THE SEX-DETERMINING MECHANISM OF DROSOPHILA MIRANDA

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INTRODUCTION

A SERIES of recent papers (DOBZHANSKY 1935; DOBZHANSKY and TAN 1936; DOBZHANSKY 1937), has described the unusual and interesting features which characterize the breeding behavior of *Drosophila miranda* and especially its method of sex determination. These papers have also reported the results of hybridization of *miranda* with the closely related and better known species, *Drosophila pseudoobscura*, and have given a detailed description, based on the study of the salivary gland chromosomes of the hybrids, of the similarities and differences in gene arrangement in these two species. In spite of these studies, there remained much that was obscure about the operation of the sex-chromosome mechanism, and particularly about the manner in which such an organization of the sex-determining material could have been derived from the more familiar sort found in *D. pseudoobscura* and, indeed, throughout the genus Drosophila.

Because of the need for more information on these interesting points, PROF. DOBZHANSKY suggested that I attempt to review and extend his observations. For this, for his kindness in furnishing me with stocks used in the investigation, and for much helpful criticism, I wish to express my gratitude to PROF. DOBZHANSKY. Helpful suggestions for the revision of the manuscript have been kindly given by PROF. F. R. IMMER and PROF. C. R. BURNHAM. To PROF. A. H. STURTEVANT, under whose direction this work was performed, I am deeply indebted for advice and encouragement.

The studies here reported have been sufficient to render more understandable the operation of the sex-determining mechanism of *Drosophila miranda*, and to indicate the manner in which it has been derived from the type present in the common ancestor of *pseudoobscura* and *miranda*.

MATERIAL

The wild-type *D. miranda* strain used most in these experiments was that designated as Olympic 1 (DOBZHANSKY 1935). By means of X-ray treatment a number of mutants have been obtained from this wild-type strain by DR. F. N. DUNCAN, working in this laboratory. Four of these mutants, crossveinless (*cv*), Truncate (*Trn*), plexus (*px*), and Smoky 11 (*Sm 11*) have been very useful in the present investigation. I wish to

thank DR. DUNCAN for kindly permitting me to use them. In one series of experiments use has been made of a strain (Wh 60) collected near Mt. Whitney by PROF. DOBZHANSKY. Another strain (Quilcene 1) has been but little used; it appears to be identical with Olympic 1.

The Texas wild-type stock of *D. pseudoobscura* has been used, and also a number of mutant strains.

Ever since the discovery of the fact that the chromosomes are the material basis of heredity, many investigators have been attracted to the comparative study of the chromosomes of related species, since such studies should yield information not merely about the evolution of a character, but indeed about the evolution of the machinery by which hereditary characters are determined. The genus Drosophila was early recognized as one between whose different species there was considerable variation in chromosome number and morphology. Thus METZ (1914, 1916) described 11 different metaphase configurations found in the 26 species the oogonial chromosomes of which he examined. But the work of METZ showed that in spite of this variation certain regularities were preserved. The haploid groups of 21 of the 26 species agreed in having one tiny dotlike chromosome and five arms, these arms being either separate or joined to form one or two V's. In *D. virilis*, for example, there are five independent rods and a dot, in *D. pseudoobscura* three independent rods, one V and a dot, and in *D. melanogaster* one independent rod, two V's and one dot.

This regularity seemed less surprising at the time it was discovered than it did some years later when the rather common occurrence of reciprocal translocations in natural populations of plants had been shown by many workers, especially by BELLING and BLAKESLEE and their collaborators (BELLING 1927, BLAKESLEE 1929). If reciprocal translocations had become established as frequently in the phylogeny of the genus Drosophila as in Datura it would be hard to understand, for example, why a dot-like chromosome should be found in so many species; why it had not lost its identity through the translocation to it of material from other chromosomes. This difficulty had not long been recognized before its explanation became apparent (STURTEVANT and BEADLE 1936). In natural populations of Drosophila there is a strong selection against translocations, this selection being due to the reduction of fertility associated with translocations in the heterozygous condition. (This would be of much less importance in a plant which, like Datura, is predominantly selffertilizing.) The same argument applies to inversions which include the spindle attachment region. But inversions which are entirely within one

arm have no appreciable effect on fertility in Drosophila, and are correspondingly common in natural populations.

Thus as two populations of the same species of Drosophila become progressively differentiated from each other until they deserve separate specific rank, one might expect that they would quite commonly come to differ by rearrangements within a single arm, only rarely by rearrangements involving a transfer of genetic material from one arm to another. Two methods are available for studying gene arrangement in related species to see whether this relation holds. The cytological method can be applied only to pairs of species which cross, and involves a minute study of chromosome pairing in the hybrid. It appears that chromosomes like those of the salivary glands of the Diptera must be available if complex rearrangements are to be analyzed by this method. The genetic method involves obtaining a large number of mutant genes in each species and determining their linkage relations. This method may be applied to pairs of species which do not cross, as well as to those which do, though in the former case allelism can only be inferred from appearance.

Of particular interest in the present connection is the application which has been made of this method (CREW and LAMY 1935; DONALD 1936; STURTEVANT and TAN 1937) to two not very closely related species, *Drosophila melanogaster* and *D. pseudoobscura*. As more mutants have become available it has grown increasingly clear that during the divergence of *pseudoobscura* and *melanogaster* each arm has maintained its separate identity, little if any genic material being transferred from one arm to another. In tabular form the correspondence may be represented thus:

pseudoobscura		*melanogaster*
arm	=	arm
XL	=	X
XR	=	III L
II	=	III R
III	=	II R
IV	=	II L
V	=	IV

One striking fact emerges from this comparison. The material which is III L in *melanogaster*, containing the loci of sepia, scarlet, javelin, veinlet, and others, is not autosomal in *pseudoobscura*, but is sex-linked, so that the genes in it are inherited like white or yellow (not like bobbed) of *melanogaster*. This means that while the *melanogaster* male is haploid for one arm, the *pseudoobscura* male is haploid for two arms. It seems clear that *pseudoobscura* is descended from a form in which the male, as in *melanogaster*, carried the III L material in duplicate. The question which demands an answer is: How could this change have taken place? It seems

impossible that it could have taken place all at once, for so large a deficiency would surely make the male inviable. This paper will suggest a plausible mechanism by which it might have taken place gradually.

