurrent mutation in evolution. Am. Naturalist **67**: 5–19.

HEITZ, E., 1933 Cytologische Untersuchungen an Dipteren III; Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. Z. Zellforsch. **20**: 237–287.

1934 Über α and β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei Drosophila. Biol. Zentr. **54**: 588–609.

KAUFMANN, B. P., 1933 Interchange between X and Y chromosomes in attached-X females of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **19**: 830–838.

KRIVSHENKO, J. D., 1952 A cytogenetic study of the Y chromosome in *D. busckii*. Genetics **37**: 500–518.

1955 A cytogenetic study of the X chromosome of *D. busckii* and its relation to phylogeny. Proc. Natl. Acad. Sci. U. S. **41**: 1071–1079.

LEVENBOOK, L., E. C. TRAVAGLINI, and J. SCHULTZ, 1958 Nucleic acids and their components as affected by the Y chromosome of *Drosophila melanogaster*. I. Constitution and amount of the ribonucleic acids in the unfertilized egg. Exptl. Cell Research **15**: 43–61.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symposia Quant. Biol. **16**: 159–174.

LINDSLEY, D. L., 1955a Heterochromatic exchange between a reversed acrocentric compound X chromosome and the Y chromosome. Drosophila Inform. Serv. **29**: 134.

1955b Spermatogonial exchange between the X and Y chromosomes of *Drosophila melanogaster*. Genetics **40**: 24–44.

LINDSLEY, D. L., and E. NOVITSKI, 1959 Compound chromosomes involving the X and Y chromosomes of *Drosophila melanogaster*. Genetics **44**: 187–196.

MATHER, K., 1944 The genetical activity of heterochromatin. Proc. Roy. Soc. London, S. B. **132**: 308–332.

MORGAN, T. H., C. B. BRIDGES, and J. SCHULTZ, 1934 Constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **33**: 274–280.

MULLER, H. J., 1918 Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors. Genetics **3**: 422–499.

1932 Further studies on the nature and causes of gene mutations. Proc. 6th Intern. Congr. Genet. **1**: 213–255.

1948 The construction of several new types of Y chromosomes. Drosophila Inform. Serv. **22**: 73–74.

MULLER, H. J., and T. S. PAINTER, 1932 The differentiation of the sex chromosome of *Drosophila melanogaster* into genetically active and inert regions. Z. Ind. Abst. Vererb. **62**: 316–365.

NEUHAUS, M. J., 1936 Crossing over between the X and Y chromosomes in the female of *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **71**: 265–275.

1937 Additional data on crossing over between X and Y chromosomes in *Drosophila melanogaster*. Genetics **22**: 333–339.

1938 A cytogenetic study of the Y chromosome in *Drosophila melanogaster*. Biol. Zhur. **7**: 335–358.

1939 A cytogenetic study of the Y chromosome of *Drosophila melanogaster*. J. Genet. **37**: 229–254.

NOUJDIN, N. I., 1936 Influence of the Y chromosome and of the homologous region of the X on mosaicism in Drosophila. Nature **137**: 319–320.

1938 A study of mosaicism of the eversporting displacement type in *Drosophila melanogaster*. Bull. Biol. Med. Exp. **5**: 548–551.

PARKER, D. R., and ALICE E. HAMMOND, 1958 The production of translocations in Drosophila oocytes. Genetics **43**: 92–100.

PATTERSON, E. K., H. M. LANG, M. E. DACKERMAN, and J. SCHULTZ, 1954 Chemical determinations of the effect of the X and Y chromosomes on the nucleic acid content of the larval salivary glands of *Drosophila melanogaster*. Exptl. Cell Research **6**: 181–194.

PATTERSON, J. T., and WILSON STONE, 1952 *Evolution in the Genus Drosophila*. Macmillan Company, New York.

PHILIP, U., 1935 Crossing over between X and Y chromosomes in *Drosophila melanogaster*. J. Genet. **31**: 341–352

SAFIR, S. R., 1930 Genetic and cytological examination of the phenomenon of primary non-disjunction in *Drosophila melanogaster*. Genetics **5**: 459–487.

SANDLER, L. M., 1954 A genetic analysis of reversed acrocentric compound X chromosomes in *Drosophila melanogaster*. Genetics **39**: 923–942.

SANDLER, L. M., and E. NOVITSKI, 1956 Evidence for genetic homology between chromosomes I and IV in *Drosophila melanogaster;* with a proposed explanation for the crowding effect in triploids. Genetics **41**: 189–193.

SCHULTZ, J., 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Natl. Acad. Sci. U. S. **22**: 27–33.

1956 The relation of the heterochromatic chromosome regions to the nucleic acids of the cell. Cold Spring Harbor Symposia Quant. Biol. **21**: 307–328.

SEREBROVSKY, A. S., 1938 Genes *ac* and *sc* in *Drosophila melanogaster* and a hypothesis of gene divergence. Compt. Rend. Acad. Sci. URSS., NS. **19**: 77–81.

SHEN, T. H., 1932 Zytologische Untersuchungen über Sterilität bei Männchen von *Drosophila melanogaster* und bei F_1 Männchen der Kreuzung zwischen *D. simulans* Weibshen und *D. melanogaster*. Z. Zellforsch. **15**: 547–580.

STERN, C., 1927 Ein genetischer und zytologischer Beweis für Vererbung im Y-chromosome von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **44**: 187–231.

1929 Untersuchungen über Aberrationen des Y-chromosom von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **51**: 253–353.

TRAVAGLINI, E. C., L. LEVENBOOK, and J. SCHULTZ, 1958 Nucleic acids and their components as affected by the Y chromosome of *Drosophila melanogaster*. II. The nucleosides and related compounds in the acid soluble fraction of the unfertilized egg. Exptl. Cell Research **15**: 62–79.

WHARTON, L. T., 1944 Interspecific hybridization in the repleta group. Univ. Texas Publ. **4445**: 175–193.