

CHROMOSOME FUSION AND SPECIATION IN DROSOPHILAE

THEOPHILUS S. PAINTER and WILSON STONE

University of Texas, Austin, Texas

Received September 3, 1934

In a paper which appeared recently Dr. J. T. PATTERSON, WILSON STONE and the Misses SARAH BEDICHEK and META SUCHE give a summary of their studies of a large number of translocations which they obtained in the spring of 1933. Most of the X-IV, III-IV, and II-IV translocations have now been analyzed cytologically, both by the usual oogonial metaphase plate and the new salivary gland methods, and it has been found that in many instances one whole arm of either the second or the third chromosome has been replaced by a fourth forming a j-shaped element, or, that the X and the fourth have united at their spindle fiber ends forming a j-shaped compound chromosome. Detailed descriptions of the exchanges involving the second and third and fourth chromosomes will be found elsewhere (PAINTER 1934b, 1935). The present paper deals with the "fusions" between the X and the fourth chromosomes. In the cases of the v-shaped second or third elements, it is not possible to determine whether there has been a mutual exchange with the fourth close to the spindle fiber attachment zone (that is, a mutual translocation) but in the case of the rod-like X and fourth there can be no such exchange if a j-shaped compound chromosome results. These cases are of very great theoretical interest both from the standpoint of the structure of chromosomes and of the behavior of chromosomes during speciation. It has been generally assumed by cytologists that number changes within a restricted group have come about largely through either a fusion of rod-shaped chromosomes or a fragmentation of j- or v-shaped elements, but little thought has been given to the question of what happens to the spindle fiber mechanism during these processes. These experimentally produced "fusions" offer an excellent opportunity for subjecting some of the questions involved to a critical analysis.

At this place the writers wish to express to our colleagues and associates our obligation and appreciation for the use of this material.

MATERIAL AND METHODS

In the original experiment, 14 translocations involving the X and fourth chromosomes, and viable in the male, were obtained. In 8 of these the gene string of the X appeared to be intact up to and including the normal allele of bobbed. The genetic data are presented in an article elsewhere by STONE (1934). A cytological study of these 8 cases shows that

they fall into three classes, (a) simple unions at the spindle fiber ends of the X and fourth forming a j-shaped element, (b) multiple unions of the X, the fourth and a segment from the right arm of the third forming a compound and essentially a Y-shaped chromosome, and (c) possibly a simple translocation during which the euchromatic area of the fourth becomes attached to the side of the X, in the inert region, or directly to the spindle fiber attachment zone.

The cytological study was made by the aceto-carmin method applied to the ovaries of young adult flies and to the salivary glands of old larvae.

Before we describe our cytological findings, it will be necessary to briefly review the general set-up found in the salivary glands and the normal topography of the X and fourth chromosomes even though this involves some repetition of facts already described in papers which are in press as this is written.

In the salivary glands the chromosomes are differentiated morphologically into euchromatic or banded areas, which carry all or nearly all of the active genetic material, and heterochromatic areas composed of genetically relatively inactive material. This heterochromatin which is carried by the X's, the Y, the second and the third chromosomes, is not organized into separate (visible) distinct parts of the individual chromosomes but unites to form a common mass of deeply-staining material which HERTZ (1933) has termed a chromocenter. The euchromatic parts of the chromosomes are connected to the chromocenter, in part at least, by thin protoplasmic processes which, directly or indirectly, connect together the two arms of the second or the third chromosomes, respectively. It is highly important, for what follows, to realize that the two arms of the second or the third element enter or are connected to the chromocenter separately, and that there is ordinarily no visible connection between the two except the deeply staining heterochromatin which is shared in common with the other elements.

The orientation shown in figure 1 is arbitrary and schematic, but the pattern of the euchromatic areas of the several elements in contact with the chromocenter, is accurately represented. In all probability the various arms or elements are all attached to one side of the chromocenter rather close together. One can visualize the probable orientation as a telophase arrangement, for the inert chromatin happens to lie at the spindle fiber ends of the chromosomes. In any given preparation the position assumed by the elements of the nucleus is the chance result of how these happen to lie with relation to the cover glass at the time the nucleus was crushed. Thus the spindle fiber ends of the third chromosome, for example, may be attached to the chromocenter close together, or they may be widely separated.

In old larvae, as PAINTER (1933a, 1934b) has shown, homologous chromosomes undergo somatic synapsis and form one apparent element. They are so represented in figure 1, but the double connections within the chromocenter indicate their dual character. Somatic synapsis is of value to us in this study because when you have a normal and an aberrant homolog united, it is easy to determine if any bands from the euchromatic area are missing.