CYTOLOGY OF MIRANDA AND PSEUDOOBSCURA

It will be well first to review briefly the findings of DOBZHANSKY (1935, 1937) and DOBZHANSKY and TAN (1936) on the cytology of *miranda* as compared with that of *pseudoobscura*. The metaphase chromosome groups of females of the two species are indistinguishable, consisting in each of a pair of dots, three pairs of rods, and a pair of V's. The corresponding salivary gland nuclei contain, as expected, one very short strand and five long ones, striking differences between the two species being here apparent. These differences are best studied in the salivary glands of the female hybrids, where each nucleus contains a complete haploid set from each species.

The results of such a study were reported by DOBZHANSKY and TAN (1937). They found that the two species differed by a great many rearrangements involving a minimum of 49 points of breakage. The great majority of the rearrangements involved a transfer of material from one point to another in the same arm. There were, however, six cases which could be interpreted as inter-arm transfers. Since *pseudoobscura* is morphologically so similar to *miranda*, and so different from *melanogaster*, it cannot but seem strange that the first pair should differ by even as many as six translocations while the second pair seem to differ by few if any. DOBZHANSKY and TAN emphasized that some sections were only very rarely seen paired; some, indeed, only a single time. Needless to say, this adds to the difficulty of an already difficult observation. It is particularly unfortunate that critical figures are so rare, for from a study of DOBZHANSKY and TAN's figure 6, it does not appear that the translocation interpretation is the only one possible. (The difficulties and uncertainities attendant on these observations may be inferred from the case of the dot (V). DOBZHANSKY and TAN had concluded that the dots in the two species were totally different. DOBZHANSKY (unpublished) has made further observations which show that they are the same in every visible detail, the earlier interpretation having been a matter of incorrect identification.)

But whether or not translocations exist, they are certainly neither numerous nor large, from which it follows that each arm of *pseudoobscura* is at least approximately homologous to one of *miranda*.

In this paper the designations XL, X̂R, II, III, IV and V will be applied to the chromosomes of both species. Following DOBZHANSKY, III of *miranda* will also be referred to as X^2. This is done because the chromosome of *miranda* which is approximately homologous to III of *pseudoobscura* is

not an autosome, but a sex chromosome. This fact does not appear from a study of the females and the female hybrids, but is shown by a comparison of the males.

In mitotic metaphases of the *pseudoobscura* male there are three pairs of rodlike elements, a pair of dots, a V-shaped X, and a Y of which the shape varies in different stocks. In the salivary gland nucleus we find three long strands corresponding to the three pairs of autosomes, two strands, also long, but paler and more slender, corresponding to the unpaired right and left limbs of the X, and one very short element, the paired dots (V). As in all species of of Drosophila heretofore described, the Y is an inconspicuous element entering into the composition of the chromocenter.

In the male of *miranda* DOBZHANSKY found the mitotic chromosomes to consist of a V (X^1), a J-shaped element (Y), a pair of dots (V), 2 pairs of rods, and one unpaired rod. The presence of an unpaired rod was totally unexpected, but an examination of the salivary gland chromosomes showed that one of the strands which in the *pseudoobscura* male was plump and darkly staining, namely III, was pale and slender in *miranda*, like XL and XR, confirming its unpaired condition. Since the approximate homologue of III was paired in the female and unpaired in the male, DOBZHANSKY referred to it as X^2. These observations seemed to point to the extraordinary fact that the *miranda* male was deficient for the entire III, which in *pseudoobscura* would certainly be lethal. DOBZHANSKY and TAN (1936) find it necessary to conclude that "*miranda* and *pseudoobscura* represent two very different reaction systems." (While the paleness and slenderness of X^2 in the male are not entirely dependable as a basis for concluding that it is haploid rather than diploid, the conclusion that it is haploid is strongly supported by observations to be presented below.)

To recapitulate, there are three strands (XL, XR, and III) which are present twice in the female *miranda* and once in the male, two of the three being joined to form a V. (From the evidence thus far presented it would not be possible to conclude, as I have tacitly done, that the two arms which compose the V are, as in *pseudoobscura*, XL and XR. Evidence to be presented below (cf. Exceptional Hybrid Males) excludes the alternative possibilities that III and XL or III and XR might form the V, XR or XL respectively being the rod.) The J-shaped chromosome is present in the male but not in the female, and may therefore be called the Y.

How would such a sex chromosome mechanism work? It is evident that in order for it to perpetuate itself without the formation of inviable gametes or zygotes a non-random segregation of X^1, Y, and X^2 in the meiotic divisions of the male would be necessary. Y would have to go to one pole and X^1 and X^2 to the other. From a cytological study of meiosis (DOBZHAN-

SKY 1935) it appeared that X^1 and Y conjugated and disjoined in a regular fashion, whereas X^2 went to one or the other pole without having conjugated with either X or Y at all. It was unfortunately not possible to say whether X^2 always went to the same pole as X^1, for X^1 and Y could not be distinguished at this stage. But since there was no evidence for the formation of non-functional sperm, and the zygote mortality was too low to account for more than a very small frequency of non-regular disjunction, it appeared that determinate segregation must be the rule (DOBZHANSKY 1935). The further study of this unusual phenomenon was a primary object of the present investigation, the results of which follow.

ACTIVE MATERIAL IN THE Y

An examination of the salivary gland chromosomes of the *miranda* male (MACKNIGHT 1938) showed that in addition to the complement of chromosomes described by DOBZHANSKY and TAN (1936) there was in each nucleus an element composed of many short, banded sections, most of them merging into heterochromatic material at both ends (fig. 1). No such element is present in females. It follows that the element in question is derived in ontogeny from the J-shaped mitotic chromosome which, by virtue of its presence in males and not in females, has already been defined as the Y.

This unexpected finding seemed from the first to resolve many of the puzzling problems connected with the operation and evolution of the sex-determining mechanism of *miranda*. In the first place, it was no longer necessary to suppose that the male was haploid for the entire third chromosome; the inference seemed justified that the *miranda* male carried most if not all the material of III in duplicate, once in X^2 and once in the Y. Such an assumption leads one to ask whether the total length of the euchromatic sections in Y is equal to that of X^2. For several reasons it is difficult to give an exact answer. The absolute and relative lengths of chromosomes vary from nucleus to nucleus depending on preexisting differences and on the amount of artificial stretching. In the present case there is also a source of systematic error, since a short section attached at both ends would not be expected to stretch in response to pressure in the same way as a long strand attached at one end. With these reservations it may be stated that the total length of euchromatin in the Y is little if at all less than that in X^2, both being about 110μ in large nuclei. This observation evidently supports the hypothesis.

