SALIVARY GLAND CHROMOSOMES IN THE TWO RACES OF *DROSOPHILA PSEUDOOBSCURA*

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INTRODUCTION

The two races of *Drosophila pseudoobscura*, known as race A and race B, produce sterile male offspring when crossed (LANCEFIELD 1929), have visibly different shapes of the Y chromosomes (DOBZHANSKY and BOCHE 1933), and differ in their physiological characteristics (POULSON 1934, DOBZHANSKY 1935a, BOCHE unpublished). They appear, however, indistinguishable morphologically. LANCEFIELD (1929) and KOLLER (1932) found, in female hybrids between these two races, a suppression of crossing over as observed in both ends of the X chromosome, while in its middle crossing over is nearly normal. Such a suppression of crossing over is usually due to the presence of inverted sections (STURTEVANT 1926, 1931). It is, therefore, probable that race A differs from race B in having two inverted sections in the X chromosome. Nothing has been reported concerning the conditions of autosomes in this respect.

The technique of studying the salivary gland chromosomes (HEITZ 1933 and PAINTER 1934a) permits a more precise comparison of the gene arrangement in the chromosomes of related species and races of Diptera than has been possible heretofore. The results of such a comparison of the chromosomes of A and B races of *Drosophila pseudoobscura* are presented in this paper. The author wishes to express his gratitude to Professors TH. DOBZHANSKY and A. H. STURTEVANT for their encouragement, advice and use of their stocks

MATERIAL AND METHODS

Strains of *Drosophila pseudoobscura* coming from different parts of the distribution area of this species were used. A collection of such strains is being kept in this laboratory. The aceto-carmines smear method for studying the salivary gland chromosomes has been applied as described by PAINTER (1934a and b). Larvae whose chromosomes were to be studied were raised at low temperature (14°C). It has been noticed by the writer, and recently also by HEITZ (1934), that chromosomes in such larvae are larger, thicker and can be better stretched in preparations than in larvae grown at higher temperatures. The slides were preserved at the same low temperature, which keeps them longer in a good condition for observation.

All drawings were made with the aid of a camera lucida. Figures 1 to 6 are really composite drawings. Each section of the chromosomes has

been copied from a camera lucida sketch showing this particular section most clearly. The banding in each section has been checked at least twice in different preparations. It is not claimed that these figures show the full number of bands present in the salivary gland chromosomes of the species, but it is hoped that they are good enough for a demonstration of at least qualitative differences in the gene arrangement in the chromosomes and as a basis for future studies.

GENERAL CHROMOSOME MORPHOLOGY

The chromosome group of *Drosophila pseudoobscura*, as seen in the spermatogonial, oögonial or nerve cell division, consists of five pairs of chromosomes: V-shaped X chromosome, three rod-shaped autosomes, and one very small dot-like autosome. In the salivary glands of adult larvae or young pupae five long strands, each embedded at one of the ends in a chromatin coagulum known as the chromocenter can be seen (figure 10). In favorable cases, a very short (sixth) element may be seen attached to the chromocenter. This is supposedly the dot-shaped autosome (figure 6). Each strand represents two closely paired homologous chromosomes, the result of somatic synapsis (HEITZ 1933).

In females all the strands are equally densely stained and similar in width. In males two of the long strands are distinctly paler and somewhat thinner than the rest. The two paler strands are the two limbs of the X chromosome. The Y chromosome, being apparently composed of an inert material, is not paired with the X, but is probably included in the chromocenter, just as it is in *Drosophila melanogaster* (PAINTER 1934b, HEITZ 1934).

Individual chromosomes can be identified in any nucleus, once one is acquainted with their distinctive characteristics, especially in the regions of their free ends. The correspondence between the respective chromosomes in the salivary gland cells and their counterparts among the linkage groups known in the species has been determined by studying the inverted sections in the different chromosomes. The autosomes were studied mostly in the preparations of male larvae, since in these the autosomes stand out distinctly from the lighter and more slender X chromosome. Figures 1 to 6 show the composite maps of the chromosomes of the race A.