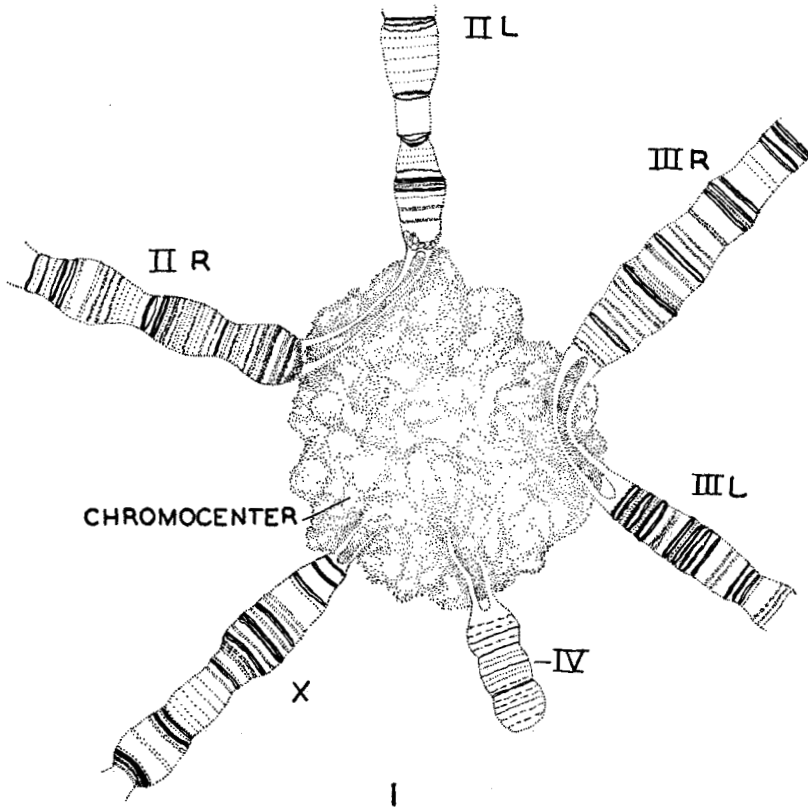


FIGURE 1.—This is a semi-diagrammatic sketch showing the general orientation of the chromosomes about the chromocenter in the salivary gland. The pattern of the ends of the various elements or arms is accurately represented for the region where they enter the chromocenter. The clear protoplasmic strands connecting the elements with the chromocenter also exist, but the way the two arms of the v-shaped II or III are connected is diagrammatic.

The normal topography of the X and fourth chromosomes, insofar as we need to consider this phase, is given in figure 1.

SIMPLE UNIONS (FUSIONS)

We have altogether 5 cases of this type, 4 of which will be considered in detail. Complete data for one case (T1-4,15A) is lacking because the stock was lost before the genetic tests were complete or cytological draw-

ings made. Fortunately, it was the first case examined cytologically and the one which gave us the key as to the nature of these experimentally produced compound chromosomes. From the genetic standpoint all these cases behave alike in the following respects: (a) All show crossing over beyond carnation and no hyper- or hypoploids live when they are tested to forked, carnation, bobbed and eyeless. In other stocks involving short pieces of the X-IV, for example, T1-4, 13A, such aneuploid flies appear. This indicates that the union is beyond bobbed. (b) All show complete linkage between eyeless and bent with no hyperploid phenomenon, (c) As compared to the normal the reduction in crossing over is slight in all cases. (For full genetic data see paper by WILSON STONE.)