In the second place, if we take the Y to be homologous not to X^1 alone, but part to X^1 and part to X^2, then we might expect that trivalent association would occur. Were this the case, the directed segregation of the sex-chromosomes, while still unexplained, would find a close parallel in the
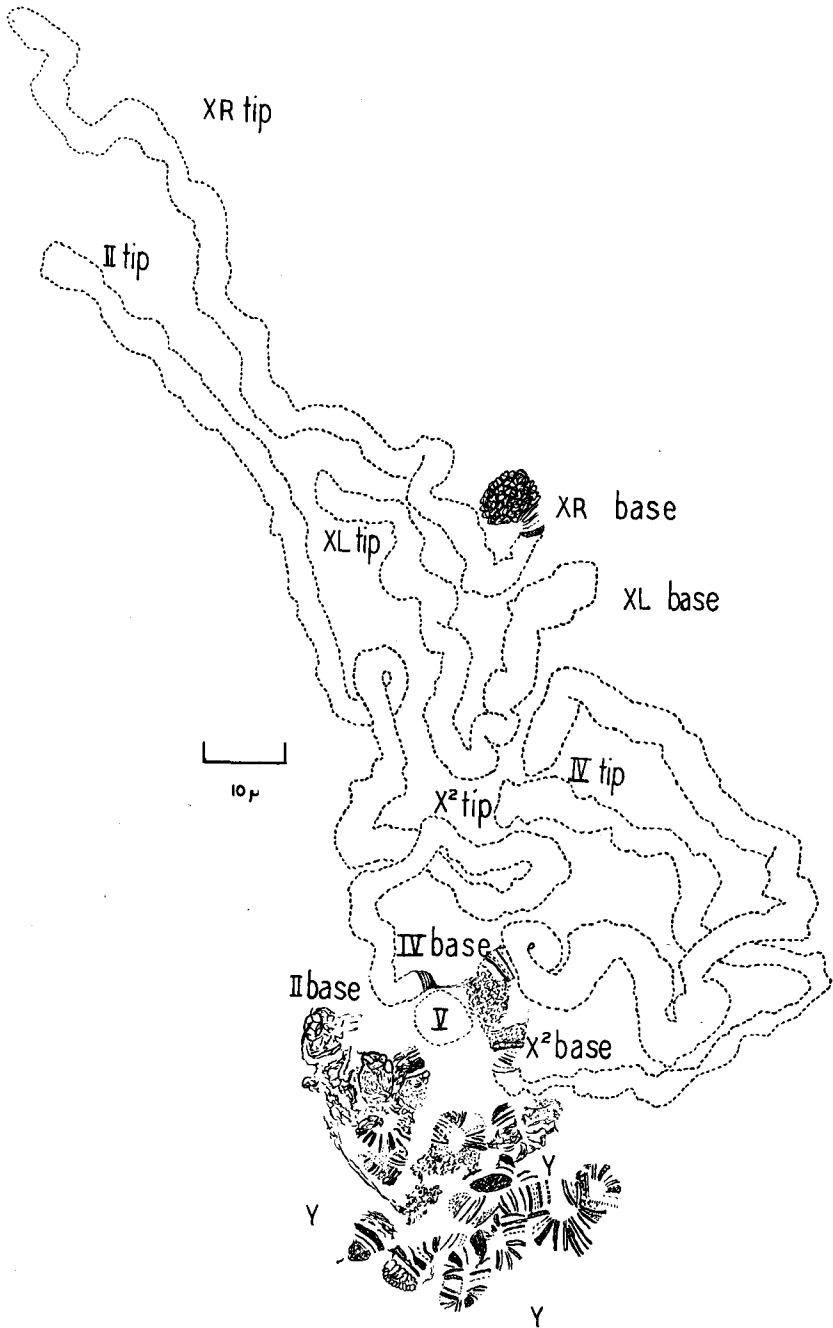
FIGURE 1.—Camera-lucida drawing of a complete salivary gland nucleus of a male of *Drosophila miranda*. The banding of XL, XR, II, X², IV, and V has been omitted except for a few bands at the bases of XR, II, X², and IV. With this exception, all the banded material shown belongs to the Y chromosome.

well-known behavior of rings in Datura, Oenothera, and Triticum. But since DOBZHANSKY (1935) found no trivalent association, it would be necessary to suppose that the association between Y and X^2 was either obliterated or in some way obscured in the stage at which DOBZHANSKY's observations were made.

If Y and X^2 conjugated in meiosis, it might be expected that pairing between these chromosomes would be seen in the salivary gland nuclei. No such pairing has been seen in the many nuclei examined. While this direct evidence of homology is therefore lacking, the lack of it, as will be seen later, does not necessarily constitute evidence against homology. In order to decide the question we must seek indirect evidence. To this end, and for the sake of the bearing it has on the question of directed segregation, as well as for its intrinsic interest, an account of the genetic studies which have been made on *miranda* will now be presented.

METHODS

Drosophila miranda is not an easy species to breed. The life-cycle requires nearly a month, if one uses the individuals whose development has been most rapid. Some individuals do not emerge until more than 36 days after oviposition. As a result the food is apt to become progressively worse in experiments in which an effort is being made to secure complete counts. Since the males develop more slowly than the females, they are exposed to less favorable conditions; this accounts for part of the excess of females over males (DOBZHANSKY 1935). As adults the males are smaller and shorter-lived than the females. It seems probable that male and female zygotes are formed with equal frequency, since from a given number of eggs one does not obtain more than half that number of females (DOBZHANSKY 1935). The tendency of these large and sluggish flies to become stuck in the food has been overcome by covering the food with a very light sprinkling of sawdust, no more than will adhere when the bottle is shaken upside down. It is even possible to drop etherized flies onto a food surface so prepared without their becoming stuck.

SEX-LINKED MUTANTS

Truncate (*Trn*) is almost completely dominant. It is often difficult, sometimes impossible, to tell whether a female is homozygous or heterozygous. The character is somewhat more extreme in the male. The marginal vein at the wing-tip is usually gone, though a scattering of hairs is sometimes left. The wing is shorter, and the veins, especially III, are still shorter, so that they do not reach even to the new margin. The scutellum is broad and blunt, and the bristles on it are usually reduplicated and irregular. There is a rough patch in the eye at the center of the anterior

margin and extending backwards from there. The viability of the hetero-
zygous females is excellent, that of the males poor. The character is in-
herited as a typical sex-linked dominant.

