THE MORPHOLOGY OF THE X CHROMOSOME IN SALIVARY GLANDS OF DROSOPHILA MELANOGASTER AND A NEW TYPE OF CHROMOSOME MAP FOR THIS ELEMENT

THEOPHILUS S. PAINTER
University of Texas, Austin, Texas

Received October 6, 1933

In a paper which appeared recently in this journal the writer has described a new method for the study of chromosome structure and chromosome aberrations and has called attention to its application to a number of problems in the field of cytogenetics. In the present paper, the normal morphology of the X chromosome will be described and a new type of chromosome map will be presented showing the approximate position of some 21 gene loci scattered well along the whole active region of the X. It is believed that this type of analysis marks a distinct advance in the technique of cytogenetics and will be of general interest from both practical and theoretical standpoints. On the practical side, the new chromosome map of the X may be used as a yardstick to determine the genetic position of new breaks cytologically, thus greatly simplifying the breeding tests which need be made and saving much time. From a theoretical standpoint, perhaps the most significant point is the demonstration that we have in our hands a qualitative cytological method of chromosome analysis which should be applicable not only to Drosophila but other types of insects and perhaps to other animals as well. For if the X chromosome of *D. melanogaster* has such distinct topographical features that we can follow a section as it is translocated here or there through the agency of irradiation, there is no obvious reason why the same method of study may not be applied to a study of all the chromosomes not only in this species but in related forms, thus opening up to a direct cytological attack the whole field of the behavior of chromosomes in phylogeny and the hosts of questions clustered about this central theme. Of considerable interest, also, is the discovery that neither the inert region of the X, nor the Y chromosome, except possibly for a short piece, which probably carries the locus for bobbed, are visible as recognizable formed structures in salivary gland cells. The X appears to be made up wholly of the genetically active region.

MATERIALS AND METHODS

Many of the breaks considered in the following pages are new ones obtained by my colleagues Professor J. T. PATTERSON and Mr. WILSON STONE, and by Miss BEDICHEK, during the course of a series of rayings carried out in their attack on the position of a primary sex factor, and

have not been described before in genetic literature. I am indebted to my colleagues for the use of this material, and especially to Mr. WILSON STONE who has carried out many genetic tests to determine the position of new breaks, and who in various ingenious ways, has built up stocks needed by the writer during the course of this study.

The exact determination of the point at which a chromosome has broken depends, among other things, on the markers used in the original experiment. For this reason the known gene loci which bound a break may be a number of crossover units apart. Under these conditions the cytologist can do no more than say that the two limiting loci are to the left and right of a given morphological point. In some cases the position of the break is known within two-tenths of one crossover unit, thus enabling us to plot the morphological position of genes with relative exactness. Realizing this limitation of our method, the writer has assigned to one of his students, Mr. OTTO MACKENSEN, a study of short deficiences of known genetic character. As this is written the results of Mr. MACKENSEN indicate that this method will allow us to locate genes within very narrow limits, perhaps on a given band. When this is done we will be able to present a more refined and accurate map of the active region of the X.

In my first paper I gave the general procedure of preparing the salivary gland for study, but since some of my readers may want to confirm or extend the observations here recorded, I am giving below a somewhat fuller account of the method.

Old larvae, about ready to pupate, should be selected for study. In younger larvae, the union of homologous chromosomes is usually not as complete nor the nuclei as large, as a rule. Dissection is made in Ringer's solution (Frog formula) with a binocular dissecting microscope, and a black background under the dish aids in finding the salivary glands. Male larvae may be distinguished by the fact that at about two-thirds the length from the head, in dorsal view, the gonads may be seen through the body wall as symmetrically placed, large clear spaces. In females the gonads are very small and are not easily visible through the body wall. Larvae are torn open with needles at about their middles. This causes the viscera to extrude. The salivary glands are transparent organs attached to the pharynx near the jaws. They may be recognized, both by their transparency and by the fact that on one side of each there is usually a narrow border of fatty tissue while the opposite side is more or less attached to the ladder-like fat body. It is desirable to free the glands of the fat, though little time should be given this process. The glands are transferred to a clean slide with a pipette, then the Ringer's solution removed. Iron-aceto-carmine is now applied. If it is flowed on from one side the glands will usually stick to the slide making subsequent manipulation easier. Cover