CYTOLOGICAL EVIDENCE

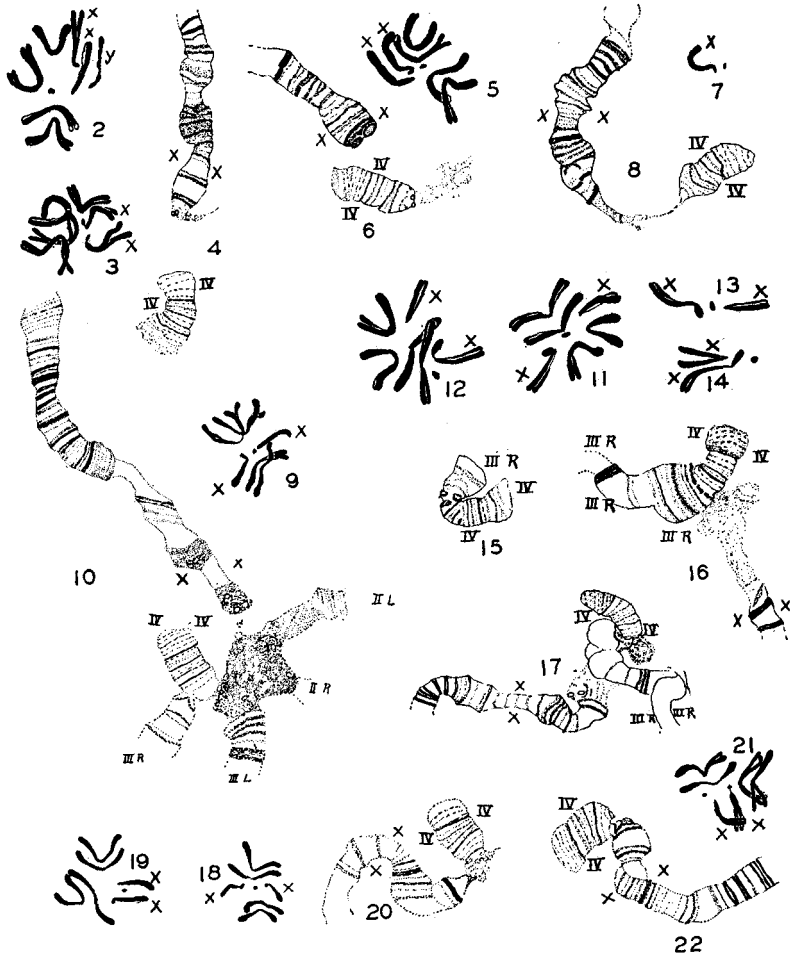
T1-4, 5A

Figure 2 is an oogonial metaphase plate taken from a fly which was heterozygous for the union between the X and the fourth. This individual carried a Y chromosome which is labelled. The figure shows one normal fourth, one normal X, and an X to which a fourth is attached forming a j-shaped element. Figure 3 is taken from a female homozygous for this aberration, and one sees that both X's have a short arm the size of normal fourth chromosomes attached to their spindle fiber ends. No free fourths are seen in the cell and the diploid number of this fly is 6 and not 8 chromosomes. A study of the salivary glands of old female larvae heterozygous for this aberration (figure 4) shows that both the paired X's and fourths are morphologically intact and are attached to the chromocenter separately. There is no suggestion of any direct union between the two. The patterns of the two X's correspond throughout their length (only the spindle fiber ends are shown) and it is quite certain that nothing is missing from the euchromatic area of either homolog. The paired fourths, in like manner, are paired throughout their lengths and nothing is missing from the euchromatic areas. Any connection between the X and the fourth must be hidden by the chromocenter, just as in the case of the two arms of the second or the third.

T1-4, 6A

Figure 5 is an oogonial metaphase plate taken from a fly which was heterozygous for this fusion. It will be seen that there is one free fourth and one attached to the spindle fiber end of the X. (We use the term spindle fiber end to indicate that part of the euchromatic area nearest the chromocenter.) Figure 6 is from a female larva heterozygous for this aberration. This figure is of unusual interest as one can see the complete morphological similarity of the normal and aberrant homologs. The figure

shows the paired X's and fourths as they lay on the slide. The X's have pulled free, a little, from the chromocenter but they were attached separately from the fourths. In all respects this case is like the preceding cytologically.



FIGURES 2 to 22.—The magnification of the oögonial plates is either 2400 \times , or 1800 \times , at the eye piece. Salivary gland drawings represented 900 \times , at the eye piece. All drawings were made by the use of a camera lucida at table level, and have been reduced about one-fourth in reproduction.

FIGURES 2 and 3 are oögonial metaphase plates of flies which were hetero- and homozygous respectively for T1-4, 5A. Figure 4 shows the condition of the X's and fourths of this translocation in salivary glands. Figure 5 is an oögonial plate from a heterozygous fly, and figure 6 a salivary gland preparation from a heterozygous larva of T1-4, 6A. Figures 7 and 8 are taken from individuals heterozygous for T1-4, 7A. Figures 9 and 10 were taken from heterozygous individuals for T1-4, 11A. Figures 11 to 14 are oögonial chromosomes from flies heterozygous for T1-4, 3A. Figures 15 to 17 were taken from salivary gland material. Figures 18 and 19 are oögonial plates from flies hetero- and homozygous for T1-4, 2A. Figure 20 is from the salivary gland. Figures 21 and 22, are from the ovary and the salivary gland of individuals heterozygous for T1-4, 14A.

T1-4, 7A

Figure 7 is from a female heterozygous for this fusion and shows a normal fourth and an X with a fourth attached to its spindle fiber end. Figure 8 is from a heterozygous female larva. In this figure the X's and fourths are quite intact in the euchromatic area; they happen to lie close together but are connected separately to the chromocenter.