Since *Trn* arose as a result of X-ray treatment, the homozygous Trun-
cate stock was crossed with wild type and the salivary gland chromosomes
of the resulting females were examined in order to find out whether any
chromosomal aberration was present. It was found that these females were
heterozygous for a very small inversion in X^2, about one-fifth of the way
from the tip. Males from the same cross had no such heterozygous inver-
sion figure in the X^2, which confirms DOBZHANSKY's conclusion that X^2 is
unpaired in males. While it seemed reasonable to assume that the loci of
Truncate and of the inversion were the same, the matter was tested by
following them genetically and cytologically in successive generations of
outcrossing. It was found that they did not separate, even though crossing
over took place nearby. Truncate is therefore in X^2.

Crossveinless, a recessive, closely resembles *cv* of *melanogaster* in appear-
ance. Its viability is poor. Males carrying both *Trn* and *cv* die, many of
them in a late pupal stage. Two have been obtained as adults. Both died
without issue soon after emergence. Females from the cross of *cv* by wild
type are heterozygous for a very small inversion in XL about one-third
of the way from the tip, though this fact was not discovered until after *cv*
had been placed in X by another method (cf. Crosses with *pseudoobscura*).
Very probably *cv* is located at or close to the inversion.

Plexus, a recessive, causes extra veins in the wing, especially extra cross-
veins in the tip of the submarginal cell. The character is less pronounced
in males than in females, almost, but not quite, overlapping normal. I
have not been able to detect any cytological abnormality in connection
with *px*. Unpublished data of DUNCAN show that *px* is linked to *Trn*,
giving about seven percent recombination with it. This is in accord with
the results of the present study. Two independent experiments have shown
that *px* and *cv* assort at random. One, involving 263 individuals, gave 46
percent recombination. Another, involving 950, gave 49.5 percent.

Vermilion (*v*) occurred spontaneously as a *v cv px* male in a linkage ex-
periment involving *Trn*, *cv*, and *px*. It has never separated from *cv*, al-
though more than 1,000 non-crossover individuals have been obtained.
This is a reason for thinking that the small heterozygous inversion seen
in females heterozygous for *cv* is at or close to the locus of *cv*.

Javelin (*jv*) makes the bristles slender and less curved than normal.
They are often broken off near the tip. It closely resembles slender of
pseudoobscura (XR) and javelin of *melanogaster* (III L). Javelin occurred
spontaneously in the Smoky 14 stock. It was crossed to *Trn*, and a re-
combination value of 49.0 percent, based on 149 individuals, was obtained.

With *cv*, it gave 51.2 percent crossing over (506 individuals). As will be shown below, it is probably in X^1, and since it shows no linkage with *cv* (XL) it is probably in XR.

AUTOSOMAL MUTANTS

The only autosomal mutants known are the Smokys, *Sm 3, 8, 9, 10, 14*. The unpublished work of DUNCAN indicates that these five are dominants, lethal when homozygous, and allelic to each other and to *Sm 11*. Smoky of *pseudoobscura*, which the character closely resembles, is situated close to the base of II.

PATROCLINOUS MUTANTS

The patroclinous characters, of which two are known, are transmitted by a male to all his sons and to none of his daughters. Thus in a true-breeding stock, the character is shown by all the males, and by none of the females. In outcrosses, the females do not transmit the character to their descendants of the first nor second generation, while the males transmit it to all their sons. In short, the distribution of the character follows that of the Y chromosome.

One of the two characters with this behavior is *Sm 11*, which phenotypically resembles the autosomal Smokys. Slightly under 2,000 flies of this stock have been systematically examined, and a great many more have been looked at in the course of breeding work, without a Smoky female or a non-Smoky male being found.

A study of the salivary gland chromosomes of a male from the *Sm 11* stock showed that the basal part of II was frequently unsynapsed, one member of the pair being attached to an unidentified heterochromatic body. A careful study has not been made, but it appears that a II-Y translocation has occurred.

The other patroclinous character, Angles, was recently obtained in an X-ray experiment. Typically the anal angles of the wings are clipped off. However, the character is rather variable. The clipping may extend irregularly all along the posterior margin of the wing. On the other hand there may be only a slight disturbance of the marginal hairs at the angle. It is not uncommon for the character to show in one wing only, and one occasionally finds males of this stock which do not show the character at all. Such males, however, so far as tested, have always produced a high percentage of Angles sons when outcrossed.

From a study of the salivary gland chromosomes of the males of the Angles stock it is clear that they carry a reciprocal translocation between the euchromatic part of IV and one of the euchromatic sections of Y. Chromosome IV is broken about one-third of the way from the free end. The euchromatic section of Y is broken about in the middle.

In neither *Sm 11* nor *An*, so far as I can see, are the mitotic chromosomes different from normal. Both these mutations, *Sm 11* and *An*, have arisen in connection with translocations between autosomes and the Y. In one of them, *Sm 11*, it is clear that the mutated gene is of autosomal origin. It is not possible to say whether the Angles gene is in the material of IV or of Y.

Both these patroclinous genes are dominant. This is not a matter of chance. On the contrary it is important to recognize that a recessive gene transmitted in a similar fashion would produce no effect at all, and would therefore not be detected. This would be true whether the recessive were in the Y or in an autosome which was involved in a Y-translocation, since in neither case could the gene become homozygous. Even though the gene were in the autosome at some distance from the point of breakage, the gene would not be able to get into the unaltered autosome, since there is no crossing over in the male. This point will be discussed further.

SUMMARY OF EVIDENCE FROM GENETIC STUDIES

Are zygotes formed in *miranda* whose chromosomal constitutions are different from those which are typical for the species? The following facts should be considered. In the first place, adults are of two phenotypic classes, normal males and normal females. There are no classes of abnormal or intersexual individuals produced, even in small numbers. Hence, if exceptional zygotes are formed whose chromosome constitution is other than normal male or female, such zygotes either die before reaching the adult stage or give rise to adults of normal appearance. From DOB-ZHANSKY'S (1935) data on mortality it is clear that the number of zygotes that die cannot be large. The number of exceptional zygotes that survive is not large either, or some would have been detected cytologically. But cytological study has been made of only a few individuals, relatively; in order to have large enough numbers on which to base our conclusions, we must use the results of genetic study.

