A COMPARISON OF THE SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER AND D. SIMULANS*

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INTRODUCTION

THE general problem of chromosomes and speciation has interested cytologists and geneticists for a long time. The earlier studies on animals dealt with comparisons of the metaphase chromosomes, and evidence for homologies in related species was restricted to a similarity in chromosome number and to the appearance of the individual elements. Following MULLER'S X-ray work and the discovery of numerous chromosome rearrangements, the judging of homologies by comparing metaphase elements appeared hazardous because it became clear that very extensive gene realignments may occur without one's being able to detect them in the condensed chromosomes. In addition to this, the presence of inert chromatin (MULLER and PAINTER 1932) could make such comparisons very misleading.

PAINTER'S discovery and development of the salivary gland method for the study of chromosomes, opened a new era for the cytologist. He pointed out in his first paper (1933) that homologous chromosomes undergo somatic synapsis, and due to this phenomenon it is possible to compare in the most exact way the "active" or euchromatic areas in salivary gland chromosomes. In closely related species which can be hybridized one should be able to detect any chromosome rearrangements which might have occurred since the related forms originated from a common ancestor.

The salivary gland chromosome method has been employed thus far in the study of three species crosses. The first of these is the *Drosophila melanogaster* and *D. simulans* cross reported by PÄTAU in 1935. He found that with a few exceptions there is usually close synapsis in hybrids in the euchromatic areas. The distal ends of some of the chromosomes do not conjugate. There are two extra lines in the X chromosome of *D. simulans* which are not seen in *D. melanogaster*, and in the right arm of the third chromosome there is a long inversion, reported earlier by STURTEVANT (1929) who based his conclusion on genetic data. PÄTAU also noted that a part of the non-inverted proximal end of the right arm of the third chromosome failed to synapse and that the fourth chromosomes were entirely different and would not conjugate in hybrids.

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KERKIS (1936, 1937) working with the same cross confirmed PÄTAU'S findings, but failed to mention extra lines in the X chromosome of *D. simulans*.

The second species cross is *D. pseudoobscura* by *D. miranda* studied by DOBZHANSKY and TAN (1936). They showed that numerous and extensive changes have taken place since these species arose. Translocations and inversions are present in the X and in the second chromosomes. In the left limb of the X, and in the third, fourth, and fifth chromosomes, the gene arrangement is very different in the two species. Even in regions where the chromosomes exhibit similar patterns, the pairing of homologues is extremely variable.

The most recent study on hybrids is by METZ and LAWRENCE (1938), dealing with a Sciara cross, *S. ocellaris* and *S. reynoldsi*. They report that in the salivary glands of the hybrids, the chromosomes are associated in symmetrical pairs, but complete synapsis is found in only a few short regions. There is an absence of conspicuous translocation and inversion configurations, but a high frequency of small differences in corresponding regions, indicating that many small chromosomal changes have occurred in the recent evolution of the two species.

The present investigation was undertaken in order to make a very detailed study of the *D. melanogaster* and *D. simulans* hybrids, and if possible to determine the origin of the extra bands reported by PÄTAU for *D. simulans*. This work was carried out under the direction of PROFESSOR T. S. PAINTER, to whom the writer wishes to express his indebtedness and thanks.

MATERIALS AND METHODS

The salivary glands were dissected from mature hybrid larvae in RINGER'S solution (cold-blooded formula). Both temporary and permanent mounts were made. PAINTER'S acetocarmine method was used in the preparation of temporary mounts, and a modification of BRIDGES' aceto-carmine-alcohol-euparal method was employed in making permanent slides. All drawings were made using a 120× oil immersion lens, a 20× eye piece, and a camera lucida at table level.

The findings of this study can be presented best by describing the individual chromosomes. For the exact location of the bands BRIDGES' nomenclature is used.