T1-4, 11A

Figure 9 is an oogonial metaphase plate taken from a fly which was heterozygous for this aberration. One of the fourth chromosomes is attached to the X. Figure 10, taken from a heterozygous female larva, shows the X's morphologically intact, as are the fourths, and these are attached separately to the chromocenter region. Incidentally some of the other elements are indicated. This aberration is cytologically just like the others considered above.

MULTIPLE UNIONS

T1-4, 3A

We have only one case of this type. Genetically it behaves as a simple fusion, like the cases described in the foregoing section, but salivary gland material shows that it is a complex case. Figures 11 to 14 are taken from oogonial metaphase plates of flies heterozygous for the rearrangement. A glance at the figures shows that one of the X's is j-shaped but that the short arm is much larger than the single fourth chromosome found in the figures. When this case was first examined in oogonia it was thought that some of the inert material from the X had in some way become associated with the fourth giving the large size. But when the salivary glands of old larvae heterozygous for this aberration were examined, it was found that the fourth was united with a short section from the right arm of the third chromosome. Figure 15, taken from a heterozygous female larva showed the paired X's (not illustrated in this figure) completely intact and attached independently to the chromocenter. The paired fourths, on the other hand, had attached to one side of one of them, right at the margin of the chromocenter, a short piece of another chromosome which was recognized at once as the spindle fiber end of the right arm of the third. Note in figure 15 that the tip of the right arm and one of the fourths are closely joined at the spindle fiber ends of the two elements. Figure 16 shows the ends of the X's below. They are normal throughout their lengths and are attached separately to the chromocenter. Above and to the left are the normal synapsed right arms of the third chromosome which become very broad where the hyperploid segment of the third joins with them. To the right the two fourths are shown attached, apparently, to

the spindle fiber ends of the right arm. Figure 17 shows essentially the same relations as figure 16, but the right arms of the paired thirds are bent over obscuring many details. The two essential features to be noted from these figures are first, that the X's and the fourths are attached separately to the chromocenter and second, that a piece of the third chromosome that is a part of the euchromatic area is closely joined to the spindle fiber end of one of the fourth chromosomes. This case has great theoretical interest, as we shall show in our discussion.

OTHER CASES

Under this heading we have to describe two aberrations which genetically show the X and fourth completely linked, as in the simple fusions, but in which crossing over is much reduced

T1-4, 2A

Figures 19 and 18 show heterozygous and homozygous oogonial plates of this aberration. In the latter figure, the fly was hyperploid for the fourth. In both of these figures the fourth chromosome appears to be attached to the side of the tip of the X, and the two are not joined at the spindle fiber ends to form a distinct *j*, as in the cases previously considered. It may be remarked that these figures were drawn and the condition noted before the explanation was apparent. When salivary gland material of heterozygous larvae was studied, at first glance it looked just like the other cases. On further study it was found that in many cells the X and fourth were closely joined at the edge of the chromocenter. Figure 20 illustrates such a case. (Of course, in mashing out the nucleus this association was frequently disturbed, as we might expect.)

T1-4, 14A

Figure 21 is an oogonial plate of a fly heterozygous for this union. We note that an X has a fourth attached to it by a chromatic bridge. Figure 22 is from the salivary gland of a heterozygous larva and shows that one of the synapsed X's is attached, at the edge of the chromocenter, to one of the paired fourth chromosomes.

DISCUSSION

The facts presented in this study show that both genetically and cytologically we are dealing with two different types of aberrations. They are all alike in that the gene strings of both the X and the fourth are intact and completely linked, likewise in that the euchromatic areas of both elements are morphologically intact. They are unlike in that the frequency of crossing over is much higher (and similar in amount) in *T1-4, 3A -5, -6, -7* and *-11* than in *-2* and *-14*, where we find a sharp reduction. Cytologically these two classes differ in the salivary gland in the way the X

and fourth are associated. In the first type (-3, -5, et cetera) the X and the fourth are attached to the chromocenter separately, and the connection between the two is, in some way, accomplished within this structure. The second type (-2, and -14) shows a much more intimate association between the X and fourth in that the two are frequently seen lying in contact on the edge of the chromocenter (they are sometimes separated when the nucleus is crushed). We think that these differences in the way the two chromosomes are associated explain the difference in the rate of crossing over of the two types, as explained below.

In his cytological study of the third chromosome PAINTER found the same two classes of exchanges between the third and fourth chromosomes, in the region of the chromocenter, as we have described in the foregoing pages. In one type, the whole arm of the third chromosome is simply replaced by an intact fourth, and such cases are entirely comparable with the simple unions of the X and the fourth, not only cytologically but genetically, because Miss SUCHE has found that crossing over is reduced very little by the fusion process (unpublished data). In the second type the right arm is broken just at the edge of the chromocenter and is attached to the fourth somewhere between the euchromatic arm of the latter and its spindle fiber. These are similar genetically to our T-2A and -14A in that Miss SUCHE (unpublished data) finds a sharp reduction in the amount of crossing over.