Since it seems clear that the eggs are all of one sort, X^1X^2A, let us consider what sort of segregation in the male would be required for the production of zygotes of exceptional constitution. The male is X^1YX^2 2A, where A represents a set of autosomes, II, IV, and V. Zygotes haploid or triploid for II or IV will be inviable. Whether haplo- and triplo-V individuals are viable is of no present interest. Let us therefore neglect the autosomes and consider only the segregation of X^1, Y, and X^2. With respect to these three chromosomes there are 2^3 or 8 possible types of sperms: (see table 1).

In the first place, types 5 and 6 are the regular male and female producing sperm respectively.

The finding that the Y contains a large amount of euchromatic material

probably homologous to X^2 leads us to expect that sperm of the sorts 1, 2, 3, and 4 will give rise to inviable zygotes, since these will carry either a deficiency (1, 3) or a duplication (2, 4) for the material of an entire chromosome. If such sperm are formed, the zygotes from them certainly die, for there is genetic evidence that they do not give rise to adults (table 1). But conversely, their failure to give rise to viable zygotes is not evidence against the production of these types of sperm; indeed, they may be produced in numbers up to the limits set by zygotic mortality, or in larger numbers if the unlikely assumption of gametic selection be made.

TABLE 1

A summary of the evidence from breeding tests within D. miranda showing that no appreciable number of individuals having exceptional sex-chromosome constitution survives to the adult stage. The table is self-explanatory, except for the two entries referring to Sm 11, which involve a few obvious and reasonable assumptions.

SPERM OF TYPE	DO NOT PRODUCE ADULT MALES BECAUSE:	DO NOT PRODUCE ADULT FEMALES BECAUSE:
(1) A	$+$ ♀ $\times$ Sm11 ♂ produce no $+^{Sm11}$ ♂	cv ♀ $\times +$ ♂ produce no cv ♀
(2) X^1YX^2 A	cv ♀ $\times +$ ♂ produce no $+^{cv}$ ♂	$+$ ♀ $\times$ Sm11♂ produce no Sm11 ♀
(3) X^1 A	cv ♀ $\times +$ ♂ produce no $+^{cv}$ ♂	$+$ ♀ $\times$ Trn ♂ produce no $+^{Trn}$ ♀
(4) YX^2 A	$+$ ♀ $\times$ Trn ♂ produce no Trn ♂	cv ♀ $\times +$ ♂ produce no cv ♀
(5) Y A		cv ♀ $\times +$ ♂ produce no cv ♀
(6) X^1X^2 A	cv ♀ $\times +$ ♂ produce no $+^{cv}$ ♂	
(7) X^2 A	$+$ ♀ $\times$ Trn ♂ produce no Trn ♂	cv ♀ $\times +$ ♂ produce no cv ♀
(8) X^1Y A	cv ♀ $\times +$ ♂ produce no $+^{cv}$ ♂	$+$ ♀ $\times$ Trn ♂ produce no $+^{Trn}$ ♀

The sperm of types 7 and 8 remain to be considered; their behavior is of particular interest. For if, as we suppose, the euchromatic material of Y is substantially equivalent to X^2, while the heterochromatic material resembles the Y of *pseudoobscura* in that deficiency and excess of it have no effect on viability, then we would expect that sperms of types 7 and 8, if formed, would produce zygotes of normal viability. Genetic evidence (table 1), and especially the inheritance of *Sm 11*, show that the expected classes of adults do not appear; from this we might conclude that such sperm are not formed. Such a conclusion would be false, as will shortly appear; there is definite evidence that sperm of type 7 are produced with a frequency of at least 1 in 268. Since the zygotes coming from sperm of type 7 are not viable, while those from type 5 are, it follows that X^2 and Y are at any rate not sufficiently alike to be interchangeable. This fact will be discussed below, but first the evidence will be presented which demonstrates the production of X^2 A sperm.

HYBRIDS WITH PSEUDOOBSCURA

DOBZHANSKY (1937) has described in detail his observations on crosses between *miranda* and *pseudoobscura*. Offspring can be obtained from each

of the two reciprocal crosses. The female hybrids from both crosses are normal in appearance, but were in DOBZHANSKY'S experiments completely sterile. It now appears that they are not invariably so. STURTEVANT (unpublished), using a somewhat different method, found it possible to obtain hybrid females which were slightly fertile. This result has also been obtained by DOBZHANSKY (unpublished) in a repetition and extension of his earlier experiments. He has found that the fertility of the hybrid females varies from very slight to almost nil, depending on the geographic races used in making the cross. Some of the most fertile female hybrids so far obtained have come from crosses of the Mt. Whitney strain of *miranda* with standard laboratory stocks of *pseudoobscura;* these hybrid females have about one percent of the fertility of females of the pure races. There will be presented below the results which have been obtained by using such females for backcrossing experiments. A simple F_2 cannot be raised since the male hybrids are totally sterile regardless of how the cross is made.

With respect to the production of hybrid males, the two reciprocal crosses give, as DOBZHANSKY (1935, 1937) has shown, entirely different results. *Miranda* females crossed to *pseudoobscura* males give male hybrids which approach the female hybrids in point of numbers, but unlike them are morphologically abnormal. It seems beyond question that DOBZHANSKY (1937) is right in concluding that they arise from the regular male-producing *pseudoobscura* sperm, the deficiency in their numbers compared with females being due to zygotic mortality. Summed data from several of my cultures show 215 males to 336 females or 32 males per 50 females.

This cross offers an opportunity to test whether a sex-linked recessive in *miranda* is in X^1 or in X^2. If the recessive is in X^1, then when a homozygous mutant *miranda* female is crossed to a wild type *pseudoobscura* male, the character should appear in the hybrid males, since most of the material of X^1 is in X of *pseudoobscura*, and since these hybrid males come from the Y-bearing sperm which lack the X. If the recessive is in X^2, the hybrid males should not show the character, since they receive the *pseudoobscura* III. The test has been applied to *cv, jv*, and *px*. The effects of *cv* and *jv* showed in the hybrid males, those of *px* did not. Hence *cv* and *jv* are in X^1, *px* in X^2, an inference which is supported by several other pieces of evidence. It was not necessary to apply this test to *v*, for a cross to *v* of *pseudoobscura* (XL) showed the two to be allelic. A cross was also made by DUNCAN between *cv (miranda)* and *cv (pseudoobscura, III)* a mutant which superficially resembles it; the female hybrids were wild type.