the glands and stain with a thin cover glass (preferably .15 mm or less in thickness so that it fits the slide snugly) at once, and crush the salivary gland by pressing on top of the cover glass with a needle. This process will free many nuclei from their surrounding cytoplasm, thus making observations later much easier. It also usually squeezes most of the aceto-carmine from under the cover glass. It is wise, after crushing, to pry up on the cover glass a trifle so as to bathe the gland tissue freely in the stain. Be careful to exclude all air bubbles. Stain for from 10 to 20 minutes. The exact time is probably not so important, but if the day is hot and dry it is perhaps wise not to allow the aceto-carmine to evaporate too much. A little evaporation seems desirable. The excess stain is now removed with filter paper and then under the high power of the dissecting microscope the individual nuclei are crushed and the chromosomes more or less spread out, by pressing on the cover glass with a needle. Again blot the preparation with filter paper using plenty of pressure and seal the cover glass with vaseline. Such a preparation may be examined at once but it is better after a few hours. These preparations are not permanent, but they last well for a week or ten days, if air is excluded.

A few comments about the preparation of the stain and the examination of slides may be helpful to those who have not used aceto-carmine for this type of work. It has been the experience of the writer that not all carmine works equally well in the preparation of aceto-carmine nor am I always successful in preparing a good lot of stain. Carmine No. 40 is very satisfactory and has been used exclusively. In preparing the stain (simmer an excess of powdered carmine in a forty-five percent solution of acetic acid and, after cooling, filter) care is taken not to allow much loss of fluid during the heating. After the stain is cool and has been filtered, the writer places a dissecting needle or an old file in the bottle of stain so that a trace of iron will be dissolved by the acid. It will be noted that after a few minutes the aceto-carmine will have turned appreciably darker. The needle is then removed.

The examination of aceto-carmine preparations is greatly facilitated by the use of artificial light tinged blue-green. For this purpose one may insert, between the light source and the microscope, a ground-glass screen and a flask containing a dilute solution of copper sulphate (*without ammonia*), or, one may use a frosted blue-green glass filter. Neutral filters of 10 and 20 percent absorption capacity, are extremely useful in controlling the intensity of illumination.

MORPHOLOGY OF THE X CHROMOSOME

For a general description of the morphology of the chromosomes in salivary glands the reader is referred to a recent article, by the author, in

this journal. Here we need mention that, in the salivary glands of old larvae, homologous chromosomes undergo somatic synapsis. Hence, in describing the X, in females, we are really dealing with two X elements which are so closely joined throughout their lengths as to appear as one chromosome. In such cases we shall speak of the X's as if they were a single body.

Figure 1 shows the typical structure of the X chromosome.[1] This is really a composite drawing, each segment representing a camera lucida drawing taken from some preparation in which the region concerned showed clearly and without distortion. My purpose has been to place before the reader a chart from which he should be able to identify any region of the X, on the examination of a good moderately stained aceto-carmine preparation. In such preparations the thickness of the X chromosomes varies a good deal depending on the state of contraction, excellence of preservation and doubtless other factors, but in general the longer the chromosome the thinner it appears and *vice versa*. In figure 1, I have sought to retain representative proportions but in putting together segments of different drawings no doubt errors have been made both in the length and in thickness. Such errors should be both plus and minus, and thus largely cancel each other. In any event they do not affect the qualitative character of the segments which is the point of primary interest. It may be said that the size of the double plate is due to the length of the right arm of the third chromosome, a map of which is in press in this journal. Thus a uniform magnification for all elements will be maintained in this series of studies.

Above figure 1 two crossover maps have been indicated. The one immediately above the figure is the new standard map which places the nor-

[1] Through the courtesy of the editor I have been allowed to substitute for the original figure 1 a new topographical map of the X, which incorporates many of the finer details of morphology which have come to light with various improvements in technique, and gives additional evidence for the morphological limits of gene loci. The present figure supercedes the earlier one published in SCIENCE (1933) from which it differs in some details and represents a map of the X as it stands at the end of March, 1934. Two aberrations, not discussed nor figured in the text, are plotted (scute-8 and X–IV, III. 12). Recent work at this laboratory (PATTERSON and STONE, in press) has shown that scute-8 is broken between the gene loci for achaete and scute. Morphologically, the point of breakage is shown at the left in figure 1. In the stock known as X–IV. III, 12, obtained by STONE, there has been an extensive rearrangement of the X chromosome. Scute and echinus are separated by one break, the gene for cut has been deleted and another break is between the genes for garnet and scalloped. These enable us to bracket the gene loci for scute, cut and garnet more closely than before. Two corrections are incorporated in figure 1. The left hand limit of the ClB inversion is not exactly the same as delta 49, but is a little farther to the left as shown. Also the point of breakage of the X–IV III, 9 is three bands farther to the right than shown in SCIENCE.