One of the most interesting features about the salivary gland is the fact that the two arms of the v-shaped autosomes enter the deeply-staining chromocenter separately. At or near the point where they are attached, the banded form ends; and the arm is visible for a short distance within the chromocenter (in favorable cases) as a thin protoplasmic strand of, presumably, matrical substance. We know little, as yet, about the nature of the connection between the two arms; some possibilities have been considered by PAINTER in his study on the third chromosome, but whatever its character it is obviously quite different morphologically from the active genetic or euchromatic (banded) part. This brings us to a new and clearer concept of the nature of the chromatic bridge (achromatic with certain stains) which connects the two arms of these v-shaped autosomes. This subject interests us here very directly because the study of the simple unions of the X and fourth chromosomes, induced experimentally, shows that morphologically (as seen both in ovaries and in the salivary glands) the relation between the joined X's and fourths is the same as that which exists between the two arms of the v-shaped autosomes, and any conclusions which we may reach concerning the origin of the j-shaped X and fourth will probably apply to the large v-shaped autosomes of *D. melanogaster* and presumably, to v- and j-shaped chromosomes in general.

We have already emphasized the fact that in these simple unions of the X and fourth chromosomes both elements are completely intact genetically and so far as can be observed, cytologically. In oögonial metaphase plates both the X and the fourth are normal-sized and differ in no visible way from the normal homologs except that they are joined at their spindle fiber ends as salivary gland studies show. The question now comes to the fore, what has happened to the spindle fiber mechanisms of the elements, are these intact also, or has one of them been lost?

Unfortunately we are not able to answer the above questions, as yet, in *D. melanogaster*. The presence of the large amount of deeply-staining inert chromocenter material obscures the details and no way has been found to get around this difficulty. In all probability the cytological answer must be sought in species of *Drosophila* in which there is little inert material and a survey of available species with this point, among others, in view is now in progress at this laboratory. In the meantime, we shall consider some theoretical ways in which the X and fourth may be joined and make pertinent comments about certain combinations.

In figure 23 we have represented diagrammatically various ways the X and fourth may have united. We are assuming that the spindle fiber mechanism is terminal and is connected to the euchromatic area in some such way as is shown in the diagrams.

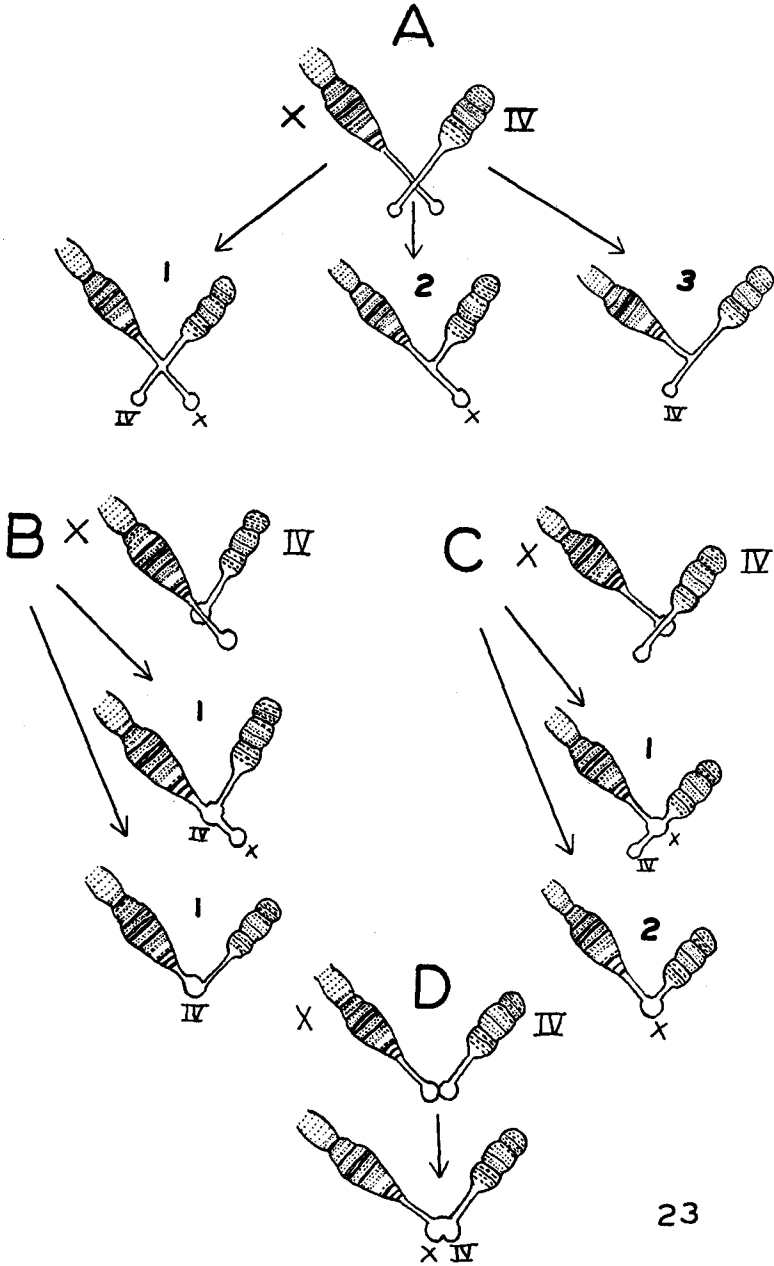
Diagram A assumes that X and fourth were joined, through irradiation, in the chromocenter region distal to the spindle fiber mechanism, but instead of separating with an exchange of spindle fibers, which would give an ordinary mutual translocation, they remain associated in some one of the three ways indicated by diagrams A1, 2, or 3.