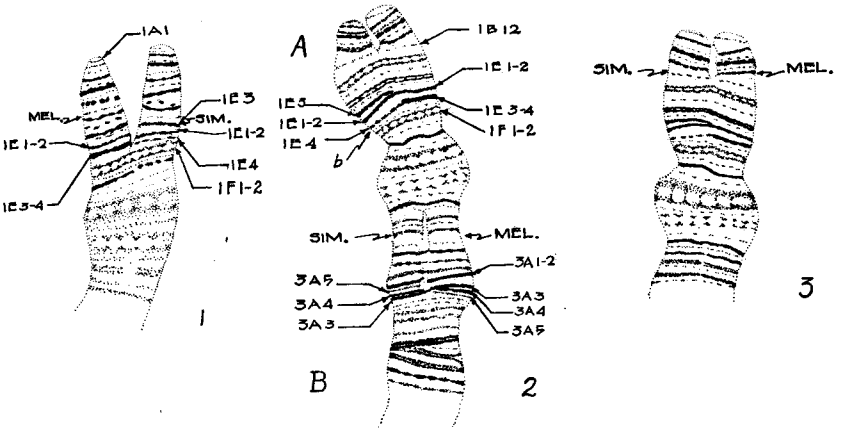
OBSERVATIONS

The X chromosome

In hybrids the proximal regions of the two homologues usually synapse intimately, but near the distal end there is a great deal of variability in the union due, mainly, no doubt, to the presence of several gene rearrange-

ments which are present here. Typically the tip ends lie separate, synapsis beginning at a line 1 F 1–2, just to the left of the puffed area (fig. 1). In some cases, however, the ends are joined up to and including lines B 12 or 13 (fig. 2), but in no case has synapsis been found beyond this point, although the bands appear alike up through the line 1 A 2 (fig. 2).

Just at the tip of the free left hand end, PÄTAU has reported that in *D. simulans* the band 1 A 1 is missing. This region has been examined with great care, and it is true that often in hybrid larvae an extra band appears in *D. melanogaster* as in figure 1. However, it is extremely difficult to determine the exact pattern in these ends, and the absence of a band in *D. simulans* does not rest on a very secure basis.



FIGURES 1–3.—Camera lucida drawings of the left hand ends of the X chromosomes of a hybrid larva. Figure 2 shows the presence of two short inversions in the region of the 1 E 1–2 band and at the 3 A 1–2 level.

Figure 2 is made from a cell of a hybrid larva in which synapsis is unusually complete. The *melanogaster* chromosome lies to the right, and the *simulans* component to the left. At the point marked A in the figure it will be noted that the *melanogaster* band pattern consists of two doublets, 1 E 1–2, and 1 E 3–4, the latter being about twice as broad as the former. In the *simulans* chromosome, the band 1 E 1–2 is continuous with its *melanogaster* homologue, but a broad 1 E 3–4 band is not seen. Instead a rather narrow band, labeled provisionally 1 E 4 is synapsed with the 1 E 3–4 band of *melanogaster*. To the left of the 1 E 1–2 band in *D. simulans* there is an extra band labelled 1 E 3 which is about equal in breadth and staining intensity to line 1 E 1–2. Essentially the same features are brought out in figure 3. Note that in each case the line 1 E 1–2 synapses in the hybrid, and that a narrow band of *D. simulans* (1 E 4) is joined with the broad 1 E 3–4 band of *D. melanogaster*. The explanation for the difference of patterns in the two species is that in *D. simulans* the bands 1 E 1–2 and

1 E 3 are inverted so that 1 E 3 lies to the left of the former. PAINTER and GRIFFEN (1937) have indicated that "double" bands may be made up of one, two, or more rows of chromomeres, and although BRIDGES (1938) represents the lines 1 E 3–4 as equal sized, we are forced to conclude that we are dealing here with a band involving at least two rows of unequal-sized chromomeres, since in *D. simulans* the band 1 E 3 is twice as broad as 1 E 4. The extra band reported by PÄTAU for *D. simulans* is, of course, the 1 E 3 band which lies separate in this species.

It is a point of unusual interest to note that although the band 1 E 1–2 in *D. simulans* is inverted, it synapses perfectly with its uninverted homologue in *D. melanogaster*.

From A to B in figure 2, there is a similarity in the patterns of the two species and synapsis is often complete through the band 3 A 1–2. In *D. melanogaster* next to the broad band 3 A 1–2, there are two broad bands 3 A 3, and 3 A 4, and then a narrow one 3 A 5. In *D. simulans* the order is reversed and bands 3 A 4 and 3 A 5 lie to the left of band 3 A 3. The 3 A 3 bands, even though one of them is inverted, often synapse in hybrids and cause a certain amount of distortion as a result (fig. 2). On the other hand, bands 3 A 4 and 3 A 5 being out of position in *D. simulans* do not unite in the hybrid. Here again the extra band reported by PÄTAU in *D. simulans* is a result of an inversion.