Two crossover maps are given. The New Standard Map values representing the X as 75.5 units long is next to the X. Above I have plotted the crossover values as given by BRIDGES and DEMEREC in their "Drosophila Information Service" Number 1, March 1934.

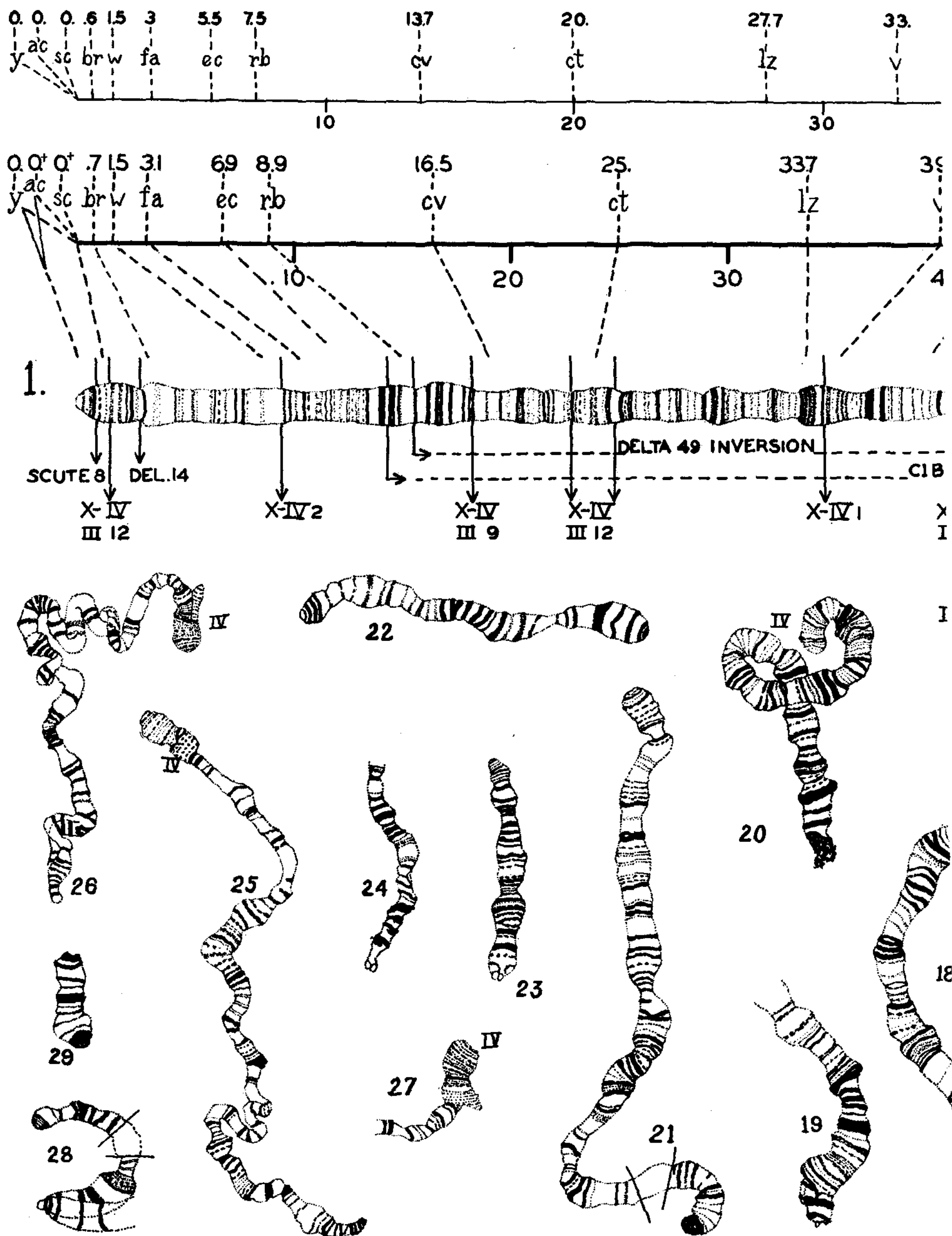
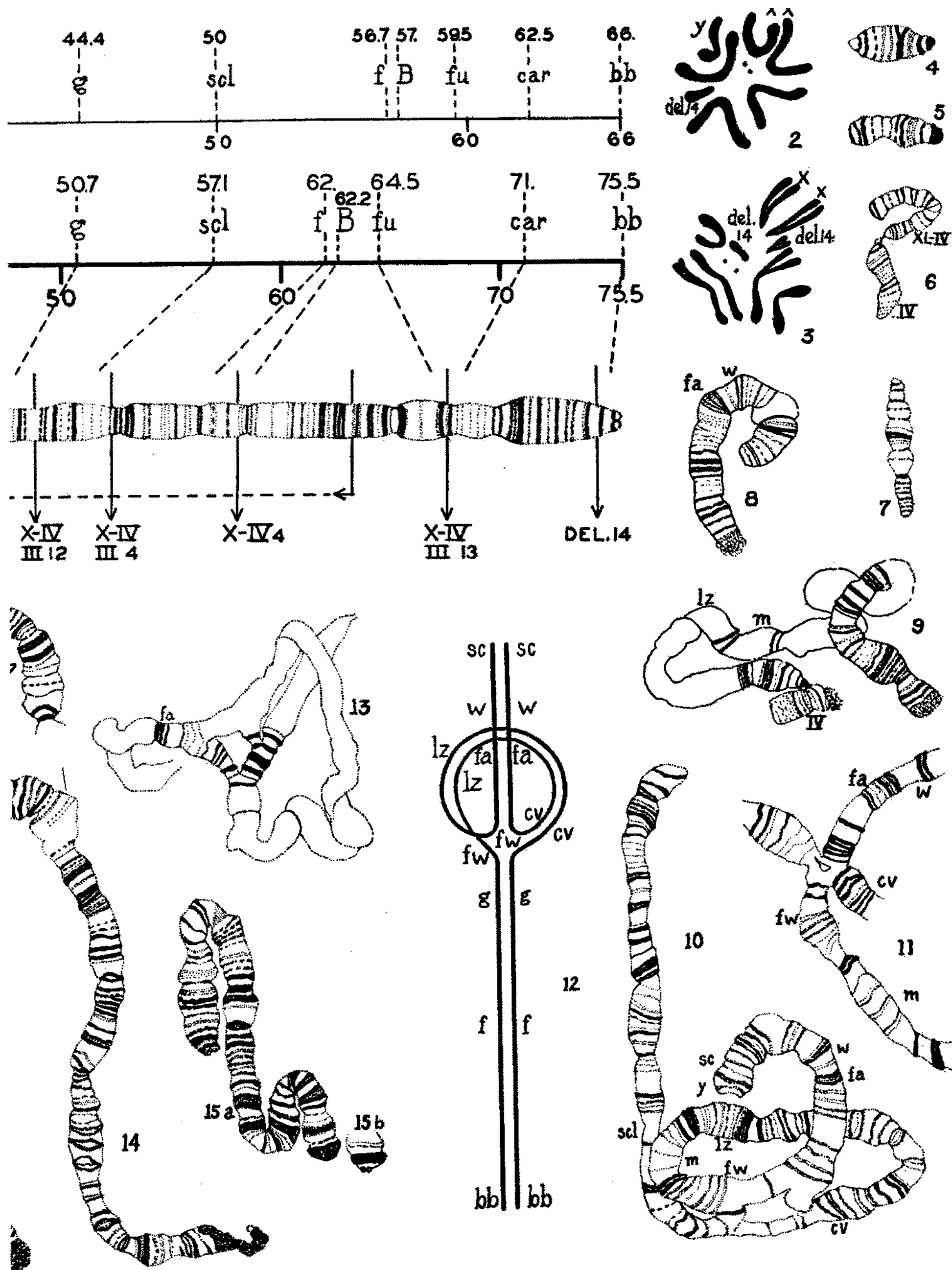