Most interesting of all, *px* was tested against *px* of *pseudoobscura* and

found to be allelic. This is striking because *px* (*miranda*) is sex-linked while *px* (*pseudoobscura*) is in an autosome (III). Thus *miranda* and *pseudoobscura* stand in the same relation with respect to *px* as *pseudoobscura* and *melanogaster* with respect to sepia, javelin, veinlet, and others. The case of *px* is discussed again in a later section.

EXCEPTIONAL HYBRID MALES

On the other hand, the reciprocal cross, *pseudoobscura* female by *miranda* male, (DOBZHANSKY 1935, 1937) produces only one male to about 268 females. Unlike the males from the other cross, these males, though small, are well-proportioned. For this reason DOBZHANSKY (1937) thought it unlikely that they came from the regular male-producing *miranda* sperm, representing the rare survivors of a class originally as numerous as the females. Having excluded the possibility that they came from no-X eggs by the observation that they showed sex-linked recessives from the mother, he sought to determine whether these exceptional males received an X^2 from the father, using the following method. He reasoned that the *miranda* male was haploid for the material of III, carrying it only in the unpaired X^2, and hence if a *pseudoobscura* female homozygous for a third chromosome recessive were mated to a wild type *miranda* male, the presence or absence of an X^2 in the hybrid male would be indicated by the suppression or appearance respectively of the recessive from the mother. He tried the experiment using orange and purple; both were suppressed in the hybrid males. From this he concluded that these males were produced by sperm carrying a set of autosomes, X^2, and probably also the Y.

The finding that the Y contained a large amount of euchromatin presumably homologous to III seemed to reopen the question. In the first place, it seemed doubtful that the hybrid males could get both X^2 and Y from the father, for these added to the III from the mother would make them carry a duplication for an entire chromosome. But what was worse, the *miranda* Y might carry the normal alleles of orange and purple. Without this being disproven, DOBZHANSKY's experiment remained inconclusive: the hybrid males might come from normal male producing sperm, YA. While their rarity argued against this, the rare survival of *Trn cv* males showed that such a thing was not impossible. Fortunately it was easy to test the matter. The objections which applied to orange and purple did not apply to plexus, for the *miranda* Y was known not to contain its wild type allele. If it did, *px* in *miranda* would be sex-linked like bobbed, rather than sex-linked like white.

Pseudoobscura females homozygous for *px* were crossed to wild type *miranda* males. The progeny consisted of 1,466 not-*px* females and six not-*px* males. This showed that DOBZHANSKY's conclusion was correct;

the hybrid males do receive X^2 from the father. This was confirmed by crossing wild type *pseudoobscura* females to *Trn miranda* males. A progeny of 2,699 females and three males was obtained. Two of them showed *Trn*, as expected. The third was unfortunately not classified for the character. The low frequency of males is probably due to the effect of *Trn* on viability.

The question remained whether or not these males received the Y as well as the X^2 from the father. This was decided in the negative by a cross of wild type *pseudoobscura* females to *Sm 11* males. The progeny consisted of 404+ females and three+ males. It might be mentioned that this is the only way in which a non-*Sm 11* son has ever been obtained from a *Sm 11* father.

Genetic evidence thus shows clearly that the exceptional males come from X^2 A sperm. This has also been shown cytologically. Two exceptional males were obtained in the larval stage and ganglion preparations made. They both had six rods and a V. In one the pair of dots was clearly seen.

The most important fact which emerges from these studies is that the exceptional hybrid males do not result from the failure of the mechanism of determinate disjunction, as suggested by DOBZHANSKY's (1937) figure 5, but from primary non-disjunction of X^1 and Y chromosomes. The frequency of this phenomenon is of the order of magnitude of the frequency of exceptional males. Combining DOBZHANSKY's (1937) data with mine, a figure of 78 males: 20,819 females is obtained, or 1:268. This figure does not include the experiments involving *Trn*, which gave a rather low value, nor an experiment in which Angles (IV-Y Translocation) males were used, which gave a rather high value of 10 males to 806 females.

Primary nondisjunction could also give rise to another class of sperm, type 8 of table 1, having X^1, Y, and A. Such sperm, if formed with a corresponding frequency, do not give rise to viable zygotes when they fertilize *miranda* eggs, (table 1), nor when they fertilize *pseudoobscura* eggs, for none were found among 4,569 hybrids from which such individuals could have been distinguished genetically, had they occurred. In fact there is no evidence at all for the formation of such sperm, though it does not follow that they are not formed. The reader will, however, recall that primary non-disjunction in the female of *melanogaster* gives rise much more frequently to no-X eggs than to 2X eggs. Loss of the heterochromosome bivalent by its failing to divide and remaining on the equatorial plate may explain both cases.

The above-mentioned exceptional males which were obtained in the larval stage were found in the course of an attempt to study zygote mortality in the cross of *pseudoobscura* females by *miranda* males. The females carried eosin, a sex-linked recessive which not only lightens the eye-color,

but also makes colorless the Malpighian tubules, which normally are yellow. They were crossed to *miranda* males of the Olympic 1 stock, and their eggs collected. Most of them produced no viable eggs while under observation, many no eggs at all. Probably most of them were never inseminated. In all, 90 larvae were obtained; these were classified for Malpighian tubule color. Yellow tubules were present in 77 of them, 11 died or were lost before they could be classified, and two had colorless tubules; these are the two exceptional males mentioned above.

In computing zygotic mortality, we must bear in mind that failure of an egg to hatch in this species cross may be due merely to failure of fertilization. If we therefore exclude those daily layings of eggs of which none or only a few hatched, we will get a better estimate of the viability of hybrid zygotes. Of the 90 larvae, two-thirds were obtained from four daily layings of 105, 5, 12 and 19 eggs; total 141. Of these, 41, 4, 6, and 9 respectively hatched; total 60. This shows that the viability of the hybrid females is good. A more significant fact shown by this experiment is that the zygotes resulting from the union of regular male-producing sperm of *miranda* with eggs of *pseudoobscura* die at a very early stage. Of the 77 to 88 of them expected, not more than 11, and probably none at all, hatched from the egg. Certainly they do not survive to the second larval instar. This unexpected finding serves to emphasize that X^2 and Y are decidedly not interchangeable. An X^2 A sperm produces an inviable zygote if it fertilizes a *miranda* egg; a YA sperm produces one which lives. With a *pseudoobscura* egg the reverse happens, and the inviable zygote dies at a surprisingly early stage.