Diagrams B and C (which are alike in principle) assume that the spindle fiber attachment zone of one element becomes attached to the connecting strand of the other in the region of the chromocenter, but distal to the spindle fiber. The various sequelae are shown

Diagram D assumes that the X and fourth have joined at the spindle fiber attachment zones and that, as a result, both elements lose nothing during the union.

Of the various theoretical possibilities we have definite evidence at this laboratory, for A2 in two translocations involving the third and fourth chromosomes. It is probable, as we shall show below, that the right-hand tip of the third and the fourth chromosomes in T1-4, 3A are associated essentially as is shown in A2. At the present time we are inclined to interpret the association of the X and fourth in T1-4A, 3, 5, 6, 7, and 11 as simple unions involving no loss as in diagram D and T1-4, 2A and 14 as like A2 or A3. The evidence will now be briefly reviewed.

The breaks in the third chromosome referred to are described by



23

FIGURE 23.—This set of diagrams represent some of the most obvious ways v- or j-shaped chromosomes may have arisen from rod-like elements. For details see the text.

PAINTER under VI 27 and VI 4. The euchromatic area of the right arm of the third was attached to the spindle fiber end of the fourth beyond its euchromatic area. In oogonal metaphase plates there were two j-shaped

elements. One was the III R-IV compound, the short arm of the *j* being the size of a normal fourth. The other *j* was formed by the left arm of the third and the inert material which belonged to the right arm but which was left in its normal position when the euchromatic area of the right arm became attached to the fourth chromosome. In salivary gland material the right arm of the third is attached to the fourth just at the edge of the chromocenter, and since the spindle fiber mechanism of the fourth lies distal to this point, this III R-IV compound chromosome must be essentially a Y as in diagram A3.

In discussing the T1-4, 3A aberration, at this point, we are confining our remarks to the nature of the association between the tip end of the right arm of the third and the fourth chromosome. The association of this compound with the X will be taken up later. Figure 15 shows that the tip of the right arm is intimately connected with the spindle fiber end of one of the synapsed fourth chromosomes. This means that the contact between the two is right at the edge of the chromocenter and either that both elements have processes running into the chromocenter or that one or the other has lost its chromocenter connection. On this point the evidence favors the latter point of view and indeed suggests that it is the spindle fiber end of the right arm which is doing double duty. In the first place, oögonial metaphase plates (figures 11 to 14) show that the short arm (made up of the III R and IV material) is far larger than we would expect judging from the size of these in salivary glands. The volume of the combined euchromatic areas of the two can scarcely be more than three times that of the normal fourth. The knob on the end of the X is far in excess of this and is just about the size it should be if the inert material carried by the right arm were incorporated. This would be the case, were the spindle fiber of the third retained.

Again, the right-hand arms of the normal third chromosomes usually synapse with the fragment of the third and the synapsis of these elements would be greatly facilitated if they had homologous spindle fiber attachments. (PAINTER, in his study on the third chromosome has pointed out that chromosomes or arms are probably attached to a definite part of the chromocenter and that homologous spindle fiber zones must be close together. See PAINTER 1935a, p. 325.)

And finally, STONE has found that the fourth component may drop off while the III R component retains its association with the X, which suggests that the fourth is loosely attached to the former.