FIGURE 1.—Typical morphology of the X chromosome, as seen in the salivary glands of female larva
morphological positions have been placed by this study. A line running from the symbol towards the
aberration, is indicated just below this drawing. FIGURES 2 and 3.—Oogonial metaphase plates to show
from a female which carried two deleted X's and a Y chromosome. FIGURES 4 and 5.—Deletion 14 as i
location. FIGURE 8.—The XL portion of the X produced by the X–IV, III. 9 break. FIGURE 9.—Th
X chromosome. FIGURE 11.—The character of the pattern at the point of divergence between nor
FIGURE 13.—Shows the way a normal and a ClB X chromosome synapse, with most details omitted.
the XR–IV component, in whole or in part. FIGURES 20 and 21.—The XR–IV and the XL–IV transloca
FIGURES 23 to 26.—The forked-Bar break. Figures 23 (female) and 24 (male) show the XR, and figures
chromosome. FIGURE 29.—The XR element of the X–IV, III. 13 break.



ı p. 451). On the crossover maps above the X, we have the symbols of the gene loci whose approximate
ıeral area of its locus. The morphological position of the break in the X, together with the names of the
on 14 as compared to the X and the other chromosomes. Figure 2 is from an attached X female, figure 3
ıry gland cells. FIGURES 6 and 7.—The XL–IV chromosome which resulted from the X–IV. 2 trans-
. FIGURE 10.—The inversion figure obtained from a female heterozygous for a normal and a delta 49
X's. FIGURE 12.—Schematic representation of the union of a normal and a delta 49 X chromosome.
—Various details of the X–IV 1 translocation. Figures 14 and 15a represent the XL piece, figures 16 to 19
ı the X–IV, III. 8 break. FIGURE 22.—The XR element obtained from the X–IV, III. 4 translocation.
–IV elements. FIGURE 28.—The XR segment of the forked-Bar break with some indication of a Y

mal allele of bobbed at 75.5. This map will be used in the following description. At the top of the plate the latest crossover values for the X are indicated as these are given in the Drosophila Information Service (DIS) No. 1. The labels immediately below the X indicate the name of the breaks considered in this study.

The X is segmental in character and it greatly facilitates descriptions to give these segments names, even before presenting the evidence to show why they are so named. The constrictions which limit segments vary in depth and are subject to some variation; some are very constant.

The left hand end of the X terminates in a highly characteristic oblong segment which shows nine lines or bands clearly, none of which is, relatively speaking, either broad or deeply staining. Next to this end segment is an area relatively free from conspicuous bands. To the left we have an enlargement of the matrix forming a bulge or vesicle. The latter is very constant in position but varies a good deal both in size and appearance. Typically, we find dash-like lines running across or around the middle, but at other times it appears only somewhat clouded or even quite clear. A narrow collar separates this vesicle from the yellow-scute segment.

The next landmarks along the X are two areas, the first consisting of two bands which, as I shall show, are near the right hand limit of the locus for white. The second is composed of five narrow bands, the last of which is dash-like, which we shall call "facet" bands because Mr. MACKENSEN (1934) has found that when facet is deleted certain of these bands are absent. These landmarks vary somewhat in appearance. In chromosomes relatively contracted and deeply stained they appear as rather broad but not sharply defined or deeply stained areas. When the X is elongated we can see the thin individual lines more clearly and the banded effect is less conspicuous.

A little distance to the right of the facet area are three prominent bands the first of which is rather narrow and deeply staining, the other two distinctly double in their make up. Next come a set of six or seven prominent bands. The first, which lies near the left hand limit of the delta 49 inversion may stain deeply, when it is conspicuous, or, diffusely, when it it not prominent, but the next six are very constant in appearance. The first appears single, but stains deeply with the sharply defined edges, the second is double and appears relatively very broad, the third is like the first, and then there are three narrow bands lying close together which bound this segment at the right. Following the "six-banded" area there are some six segments all more or less banded but with no outstanding topographical features. This region is bounded at the right by what will be called tentatively the "lozenge" segment. Here I wish to point out that the appearance of this segment may vary in different larvae. Usually, a

number of individually narrow lines are crowded together at the left end of this segment forming a very broad banded area, but sometimes, in what are presumably normal X chromosomes, the lines at this end are pushed to the right and the banded effect is lost.

At the right of the "lozenge" segment we have, perhaps, the most easily identified section of the whole X. At the left of the segment are two broad and heavily staining bands, the first about twice as thick as the second. At the right end are two much narrower but sharply staining bands. In between these boundaries the lines are thin and faintly staining. This segment has been named the "miniature" segment and its right end seems to be structurally a weak spot in the X, for there is a very pronounced tendency, with the technique employed, for the chromosome to break here.

The segment just to the right of the miniature area varies in different preparations. Typically, it is as shown but in some slides there is a marked tendency for the lines to crowd towards the left end forming a conspicuous band.

The next conspicuous area lies just to the right of the X-IV-III.4 break. There is a segment here bounded at the left by four narrow but sharply staining lines. Just to the right of this four banded area the next segment usually shows two conspicuous dash-like bands. This is called the "forked" segment because a break between forked and Bar breaks the chromosome at the right end of this segment.

The whole right end of the X chromosome beyond the forked Bar area has a highly characteristic and conspicuous topography. First, we have five deeply staining and broad banded areas with a definite number of lines forming each band. To the right of this area we have a clitellar-like enlargement of the matrix, then a narrower neck region followed by a greatly enlarged right-hand end terminal segment. This end segment is much subject to distortion and, hence, variation in appearance. One of the commonest distortions is for the segment to tear apart in the region just to the left of the three last bands. In males the fainter lines of this segment usually do not show distinctly. The paired X's terminate to the right either in two ring-like stumps, or in two fine protoplasmic strands which connect them with the chromatic coagulum or chromocenter.