THE X CHROMOSOME

The X chromosome (figures 1 and 2) is recognized by being thicker and more intensely stained in females than in males (see above). The two arms are of unequal length in salivary gland cells, one arm (figure 2) being about two-thirds of the length of the other (figure 1). Since in cells other than those of the salivary glands, the X chromosome is equal-armed

FIGURES 1-6.—Composite drawings of the salivary gland chromosomes in race A of *Drosophila pseudoobscura*. Figure 1—the right limb of the X chromosome (the figure cut in two; 1A—the distal part, 1B—the proximal part). Figure 2—the left limb of the X chromosome. Figure 3—the second chromosome (3A—distal, 3B—proximal part). Figure 4—the third chromosome. Figure 5—the fourth chromosome. Figure 6—the fifth chromosome. The distal ends are directed upwards in all figures. The approximate lengths of the inversions distinguishing races are shown by cross bars. The left limb of the X chromosome has three inversions, the distal one being relatively somewhat longer than suggested by the drawing. The scale below represents 30 micra.

(DOBZHANSKY and BOCHE 1933), the behavior in salivary gland cells suggests that one of the arms contains more inert material than the other. In most salivary gland preparations, there is no indication that the two arms of the X chromosome belong together, but occasionally, on crushing the nucleus, they are separated from the rest of the chromosomes together with a part of the chromocenter adjoining them.

Race A females carrying the dominant sex-linked gene *Pointed* have been repeatedly crossed to B race males. After many such crosses flies were obtained that carried *Pointed* and a section of the X chromosome adjacent thereto from race A, and had all other chromosomes replaced by those of race B. Linkage tests show that females carrying this X and a race B X give normal crossing over in the right end of X, and greatly reduced crossing over in the left end. The salivary gland cells of such females heterozygous for *Pointed* were studied (the flies were supplied by Dr. A. H. STURTEVANT). A long and two very short inverted sections have been found in one of the limbs of the X chromosome (figure 11). Since the locus of *Pointed* lies at the extreme left end of the known map of the X chromosome, the limb showing these inversions must be the left limb of the X. This limb appears, in the salivary gland cells, shorter than the other limb, which, consequently, must be the right limb of the X chromosome.

Race A females homozygous for the sex-linked recessive genes *beaded*, *yellow* and *short* were crossed to race B wild-type males. The F_1 females were backcrossed to *beaded yellow short* males, and in the offspring some *beaded non-yellow non-short* females were selected. Since *beaded* lies near the left end (not very far from *Pointed*), *yellow* about in the middle, and *short* at the right end of the known map of the X chromosome, these females presumably had the right limb of one of their X chromosomes from race B, and the rest of the X chromosomes from race A. A part of the offspring of such females (crossed to race A males) showed an inverted section in the longer limb of the X chromosome (figure 12). The longer limb of the X chromosome in the salivary gland cells is, accordingly, the right limb.

Besides the difference in length, the two limbs of the X chromosome can be distinguished in salivary gland preparations by the characteristics of their free end portions. The free end of the right limb (figure 1) is inflated to form a disc-like structure. Just beyond the disc there is a light area, followed by about twelve heavy and light bands lying rather close together. Next follow an inflated lightly staining region, and a group of about six or seven heavy bands and a second inflated region and a constriction which usually makes a good landmark. The left limb (figure 2) terminates in an oblong, square-headed bulb, showing a number of dis-

tinct bands and vesiculated areas, among which one band, lying at about the middle of the bulb, is particularly heavy. Proximally the bulb is separated from the body of the chromosome by a neck-like constriction, the distal end of which is also marked by two heavy bands.

THE SECOND CHROMOSOME

The second chromosome (figure 3) is the carrier of the Bare-Smoky linkage group. In the salivary gland cells the second chromosome is the longest in the nucleus (considering each limb of the X as a separate chromosome). It has been identified by means of an inversion, present in the

FIGURE 7.—Inversion figure in third chromosome in the hybrid San Bernardino-1 ♀ × *Sc pr* ♂.