If the two elements of the III R-IV complex are joined at their spindle fiber ends, then in their association with the X they must make a y-shaped or forked chromosome complex. This is not visible, however, in oögonial metaphase plates, a fact which should not be surprising. It is well known

that at metaphase all chromosomes are split into two daughter elements and yet they appear as one. Under the circumstances we would not expect to see the fork between the short III R and IV elements. Attention should be called also to the translocation which DOBZHANSKY and STURTEVANT (1931) have reported, in which, judging from the genetic data, a piece of the second chromosome was attached to the side of the third chromosome in the region of hairy. In metaphase plates they were unable to see this association, but it should be visible by the salivary gland method.

In concluding that the connection between the X and fourth chromosomes in T1-4, 2A and 14 are different from the other X-IV unions considered below, we are guided by a number of considerations. First, in oogonial metaphase plates it is noted that the fourth seems to be attached to the side of the X (figures 18, 19 and 21) close to the tip and not directly to the tip. In the second place, in salivary gland material, we find the X and fourth, as a rule, connected right at the edge of the chromocenter, a fact which we have stressed. This is the same sort of association which PAINTER found in VI 27 and VI 4, and like these the T1-4, 2A and 14 show a marked reduction of crossing over as compared to simple fusions. T1-4, 2 and 14 must therefore be essentially y-shaped chromosomes like diagrams A2 or A3.

We have now to consider the evidence which causes us to believe that the simple unions of the X and fourth chromosomes are real fusions during which the spindle fiber areas of neither is lost. It may be said at once that we have no direct cytological evidence to offer on this point and that we are influenced by indirect evidence and by general cytological considerations.

As we have already outlined in connection with figure 23, there are three general ways in which the X and the fourth might have united, illustrated by A, B, or C, and D. In the first case we would have an X-like figure or, by derivation, a y. In the second a y-like arrangement or secondarily a v. In the third a v with two spindle fibers would result. The first two of these possibilities seem less likely because we actually have cases like A2 or A3 in which there is a great reduction of crossing over, and were the union of the X and fourth like B or C, we would expect the same effect. This leaves D as the most likely method of union, as we now interpret our results.¹

¹ The theoretical interpretations given in figure 23 are based on the assumption that both the X and fourth chromosomes are rod-shaped, but recently KAUFMANN (J. Morph. 56, 1934) and PROKOFIEWA (Z. Zellf. Mic. Ana. 22, 1935) have figured and described the X as essentially j-shaped and KAUFMANN thinks that the fourth also has this form. Granting the correctness of the interpretation given for the tiny terminal chromomeres seen with special technique (though other explanations are possible) then our cases -5, -6, -7 and -11 might have resulted from mutual translocations, but cases -2, -3 and -14 cannot be explained in this way.

It seems probable that two of the three possible types of union between rod-shaped chromosomes have been obtained experimentally. It now is pertinent to consider the structure of the v-shaped second and third chromosome. As far as the cytological evidence is concerned, we have no direct evidence to offer; they may have arisen in any one of the three ways we have considered. It will be possible, however, to test out the nature of the connection between the two arms with breaks we now have, and the building up of the necessary testing stocks is in progress at this laboratory. So far as we know, no one has ever made a critical experiment covering this point.

Parenthetically, it may be remarked that crossing over is much less in the euchromatic area adjacent to the spindle fiber, per morphological unit of the salivary gland chromosome in the case of the second and third chromosomes than it is in the case of the X. PAINTER suggested that the presence of such a large amount of inert material in the latter may cancel out the inhibiting effect of the spindle fiber region. It is possible, however, that the second and third chromosomes are essentially y-shaped in structure.

The theoretical cytological background which leads us to believe that the compound X and fourth chromosomes have two spindle fiber attachments will be briefly filled in. It has been quite generally assumed, by cytologists, that chromosome numbers have been changed by the fusion of rod-shaped elements or a fragmentation of v- or j-shaped chromosomes. Scattered through the literature there are many cases which support these ideas (see WILSON 1928, or REUTER 1930, p. 89, for examples). But we have known nothing about what happened to the spindle fiber mechanism when fragmentation or fusion occurred. In the meantime, experimentally-induced fragmentation following irradiation has taught us that regardless of how the bio-physicists may interpret the nature of the spindle fibers in dividing cells (be they optical illusions or what not), the fact remains that unless a piece of or a whole chromosome, does not carry what we may non-committally call a "spindle fiber attachment zone" it is lost sooner or later from the chromosome complex. If fragmentation has occurred in nature, we must assume that the spindle fiber attachment zone was divided and was essentially plurivalent in the first place. (We regard the *de novo* origin of the attachment zone as very improbable.)