Farther to the right along the X chromosome in hybrids, certain regions characteristically fail to undergo intimate synapsis, namely regions 7 C, 10 B and F, 12 D to F, and 18, 19 and 20. But in each instance cells have been found in which synapsis is completed, indicating that these regions are similar in nature in the two species.

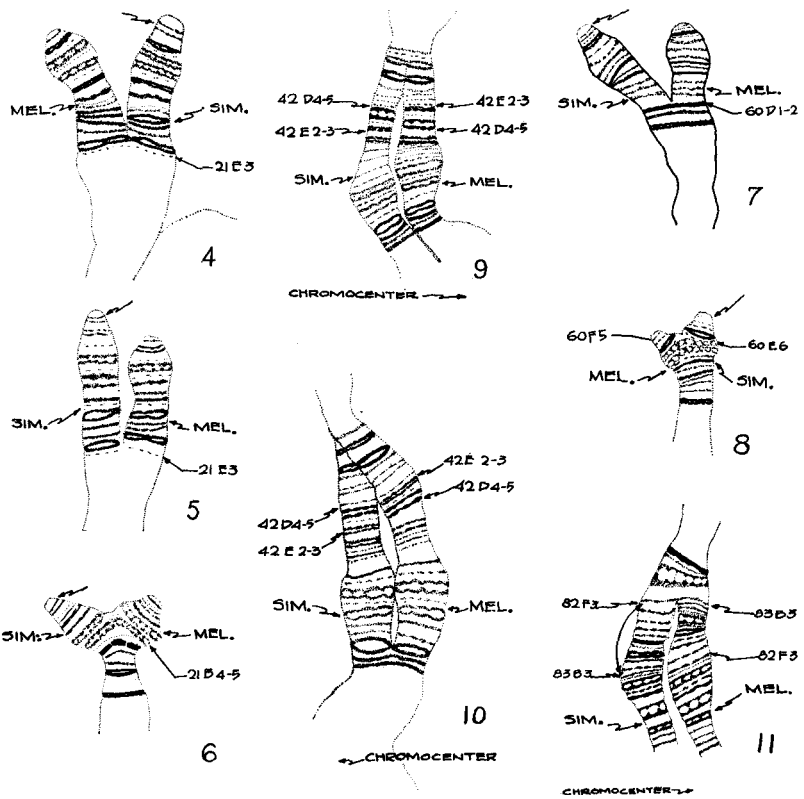
Second chromosome

As KERKIS has pointed out, the distal ends of the left arm of the second chromosome never completely join in synapsis. Typically the union ends at about the band 21 E 3 (figs. 4 and 5) but in exceptional cases may extend to 21 B 4–5 (fig. 6). There is a consistent tendency for the *melanogaster* chromosome to be shorter and thicker at the end region, and for the *simulans* component to be extended, especially the extreme tip. Otherwise there is no detectable difference in the band pattern of the two species until the free tips are reached. Here *D. simulans* shows one band that can not be seen in *D. melanogaster* (figs. 4 and 6).

In regions 33, 34, 35, 38 and 39 in the left arm conjugation is usually incomplete, but in all instances cells have been found in which synapsis is completed and we have no morphological evidence for any changes in these regions for the two species except their failure to synapse regularly.

The free end of the right arm of the second chromosome behaves very

much like the tips of the left arm. Figures 7 and 8 show the normal range of synaptic union. It will be observed that the *simulans* chromosome has a tendency to be more elongated than the *melanogaster* component and again we see in the figures the presence of an extra band in *D. simulans* right at the tip end.



FIGURES 4–11.—Figures 4–6 show the range of synaptic union in the distal end of II L and the presence of an extra band in *D. simulans*. Figures 7 and 8 are from the distal end of II R. Figures 9 and 10 show the presence of a short inversion in the right arm of chromosome II near the chromocenter region. Figure 11 shows the presence of a short inversion in the right arm of chromosome III near the chromocenter.

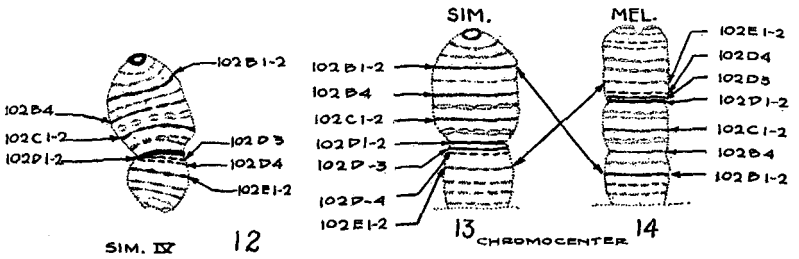
Although there are short areas along the right arm which frequently do not join in synapsis, notably at 58 D and E, no other evidence of a change in pattern or of homology has been found until the region of the spindle fiber is reached. Here at region 42 (figs. 9 and 10) there is a very short inversion in *D. simulans* which involves bands 42 D 4–5 to 42 E 2–3. As often happens, even though the inversion is quite short, the chromosomes in hybrids usually remain apart for some distance on either side of the rearrangement.