COMPOSITION OF THE MIRANDA Y

We have already seen another respect in which the *miranda* Y differs from the X^2, namely, that it has either a recessive allele or a deficiency at the *px* locus. If other recessives in X^2 were available, it would be possible to test whether or not the Y carried their wild-type alleles by simply seeing whether the character showed in males. Unfortunately *px* is the only X^2 recessive thus far found. Many recessives are available in III of *pseudoobscura*, but it is not possible to determine whether their wild type alleles are present in the *miranda* Y simply by crossing to a *miranda* male, since the few males produced receive the X^2, and not the Y, from the father. I have tried to make use of the slight fertility of the hybrid females in order to overcome this difficulty. By backcrossing to *Sm 11* males for several generations it has been possible to obtain a male with the *miranda* Y and a part of the *pseudoobscura* III carrying orange and Blade. The male's eyes were orange; Blade appeared as it does when heterozygous. From this it may be concluded that the miranda Y lacks the wild type

allele of orange, as it does of *px*, but very probably carries that of Blade. These experiments are still in progress, and may furnish similar information about other *pseudoobscura* genes.

DISCUSSION

The purpose of this discussion is twofold; first, to present and criticize the view of the construction, operation, and evolution of the sex-determining mechanism of *Drosophila miranda* to which the observations reported in this paper have led, and, second, to suggest some bearings which they may have on the evolution of sex-determining mechanisms in general.

It seems likely that the first step in the origin of the *miranda* Y was the
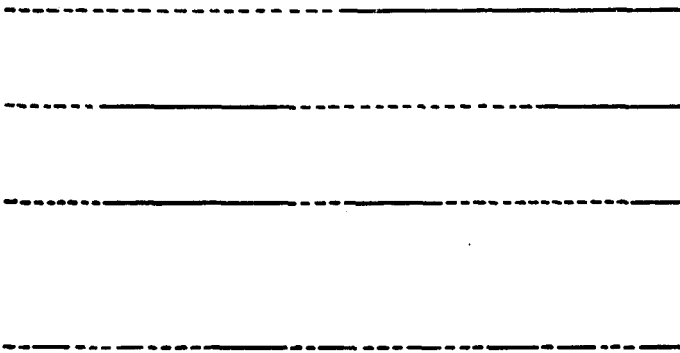

FIGURE 2.—Heterochromatin dotted line, euchromatin solid line. Depicted are the results of fusion of Y and III, and of one, two, and several subsequent inversions. At the bottom, the configuration which would be produced by the non-specific association of heterochromatin, and which would therefore be expected in the salivary gland nuclei.

fusion of III and Y at the spindle attachment region. Had the Y possessed two arms, this might have involved the loss of one of them. However, wide variations in the size of the Y are known to exist in different strains of *pseudoobscura* today, without apparent effect on the fertility of the male. Perhaps a rod-shaped Y may have been present in the male in which the fusion took place. After the two chromosomes had become one, extensive rearrangement must have taken place within it, in the course of which the euchromatic material came to be in the form of many short segments inserted at various points in the heterochromatin. Of course, one might equally well think of the heterochromatin being scattered through the euchromatin. By way of example, figure 2 shows how successive inversions might have led to such a condition.

Such rearrangements would not have resulted in any difficulties connected with crossing over, since the chromosome would have been present only in males. How the pairing relations between Y and III would have been affected is less clear. It is hoped that DR. KOLLER's current investigations may shed light on this question. In the salivary gland nuclei, the non-specific affinity of heterochromatin (BAUER 1936) would lead to the association of all the heterochromatic regions in the Y with each other. Such is my interpretation of the condition seen in figure 1. The extensive rearrangement of the euchromatic material in Y, perhaps also its position in the nucleus, would result in the observed rarity or absence of pairing between it and X^2. This observation, and other considerations as well, suggest that meiotic pairing between X^2 and Y, if it occurs, may involve a heterochromatic region.

This view of the sex-determining mechanism of *Drosophila miranda* is, I believe, consistent with all known facts concerning it. DARLINGTON (1936) has put forward a different theory which is at variance with the facts available at the time of its publication (DOBZHANSKY 1935) as well as with those that have appeared since (DOBZHANSKY & TAN 1936, DOBZHANSKY 1937, MACKNIGHT 1938, and the present paper). Strangest of all, it was because of DARLINGTON's idea that directed segregation "almost uproots genetics" that he felt driven to put forward an alternative theory. He apparently did not realize that his own theory also demanded directed segregation, since whatever he might assume about the internal composition of Y and X, the fact remained that they were visibly different, and that the Y occurred only in males. He apparently also did not realize that a well-authenticated case of the directed segregation of chromosomes not in contact at metaphase had long been on record. In *Gryllotalpa borealis* there are present an unequal bivalent and a univalent (BAUMGARTNER 1911). (These observations are not to be confused with the dubious and in any case irrelevant studies made by VOINOV (1914, but compare 1912) on a different species, *G. vulgaris*.) The univalent and the larger member of the bivalent always go to the same pole (PAYNE 1912, 1916). The other pole receives the smaller member of the bivalent, which we may call Y. Sperm receiving Y give rise to males. The sperm which receive the two X chromosomes (we may call them X^1 and X^2) give rise to females. However, it is interesting, and perhaps important, that one of PAYNE's (1916) figures shows a connection between the unequal bivalent and the univalent. An examination of the prophases might prove fruitful, especially in view of the work of CAROTHERS (1913) who found a bivalent which divided unequally in the first meiotic divison, after having been associated with the accessory in prophase. At metaphase the bivalent was no longer associated with the accessory, and disjoined at random with

respect to it. But the unequal bivalent was present in all the twenty males examined. CAROTHERS assumed that the female also carried the unequal bivalent and that strict selective fertilization took place. I would prefer to suppose that zygotic mortality was the explanation.

Be that as it may, it is clear that in *miranda* both directed segregation and zygotic mortality are involved. Directed segregation makes exceptional zygotes rare. When they occur, zygotic mortality eliminates them.