The foregoing description of the morphology of the X is based on a study of female larvae in which the X's are paired and as thick in diameter and as deeply staining as any of the autosomes. In males the X shows the same regional differentiations as in the female but the chromosome is much thinner, stains much less deeply and is more apt to be contorted by the technique. It should be remembered that in the female the X is paired while in the male the X is single, the Y not being visible

with the technique employed. Many of the drawings given below are from male larvae, which accounts for the thinness of the chromosomes as compared to the paired element shown in figure 1.

Deletion 14

This aberration has proved to be the most illuminating of all those studied and will be considered in some detail. It has already been described both cytologically and genetically (PAINTER and MULLER 1929, MULLER and PAINTER 1932). It is a case in which nearly the whole of the genetic map has been deleted leaving the two ends of the X joined together. The break at the left end is between scute (0.1) and broad (0.7) and at the right hand end between carnation (71) and the normal allele of bobbed (75.5), the latter being carried by the deletion according to the genetic analysis of Dr. MULLER. In figures 2 and 3 oogonial plates are shown from which the size of the deletion, as compared with normal X's, may be seen. An explanation of the figures is given in the legend. The deletion has a volume of between a third and a fourth of that of the normal X, its large size being due to the fact that it carries a large amount of relatively inert material in its right hand end, and it is this inert material which makes it appear so large in oogonial plates (PAINTER 1931b, MULLER and PAINTER 1932). A study of deletion 14, therefore, should show us a number of interesting points: first, the contribution made from the left hand end of the X, thus giving us morphological limits for the loci of scute and broad, second, the general position of the normal allele for bobbed, and third, that region of the X which is genetically inert, since a considerable portion of this is represented in metaphase plates.

Figures 4 and 5 represent deletion 14 as it appears in salivary gland cells. By comparing these figures with the left hand terminal segment of the X of figure 1 the reader will have no difficulty in identifying that portion of the deletion which comes from the left end. The whole terminal bulb is represented with the exception of the collar which separates this bulb from the vesicular area to the right. The two dark bands of the nipple-like projection, in figure 4, are interpreted as the two terminal bands from the right hand end of the X, as may be seen by an examination of figure 1. From this evidence, it is clear that scute lies in or on the left terminal bulb, and that broad must be to the right of the break, as shown in figure 1. Likewise, the normal allele for bobbed must lie on or about the two terminal bands at the extreme right-hand end of the X. The inert region either is not present or it is represented by the nipple-like right tip of deletion 14. In passing, it may be noted that the volume of deletion 14, as seen in salivary glands, is about 1/20th of the X, while in metaphase plates it is fully 1/4, or more, of this element. The bearing of these facts

will be discussed later in this paper, in connection with other data.

Translocation X-IV 2

This stock, commonly known as 'mottled-5' at this laboratory was isolated by MULLER and analyzed genetically by BOLEN (1931). A mutual translocation between the X and a fourth chromosome is involved. The X is broken between the loci of white (1.5) and facet (3.1). In salivary gland nuclei, the break in the X is to the right of the first two fairly prominent bands in the sub-terminal segment (figures 6 and 7). In figure 6, which shows the XL-IV element in close association with a normal fourth, these bands can be seen, but in figure 7 the emphasis of the lines is not so clear. In figure 1, the approximate location of this break is shown. It is possible that the locus for white may lie close to or on the first of the "facet" bands because MACKENSEN (1934) has found that a short deletion removing this line also removes the locus of white.

Translocation X-IV III-9

This is one of the translocations recently isolated by my colleagues, PATTERSON, STONE and Miss BEDICHEK, and incidentally was analyzed cytologically before a genetic analysis was made. From cytological data, I came to the conclusion that the break was close to crossveinless, and genetic data since obtained by STONE show that it is between the gene loci of echinus (6.9) and crossveinless (16.5). Cytologically, this proves to be a mutual translocation in which most of the fourth chromosome is attached to the left end of the XR component. Figures 8 and 9 were taken from homozygous females. Figure 8 shows the XL-IV piece and we note the presence of the three heavy and two light lines of the six-banded area. Figure 9 shows the IV-XR piece and by following the pattern in the region of the fourth chromosomes we can easily identify the peculiar bands which lie under 20 of the crossover scale on figure 1, with several additional bands to the left. From these figures it is clear that the original X was probably broken between or very close to the fifth and sixth bands of the six banded area, as is shown in figure 1. This places echinus to the left of the break, while crossveinless must lie somewhwere to the right of it. Here it may be pointed out that the crossover value of crossveinless is close to its morphological left limit. That is, morphologically the left hand limit of crossveinless is about 18 units from the left end while its crossover value is 16.5. In contrast to this, we note that the other gene loci at the left end of the X have morphological values in linear units far in excess of their crossover values. Thus the left limit of facet is some ten linear units from the left end morphologically, while its crossover value is 3.1. From crossveinless on to the right hand end of the X, there is a close agreement of crossover and cytological values.