Third chromosome

The left arm of the third chromosome shows complete synapsis in hybrids and no differences in band pattern have been found. The distal end of the right arm also appears to be similar in the two species. There are, however, two inversions in the right arm of the third chromosome. A large inversion (described by PÄTAU) extends from band 84 B 3 to band 92 C 3. A second inversion, which is relatively small, is nearer the spindle fiber end and is not quite so obvious (fig. 11). As indicated in the figure the inverted section of *D. simulans* extends from 82 F 3 to 83 B 3 on the *melanogaster* chromosome, and involves some ten or twelve bands.

Fourth chromosome

The outstanding features of the fourth chromosome of *D. simulans* (figs. 12 and 13) are: A rather narrow basal portion next to the chromocenter which is limited distally by three deeply staining bands, 102 D 1–2,



FIGURES 12–14.—Figure 12 is a drawing of a normal chromosome IV taken from *D. simulans*. Figures 13 and 14 are semidiagrammatic drawings of the fourth chromosomes of *D. melanogaster* and *D. simulans* and the arrows indicate the *minimum* limits of an inversion present in the latter species.

3 and 4. The distal two-thirds of the chromosome is typically much broader than the basal third, and ends in a deeply staining ring. In contrast to *D. melanogaster* the distal end of the chromosome has never been observed attached to the chromocenter. The salient features of the fourth chromosome of *D. melanogaster* are shown diagrammatically in figure 14. Two banded areas are conspicuous. The first lies near the chromocenter and consists of the deeply staining band labeled 102 B 1–2. The second is made up of three bands (102 D 1–2, 3 and 4 which are found about two thirds of the length from the chromocenter. Arrows in figures 13 and 14 indicate the minimum limits of an inverted area in *D. simulans*. Starting with the band 102 B 1–2 in both species and reading in opposite directions, the figures show that we have exactly the same sequence of band pattern in reverse order. In *D. simulans* the homologies of the lines beyond the arrows, at either end, are uncertain because somatic synapsis has never been observed here in hybrids. In *D. melanogaster*, there are two distinct

bands between line 102 B 1–2 and the chromocenter. In *D. simulans* there are three bands of similar breadth and intensity of staining. At the distal end of the *melanogaster* chromosome there are four distinct bands and the nipple-like ends with their faint lines. In *D. simulans* there are only two distinct lines beyond 102 B 1–2, and the ring-like terminal band has no obvious homologue in *D. melanogaster*. It is quite possible that the inversion in *D. simulans* involves bands beyond the points indicated by the arrows, and thus that the three bands next to the chromocenter in *simulans* are really 102 E 3, 4, and 5. If this is true, then the two bands next to the terminal ring of *D. simulans* have their homologues in the basal part next to the chromocenter in *D. melanogaster*. In any event, we are forced to conclude that in addition to a relatively long inversion, other changes have occurred in the fourth chromosomes of the two species which have affected the distal ends. The origin of the terminal ring in *D. simulans* is obscure.

DISCUSSION

The present study has given evidence of at least ten gene rearrangements which have occurred in the chromosomes of *D. melanogaster* and *D. simulans* since they arose from a common ancestor. Of these rearrangements, six are demonstrable inversions (five hitherto unreported) and four involve changes in the band pattern at the distal ends of certain chromosomes.

The five newly described inversions are all small, involving two (one and a part of a doublet), three, three, ten and ten fairly conspicuous bands respectively. The very faint lines are not considered here. This raises the very interesting question of how such small inversions could have been produced. On the basis of SEREBROVSKY'S hypothesis, at first sight, it is difficult to visualize loops in the chromonema thread so short as to involve only two or three chromomeres, as this would seem to imply that the thread was greatly elongated and tangled when the lesions producing the change occurred. This difficulty, however, is largely removed if we assume that in animals, as in plants, the chromonema is coiled in a minor spiral as well as in the more easily observed major spiral. The minor spiral coils would favor small intrachromosomal rearrangements for, due to the minor coiling, adjacent regions of the chromonema would be in close physical contact. On this basis we would expect that small rearrangements would be the rule and this seems borne out in the present study as well as by the work of DOBZHANSKY and TAN dealing with *D. pseudoobscura* and *D. miranda* and the Sciara cross of METZ and LAWRENCE.