FIGURE 8.—A complex configuration in the third chromosome given by two overlapping inversions (A cross San Bernardino-1 ♀ × Seattle-4 ♂).

FIGURE 9.—An inversion figure in the second chromosome (A cross Santa Lucia-7 ♀ × Bare-Smoky ♂). The scale above represents 30 micra.

strain Santa Lucia-7, which has been found by Dr. STURTEVANT to reduce very markedly the crossing over between the genes Bare and Smoky. One half of the larvae from the cross (Santa Lucia-7 females × Bare-Smoky males) F_1 females × cinnabar males were found to possess in their salivary gland cells the inversion figure, an example of which is shown in figure 9.

Cytologically, the second chromosome resembles the right limb of the X chromosome. Both have terminal bulbs carrying rather heavy bands. However, in the second chromosome there exists a large vesiculated area not far from the free end; this area carries a heavy band in its middle, and is preceded by four very heavy bands. Distally, the free end is more or less club-shaped rather than disc-shaped as in the X chromosome.

THE THIRD CHROMOSOME

The third chromosome (figure 4) carries the orange-Scute-purple linkage group (CREW and LAMY 1934). STURTEVANT (unpublished) observed that a large number of wild strains have an inverted section in this chromosome (as judged by a reduction of crossing over in the hybrids between these strains and *or Sc pr*). Two of the strains in which such a reduction of crossing over has been observed, namely, Santa Lucia-37 and San Bernardino-1, were crossed to *or Sc pr*. The salivary glands of the F_1 larvae were examined, and inversion figures, an example of which is shown in figure 7, were found in every cell.

The free end of the third chromosome resembles somewhat that of the left limb of the X. However, in the third chromosome there exists a rather prominent inflated region very close to the free end instead of the constriction observed in the left limb of the X in approximately the same position. Distally from this inflated area, which is devoid of prominent bands, is located a knob-like end portion with heavy bands.

THE FOURTH AND FIFTH CHROMOSOMES

The two remaining autosomes (figures 5 and 6) are extremely unlike in size. The fifth obviously corresponds to the dot-like chromosome in the metaphase plate. The correspondence between these chromosomes and the linkage groups is a matter of conjecture since no inversions have been as yet discovered in either.

The fourth chromosome (figure 5) is shorter than any other chromosome in the salivary gland cells except the fifth, which is very small. The fourth is easily distinguished from the rest by a conspicuous terminal inflation. The fifth (figure 6) is frequently invisible due to its smallness and to being imbedded in the chromocenter. It is elliptical in shape, and shows five heavy and several light bands.

CHROMOSOME DIFFERENCES BETWEEN
RACE A AND RACE B

Salivary gland cells of hybrid larvae from the crosses of race A and race B show one large and two very small inverted sections in the left limb of the X chromosome (figure 11), and rather long inversions in the right limb of the X chromosome, in the second and in the third chromosomes (figures 12, 13 and 14). In figure 10 all six inversions can be seen.

These inversions have been observed in the following interracial crosses:

Texas(A) ♀ × Seattle-4(B) ♂	<i>or Sc pr</i> (A) ♀ × Cape Flattery-3(B) ♂
Texas(A) ♀ × Seattle-6(B) ♂	<i>or Sc pr</i> (A) ♀ × Campbell Riv.-3(B) ♂
La Grande-2(A) ♀ × Seattle-4(B) ♂	<i>or Sc pr</i> (A) ♀ × Quesnel-5(B) ♂
La Grande-2(A) ♀ × Seattle-6(B) ♂	<i>or Sc pr</i> (A) ♀ × Greenhorn Mt.-3(B) ♂
La Grande-2(A) ♀ × La Grande-4(B) ♂	<i>or Sc pr</i> (A) ♀ × Lassen-7(B) ♂