The presence of two spindle fiber attachment zones on one chromosome raises the question of whether this would make a stable complex. We share the opinion already expressed by MULLER and PAINTER (1932), NAVASHIN (1932) and others, and supported by the recent work of Mrs. L. V. MORGAN (1933), that when the attachment zones are far apart such a compound chromosome would probably break down. On the other hand, we

must assume a definite structural polarity within the chromosome and if the two centers were properly orientated with regard to this and close together, the compound element would probably be stable. As a matter of fact, we appear to have in the attached-X race of *Drosophila*, a compound element such as we are postulating and it is to be noted (ANDERSON 1925) that crossing over is not appreciably reduced, just as STONE finds in T1-4, 5A-6A et cetera. We must also remember the classic case of *Ascaris* in which there are possibly as many spindle fiber attachment zones in the chromosomes of germ and early cleavage cells as there are dot-like elements in the somatic tissue.

One further comment may be pertinent, and that is, in our discussions, we are apt to think in diagrammatic terms and may thereby get far away from the physical structure of the chromosome. Thus, while in plants we have many instances of attachment zones which appear to be discrete bodies (see TRANKOWSKY 1930, for instances, and the literature) nevertheless, botanical cytology is filled with careful drawings showing broad attachment zones with a whole tuft of fibers running from the chromosome towards the two poles. Here the attachment zone is certainly very long and is, presumably, capable of subdivision. In animals, in general, the attachment zone seems to be more localized, but after all it may be capable of fusion or of subdivisions and still remain functional.

From the phylogenetic standpoint the assumption of plurivalent spindle fiber attachment zones (it makes no difference whether a general area is divisible, or is made up of several closely connected centers) would readily explain what has been often found when the chromosomes of a restricted group of animals are studied. While most species have the same or nearly the same number of chromosomes, here and there we encounter a species which has a much higher number than we would expect. In addition, CROSS (1931 and unpublished data) has found that the species with the higher numbers may have as many v- and j- shaped elements as the generic type, showing that the higher number could not have been derived by a simple splitting of v- or j-shaped elements. The article by Dr. Cross cites much pertinent literature.

T1-4, 3A is interesting from the phylogenetic point of view. In the homozygous female the genes of the duplication of the third chromosome are in the tetraploid condition, yet these females are perfectly viable and fertile. If this case is one that involves two spindle fibers, this fragment could later become free and furnish a new spindle fiber attachment zone to which, by translocation, other chromatin could be added. This would raise the chromosome number of the race.

LITERATURE CITED

- ANDERSON, E. G., 1925 Crossing over in the case of attached X-chromosomes in *Drosophila melanogaster*. *Genetics* **10**: 403-417.
- CROSS, J. C., 1931 A comparative study of the chromosomes of rodents. *J. Morph.* **52**: 373-402.
- DOBZHANSKY, TH. and STURTEVANT, A. H., 1931 Translocations between the second and third chromosomes of *Drosophila* and their bearing on the Oenothera problem. *Pub. Carnegie Instn. Washington* **421**: 39-59.
- HEITZ, E., 1933 Die somatische Heteropyknosis bei *Drosophila melanogaster* und ihre genetische Bedeutung. *Zeit. f. Zellf. u. mikr. Anat.* **20**: 237-287.
- MORGAN, L. V., 1933 A closed X chromosome in *Drosophila melanogaster*. *Genetics* **18**: 250-283.
- MULLER, H. J. and PAINTER, T. S., 1932 The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. *Z.I.A.V.* **62**: 316-365.
- NAVASHIN, M., 1932 The dislocation hypothesis of evolution of chromosome numbers. *Z.I.A.V.* **62**: 224-231.
- PAINTER, T. S., 1933 A new method for the study of chromosome rearrangements and the plotting of chromosome maps. *Science* **78**: 585-586.
- 1934a A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. *Genetics* **19**: 175-188.
- 1934b The morphology of the X chromosome in salivary glands of *Drosophila melanogaster* and a new type of chromosome map for this element. *Genetics* **19**: 448-469.
- 1935 The morphology of the third chromosome in the salivary gland of *Drosophila melanogaster* and a new cytological map of this element. *Genetics* **20**: 301-326.
- PATTERSON, J. T., STONE, WILSON, BEDICHEK, SARAH and SUCHE, META, 1934 The production of translocations in *Drosophila*. *Amer. Nat.* **68**: 359-369.
- REUTER, E., 1930 Beiträge zu einer Einheitslichen Auffassung Gewisser Chromosomenfragen. *Helsingfor.*
- STONE, WILSON, 1935 Linkage between the X and IV chromosomes in *Drosophila melanogaster*. *Genetica* **16**: 506-519.
- TRANKOWSKY, D. A., 1930 "Leitkörperschen" der Chromosomen bei einigen Angiospermen. *Zeit. f. Zellf. u. mikr. Anat.* **10**: 736-743.
- WILSON, E. B., 1928 *The cell in development and heredity*. 3rd Ed. New York.