Changes in the free ends of the salivary gland chromosomes are considerably more difficult to determine exactly, than in the body of the

chromosome, and for this reason we cannot be so sure in interpreting our observations. Nevertheless, it seems clear that in *D. melanogaster* there are three bands of moderate breadth and intensity (one extra band in the X and two extra bands in the fourth chromosomes) not seen in a corresponding position in *D. simulans*. Conversely in *D. simulans*, there are three bands (one extra band in the left arm of the second, one in the right arm of the second, and the terminal ring on the fourth chromosome) not observed in a corresponding position in *D. melanogaster*. The three extra bands of *D. melanogaster* are offset by the three extra bands of *D. simulans* and except for changes in the tip of the fourth chromosome which probably have another explanation, no evidence has been found that there has been the loss or the addition of any band in either species.

The consistent failure of the tip ends of the X and the second chromosomes to synapse in hybrids would seem to suggest that mutual translocations have occurred involving the X, the left and the right arms of the second, and the fourth chromosomes. But this would scarcely explain the relatively deeply-staining ring at the end of the fourth chromosome in *D. simulans*. Regarding the latter, it will be recalled that whereas the distal end of the fourth chromosome in *D. melanogaster* is connected with the chromocenter, in *D. simulans* the free tip has not been observed behaving in this manner, and the ring may have been produced in some way when the fourth chromosome became disconnected from the inert chromatin, or became connected with it.

One of the most interesting features brought out in this study is the fact that the inversion of a band does not prevent it from synapsing in its reversed position directly with a homologous band in another chromosome, and thus it is clear that somatic synapsis does not depend entirely on the orientation of the band in the chromosome. On the other hand, a very short inversion, involving two or more bands ordinarily disturbs somatic synapsis for some distance on either side of the rearrangement. The change in some way seems to interfere with a complete union of the adjacent bands. The nature of this effect is unknown.

The fact that an inverted band occasionally will synapse with its homologue would effectively prevent us from determining "one band" inversions. From morphological observations, the only indication that an inversion has occurred would be the frequent failure of a limited area, along a chromosome, to synapse. Both PÄTAU and KERKIS have indicated several such regions in *D. melanogaster* and *D. simulans* hybrids, and we have recorded above a considerable number of such areas, where the morphological pattern is the same in both chromosomes, but usually the chromosomes lie separated for a short distance. Exceptionally the union is complete. It seems probable that this failure to synapse regularly is due to

some undetected rearrangement, perhaps the inversion of a single band, or to a change in some extremely narrow and lightly staining line.

There are altogether some 14 regions (seven in the X, five in the left arm and two in the right arm of the second chromosomes), where the visible morphological pattern of the bands of homologues are similar and yet do not ordinarily undergo somatic synapsis. If we grant that a consistent failure to synapse is *prima facie* evidence for a change in the band pattern, and add these 14 areas to the ten regions of demonstrable changes, we get a total of some 24 chromosomal rearrangements which have occurred in *D. melanogaster* and *D. simulans* since they arose from a common stem. Of these changes only one involves many bands.

SUMMARY

1. In the salivary chromosomes of hybrid larvae between *D. melanogaster* and *D. simulans*, ten clear chromosomal rearrangements have been found. Six of these are inversions, five of which are very short, and four involve changes of one or a few bands at the free ends of certain chromosomes. In addition to these demonstrable rearrangements, there is a total of 14 short areas where the chromosomes do not synapse ordinarily.

2. Very short inversions show that an inverted band will occasionally synapse directly and intimately with its homologue, thus proving that synapsis is not entirely dependent upon the band's orientation. This fact indicates that single band inversions can not be detected cytologically.

3. Ordinarily, a very short inversion prevents somatic synapsis for some distance on either side of the rearrangements, and it seems probable that the 14 short areas where somatic synapsis does not usually occur, are due to cytologically undetectable rearrangements.

4. The evidence suggests that as many as 24 rearrangements have occurred in the chromosome of *D. melanogaster* and *D. simulans* since these arose from a common stem.

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