Zion-1(A) ♀ × Seattle-4(B) ♂	or <i>Sc pr</i> (A) ♀ × Humboldt-1(B) ♂
San Bernardino-1(A) ♀ × Seattle-4(B) ♂	or <i>Sc pr</i> (A) ♀ × Reedsport-3(B) ♂
Lassen-16(A) ♀ × Seattle-4(B) ♂	or <i>Sc pr</i> (A) ♀ × La Grande-4(B) ♂
Aspen(A) ♀ × Seattle-4(B) ♂	or <i>Sc pr</i> (A) ♀ × Seattle-4(B) ♂
Idaho-Falls(A) ♀ × Seattle-4(B) ♂	Shuswap-1(A) ♀ × Seattle-4(B) ♂
<i>bd y sh</i> (A) ♀ × Seattle-4(B) ♂	

Since inverted sections are by no means infrequent in different chromosomes in various strains collected in nature (unpublished data of Dr. STURTEVANT, see above), it was necessary to establish whether these inversions observed in the interracial hybrids are racial characters or char-

FIGURE 10.—A—a microphotograph of the salivary chromosome complement of A × B hybrid showing all interracial inversions. B—an outline sketch of the figure on the left with each chromosome and inversion labelled.

acters of individual strains. In the crosses listed above both A race and B race strains were used coming from diverse parts of the distribution area of each race. Likewise, strains of race A and race B of different “strength” and having different types of Y chromosomes (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1935b) were used. Since in all crosses with the apparent exception of the cross or *Sc pr* ♀ × Greenhorn Mt.-3 ♂, all the inversions mentioned above were cytologically identified, these inversions may be safely considered to constitute distinctive properties of races. In the exceptional cross the third chromosome of the hybrid had no inverted section.

The three inversions observed in the hybrid in the left limb of the X chromosome are shown in figure 11. One of these inversions, located most distally, is the longest in this chromosome. The other two, one at the middle and the other in the proximal portion of the chromosome, are exceedingly short. Each of these, especially the proximal one, includes very few bands. In view of this the identification of these two inversions is based on the consistent lack of pairing in those regions rather than on a definite alteration of the sequence of the bands (these regions pair normally in pure races). However, the possibility is not excluded that these are not inversions at all. The inverted section in the right limb of the X, in the second and in the third chromosomes are shown respectively in figures 12, 13 and 14. All of these involve relatively long sections of chro-

mosomes (their limits are marked in the composite chromosome maps in figures 1, 2, 3 and 4).

None of the inverted sections found within a race (see above) seem to be identical with the inversions distinguishing races. As stated above, a number of A race strains found in nature have an order of genes different from that characteristic for the *or Sc pr* strain of the race A, and show inversion figure when crossed to the latter. One of these strains, namely San Bernardino-1, has been crossed to race B (Seattle-4). In the hybrid the third chromosome showed a peculiar configuration (figure 8) which

FIGURES 11-14.—Inversions in the A×B hybrids. Figure 11—left limb of the X chromosome; Figure 12—right limb of the X; Figure 13—the third chromosome; Figure 14—the second chromosome. Arrows in figure 11 point at the inversions. The scale below represents 30 micra.

can be accounted for on the assumption that this hybrid had two different inversions in this chromosome. The non-identity of other interracial inversions with the intraracial ones has been established by observing that they involve different regions.

Some interesting observations on the pairing of inverted chromosome segments in the salivary gland preparations were made. A complete pairing of all sections of chromosomes is, on the whole, infrequent in such preparations. Very short inversions, such as the two smaller ones in the left limb of X, apparently never pair, although the non-inverted parts adjacent to the inversion usually show complete pairing. In somewhat longer inversions, such as the longest inversion in the left limb of the X

chromosome, pairing of the inverted parts is only seldom observed. Finally in cases of long inversions, such as both interracial and intraracial inversions in the second and third chromosomes and in the right limb of the X, the inverted segments are apparently always paired at least in a part of their lengths, and occasionally are paired completely. On the other hand, in these long inversions the non-inverted parts of the chromosomes adjacent to the inversions almost invariably fail to pair for a certain distance.