We have formed a picture of the sex-chromosome mechanism as it exists in *miranda* today, and of the stages through which it must have gone in the past, since the time when it diverged from the *pseudoobscura* type. If we have said nothing about the possible causes of its divergence and subsequent evolution, it is for reasons not far to seek. But where information is lacking, it is tempting to speculate. There is present in wild populations of *pseudoobscura* a gene, "sex-ratio" (STURTEVANT and DOBZHANSKY 1936) which, by virtue of its effect on its own distribution at meiosis, tends to displace its normal allele. It is sex-linked, and a male carrying it produces almost all daughters, so that a population homozygous for it would die out. But if a III-Y fusion chanced to occur and to spread through a small population, that population would then be protected against such a danger, for "sex-ratio" would have much less tendency to spread in it. This follows from the fact that the Y is eliminated during the maturation divisions of the "sex-ratio" male. If a III-Y fusion were present, the fertility of the male would be halved. Again, a population carrying a III-Y fusion might be at an advantage in that half its members would benefit from whatever heterosis resulted from III being kept heterozygous.

It is necessary to seek some reason for the spread of this fusion, for there can be no doubt that it is, and has been from the first, responsible for a certain amount of zygote mortality. But no zygote mortality need have occurred during the transition from a *melanogaster*-like condition to that found in *pseudoobscura*, except perhaps at first. Let us suppose that in the common ancestor of *melanogaster* and *pseudoobscura* the material of III L (*melanogaster*) (=XR *pseudoobscura*) was in the form of paired rods. In the *melanogaster* line of descent it would undergo no change except to become attached to III R. In the *pseudoobscura* line of descent we would suppose that a fusion of one member of the pair with Y took place, and, perhaps simultaneously, perhaps not, a fusion of the other member of the pair with X. (Similar fusions have actually been obtained in Drosophila by PAINTER and STONE (1935).) The *pseudoobscura* condition would thus be reached, with the very important exception that the male, instead of being haploid for the material of III L (=XR), would carry it in duplicate, once in the X and once in the Y. (It should be mentioned here that with

this point in view I carefully examined the salivary gland chromosomes of a *pseudoobscura* male of the Oaxaca 4 race, in which the largest Y occurs. I could find no euchromatin in the Y.)

Thus we arrive at what is perhaps the most interesting question of all. Can we suppose that euchromatic material in the Y would degenerate and ultimately disappear as a result of its being kept heterozygous? Such and idea was propounded by MULLER and STURTEVANT (MULLER 1914) to account for the absence of genes in the Y, and has since been elaborated by MULLER (1918), and, among others, by HALDANE (1933). Opposed to this view is FISHER (1935), who believes he has shown by mathematical considerations that no accumulation of deficiencies, lethals or deleterious genes, even though recessive, could take place; that it would be effectively prevented by the occasional presence of a mutant gene at the same locus in the homologue. However, FISHER's mathematical treatment is based on a number of premises. These are not all correct, and one might expect the same to be true of his conclusions. The present study has shown that deleterious genes (deficiences or mutant alleles at the *or* and *px* loci) have in fact become established in the material of III since it became a part of Y. The time required for this process has been so short, geologically speaking, as scarcely to permit *pseudoobscura* and *miranda* to become visibly different in external morphology. I think it not at all unreasonable to suppose that *pseudoobscura* and *melanogaster*, which are very different externally, have been separated long enough for an entire chromosome-arm, attached to the Y, to degenerate and disappear.

To be sure, the *or* and *px* loci are the only ones for which the *miranda* Y is known not to carry a normal allele. But they are two of the three loci which it has so far been possible to test. There can be no doubt that the material of III in the Y has been greatly altered. How different the Y is from X^2 in total effect is well shown by the fact that hybrid males carrying X^2 survive to the adult stage, while those carrying Y, the other chromosomes being the same, die before hatching from the egg. On the other hand, individuals of pure *miranda* having the male set, except for Y being replaced by X^2, do not live to be adults, but die in an immature stage, though precisely which stage it is not possible to say.

I believe that positive selective action has aided passive mutation in making Y and X^2 different. $X^1_2 X^2_2 A$ individuals would have been sterile from the time when the Y-III fusion originated, since they would have lacked the Y (which in *pseudoobscura* as in *melanogaster* carries factors necessary for the normal development of the spermatids into spermatozoa). Such sterile males would have been disadvantageous to their blood relations. Since Drosophila is not a social animal, sterile individuals are entirely worthless; indeed they compete with fertile individuals for mates

and food. Consequently some geographic races, some local populations of what has since become *Drosophila miranda* would have been at a selective advantage if they possessed such a gene complex that $X^1{}_2X^2{}_2A$ males were weak or short-lived as adults, or survived to that stage less often. Such selective action would continue till $X^1{}_2X^2{}_2A$ zygotes did not hatch from the egg. Since there is no competition between unhatched zygotes, selection would not tend to make zygotes die at a still earlier stage, unless perhaps by way of insuring against even their occasional survival. I shall attempt to find out how far this process has actually gone. Of course there would be a concurrent selection of the same nature against the *production* of $X^1{}_2X^2{}_2A$ zygotes, that is, for regular segregation in the male.

Probably a selection of similar nature would act to make hybrids between *miranda* and *pseudoobscura* sterile or inviable (STURTEVANT 1938). DOBZHANSKY and TAN (1937) have suggested one possible reason why the hybrids of the heterozygous sex are, as HALDANE (1933) had pointed out, usually the less fertile and the less viable. In the present connection I should only like to add that, in the cross of a *pseudoobscura* female by *miranda* male, selection against the survival of X III A (*pseudoobscura*) YA (*miranda*) zygotes would be about 267 times as strong as that against the survival of X III A (*pseudoobscura*) X^2A (*miranda*) individuals.

It is only on the recent evolution of the *miranda* sex mechanism that this comparative study has cast any light. What the series of changes may have been which led to the condition present in the common ancestor of *miranda*, *pseudoobscura*, and *melanogaster* is a question which must be answered by further study. Within the genus or near it there are surely species by the investigation of whose sex-mechanism this question could be at least partially answered, and a material contribution to our understanding of the evolution of sex-determination thereby made.

SUMMARY

1. *Drosophila miranda* and *Drosophila pseudoobscura* are two closely related species whose most striking difference is in the structure of their sex-determining mechanisms. The nature of this difference is elucidated in the present paper.

2. An attempt is made to reconstruct the recent evolution of the *miranda* mechanism. It is shown that degenerative changes have been retained in chromosomal material kept heterozygous by being attached to the Y.

3. On the basis of this study a better understanding of other variations in the sex-determining mechanism within the genus Drosophila is possible. Some bearings of this study on the general question of the evolution of sex-determining mechanisms are discussed.

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