The delta 49 inversion

This inversion has been analyzed genetically by MULLER and STONE (1931), with some additional data presented by GLASS (1933). The left hand limit of the inverted area is between ruby (8.9) and carmine (22.5), and the right hand limit was first reported as being between furrowed (43.4) and forked (62). Subsequently, it was determined (unpublished data) that the right hand break was between furrowed and garnet (50.7). In spite of these rather wide genetic limits, the rearrangement is of value when considered along with the other observation described in this paper.

Female larvae heterozygous for a normal and a delta 49 X were studied. Figure 10 shows the way these two X chromosomes are joined. Figure 11 is given to show the morphology of the inverted area, and figure 12 is a simple diagrammatic interpretation of this case. The left hand limit of the inversion (figures 10 or 11) is in the clear or achromatic space between the three-banded and the six-banded areas. In figure 11 we see that the two X's synapsed both at the three-banded and the six-banded areas while between the elements diverge. Figure 10 shows the same thing somewhat less clearly because some of the lines are stretched and thus appear less deeply stained. From figure 1 we see that the point at which the delta 49 chromosome is inverted is to the left of the III-9 break already described, hence it is clear that genetically the break is to the left of crossveinless. From genetic data we know that ruby (8.9) is outside of the inversion, so that by combining genetic and cytological data we can say that the delta 49 chromosome is inverted very close to 12 crossover units from the left end.

The right hand limit of this inversion, morphologically, is at or very near the end of the segment which lies immediately to the right of the miniature segment (figure 10) and which is shown diagrammatically in figure 1. Since furrowed lies within the inverted area, we have the right-hand limit for this locus, and since garnet lies outside of the inversion, we know that its locus is to the right of the break.

The ClB inversion

A partial genetic analysis has been made of this gene rearrangement by MULLER and STONE (1931). They concluded from a study of a partially reinverted chromosome, that the left limit of this inversion was somewhere to the left of cut (25). Nothing very definite is known about the genetic right hand limit of this inversion, although some unpublished data recently obtained by STONE suggests that carnation lies outside of the inverted area.

Females heterozygous for the ClB chromosome were studied cytologically. The left limit of the inverted area is very close to or between the two

heavy bands of the three-banded area thus lying a little to the left of the point where delta 49 was inverted as is shown by figure 1. Figure 13 shows the points of the inverted areas. From the evidence presented for the delta 49 chromosome we are able to say that the left limit of the ClB inversion is very close to 12 crossover units from the left end.

The right-hand limit of the ClB inversion is near the right-hand end, cytologically, and comes between the second and the fourth of the five heavily banded areas, as shown by figure 13, or diagrammatically in figure 1. From evidence to be presented, this point is genetically between Bar and carnation.

Parenthetically, it may be pointed out that the union of the two elements in a larvae heterozygous for a normal and a ClB chromosome, may be very complete. This raises the question, is the union in meiosis as intimate, and if so, why does not more crossing over occur in the very long inverted section? If we assume that the meiotic pairing is like that we see in the salivary gland, then the following observations may throw some light on these questions. In any given preparation of a salivary gland, the union of homologues will be seen in many different stages, from cases with complete union, except at the points of inversion, to young cells in which only the spindle fiber ends are joined. It is not unusual to find cells in which both right and left ends of the X chromosomes are joined for some distance before the middle area is synapsed at all. With the two ends anchored in this way, there would be little opportunity for the strands of homologues in the inverted area to twist about each other. In so far as crossing over is dependent upon a twisting of the strands, this would seem to be a factor in reducing the amount of crossing over.

Translocation X-IV 1

The case was found by MULLER in 1928 and was analyzed by him genetically in collaboration with STONE. From the genetic evidence, it was supposed that a long section from the middle of the X had been deleted, with the subsequent attachment of this deleted area to a fourth chromosome. The left-hand break was between lozenge (33.7) and vermilion (39.7) and the right hand between the normal allele for bobbed and the spindle fiber attachment. Thus, the gray or left-hand part of the X was supposed to have the spindle fiber of the original X from which this aberration arose, while the deleted area carrying Bar was supposed to have obtained its spindle connection from the fourth chromosome to which it became translocated. Cytological evidence would indicate that these inferences, drawn from genetic data, regarding the right-hand end are not well founded. This case will be considered in some detail.