DISCUSSION

Comparisons of chromosome groups in closely related species frequently show dissimilarities both in the number and in the morphology of chromosomes. It seems probable that these cytologically observable dissimilarities are merely visible manifestations of the differences in the internal structure of the chromosomes, or more precisely, in the gene arrangement. STURTEVANT (1921, 1929) was the first to show, in the case of *Drosophila melanogaster* and *Drosophila simulans*, that such differences between species really exist. The third chromosome of *Drosophila simulans* has an inverted section as compared with *Drosophila melanogaster*.

As shown by the sterility of their hybrids, race A and race B of *Drosophila pseudoobscura* may be regarded an incipient species. The data presented above show that rearrangements of the genic material within the chromosome took place during the processes that led to the separation of these two races. Six inverted sections, four in the X chromosome, and one in each of the second and the third chromosomes, are present in the interracial hybrids. Since a rather large number of strains coming from different parts of the distribution area were involved in the hybrids in which the inversions were discovered, it is reasonable to assume that these inversions are racial characters.

Whether the inverted sections distinguishing the races are directly responsible for the sterility of the interracial hybrids is at least doubtful. DOBZHANSKY (1933a, 1934b) adduced a number of arguments to prove that the sterility of the *Drosophila pseudoobscura* hybrids is due to an interaction of complementary factors rather than to differences in the gross structure of the chromosomes. Moreover, judging by analogy with *Drosophila melanogaster*, an individual heterozygous for several inverted sections in different chromosomes need not be sterile. There still, however, remains a possibility that sterility is somehow indirectly connected with these intraracial inversions. The gene mutations that cause sterility might have been in some manner connected in their origins with the origin of the inversions, just as some mutations, or position effects, are connected with translocations and inversions.

Whether or not the variations in the gene arrangement are causally

related to the hybrid sterility in *Drosophila pseudoobscura*, it remains a fact that racial differentiation partook in this case of both sources of evolutionary variability—gene variability and variability in the gross structure of chromosomes. It is, indeed, probable that the hybrid sterility may be in some cases directly caused by structural differences in the chromosomes of the parental species, especially in the hybrids that produce fertile allotetraploids.

The pairing in the salivary gland cells of the homologous strands carrying inversions deserves some consideration. In case of relatively long inversions, the parts of the chromosome adjacent to the loci of breakage mostly fail to pair with their homologs, while the inverted sections usually pair along most of their lengths, and form rings or similar configurations. On the other hand, short inverted sections frequently fail to pair completely, while the non-inverted parts are regularly paired. These facts are especially interesting if the meiotic pairing in the female germ cells is really analogous with the somatic synapsis in the salivary gland cells. For in this case, the disturbances of crossing over produced by the inverted sections may find their causal explanation, as suggested by the hypotheses of competitive pairing (DOBZHANSKY 1933b, 1934a). Indeed, similar phenomena have been observed by McCLINTOCK (1933) in the meiosis of individuals of *Zea mays* carrying inversions.

SUMMARY

1. Five long chromosome strands, each attached at one of its ends to the chromocenter, and one small chromosome imbedded in the chromocenter, are visible in the nuclei of the salivary gland cells of the adult larvae of *Drosophila pseudoobscura*.

2. Individual chromosome strands can be identified by their morphological peculiarities—distribution of the “bands,” inflated areas, and the shape of the free ends. In the salivary gland cells of the adult larvae, the Y chromosome is invisible, and, in the males, the X chromosome is paler than the autosomes, due to being single instead of composed from two paired homologs.

3. The correspondence between the chromosome strands observed in the salivary gland cells and the linkage groups of genes known in *Drosophila pseudoobscura* has been established by observing individuals carrying inverted sections in one or other of the chromosomes.

4. The two limbs of the X chromosome appear similar in length in the spermatogonial or nerve cell metaphases, but they are unequally long in the salivary gland cells. This is probably due to one of the limbs containing more inert material than the other limb.

5. The two races of *Drosophila pseudoobscura* differ in six inverted sec-

tions: four in the X chromosome, one in the second, and one in the third chromosome.

6. Inverted sections within a race have also been demonstrated cytologically. These inversions occur in nature.

7. Apparently none of the intraracial inversions are identical with the interracial ones.

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