The reader will doubtless find it easier to follow the description given

below if he will first study the morphology of the two segments of figure 1 which lie between the labels of *lz* and *m*. It will be noted that in the lozenge segment a number of rather narrow bands are crowded towards the left end forming a broad band, while the miniature segment is characterized by the presence of four heavily stained bands, a pair to each end.

Figures 14 to 19 were all taken from female larvae homozygous for the X-IX 1 translocation. Figures 14 and 15a show the morphology of the XL or gray component. By comparing these with figure 1 we see that all the typographical features of the left end of the X are represented up to and including the broad band of the lozenge segment. Here the gray or XL element ends in a spindle fiber attachment vesicle or granule, shown in figure 15b, with no trace of any bands such as we see at the extreme right hand end of the X chromosome (figure 1).

The Bar component (above in figures 16 and 17) carries all of the banded part of the fourth at its left-hand end. Following the fourth chromosome part, in figures 16 and 17, we have the remainder of the lozenge segment and then the striking pattern of the miniature segment. In figure 18 the fourth chromosome part is not shown but the rest of the XR piece is present and by comparing this with figure 1 we are able to recognize all features up to and including the bands which are adjacent to the point of spindle fiber attachment. The last feature is best seen in figure 19. As far as I can determine, the spindle fiber area of the XR chromosome carries all the bands it should but it generally is somewhat distorted so that in the end it may have suffered some changes.

This X-IV 1 translocation allows us to give morphological limits to the loci of the lozenge (33.7) and vermilion (39.7) as shown in figure 1, but it does not help us place the normal allele of bobbed. From the cytological evidence, this aberration does not appear to be a long deletion accompanied by a translocation, such as MULLER and STONE first thought (see page 337, MULLER and PAINTER 1932). Rather it looks as if this is a mutual translocation in which just the spindle fiber part of the fourth chromosome has become attached to the XL component, while the rest of the fourth chromosome is attached to the left end of the XR element. In this process some of the fourth chromosome, perhaps, has been lost. This case deserves more study both genetically and cytologically.

Translocation X-IV III-8

This aberration was isolated recently by my colleagues. The genetic analysis by STONE shows that a break occurred between the loci of miniature (42.8) and garnet (50.7). Both cytological and genetic evidence show it to be a mutual exchange. Figure 21 was taken from a female homozygous for this translocation. The XL piece is represented and by comparing this

with figure 1 we see that all the left hand portion of the X is present up to and including the four bands of the "miniature" segment. The fourth chromosome has made very little morphological contribution to this XL piece except the spindle fiber area. The XR piece, figure 20, shows almost the whole of a fourth chromosome attached to the left-hand end. In other respects this piece is normal in topography from the point of break to the spindle fiber. The point of this break is shown diagrammatically in figure 1, and gives us limits for the loci of miniature and garnet.

Translocation X-IV III-4

This is a new translocation recently obtained by my colleagues and the genetic analysis by STONE shows that the X is broken between scalloped (57.1) and forked (62). Cytologically, we find that the break occurred just to the left of the four-banded area. Figure 22 shows the X R component. By comparing this with figure 1 we see that from the point of breakage on to the spindle fiber, the XR piece is normal. This case gives us morphological limits for scalloped and forked.

Translocation X-IV 4

This most interesting case was found by Mr. STONE and analyzed by him. The X is broken between the loci of forked (62) and Bar (62.2), the forked or gray section being translocated to a fourth chromosome. Figure 23 is from a homozygous female, and figure 24 from a male, showing the XR section of the X chromosome. It will be seen that the X is broken just to the left of the segment which is characterized by the five broad-banded areas. The segment where the break occurred (figure 1) shows two dash-like bands. As far as I can tell, the break was to the right of these bands, but a little of the achromatic section is still visible, to this a fragment of a fourth chromosome is attached. The XL-IV chromosome is shown in figures 25 and 26, with an additional terminal area shown in figure 27. These figures were from male larvae, and while it is somewhat difficult to identify all portions of the X up to the point of breakage, we get an excellent idea of the relative lengths of the XR and XL components. In addition we can see very clearly just how much of the fourth chromosome is missing. This case allows us to place morphological limits to forked and Bar and is further illuminating for several other questions which will be taken up later.

Translocation X-IV III-13

This is another case found recently by my colleagues and analyzed by Mr. STONE. It is of unusual interest because it enables us to place carnation. The break occurred between the loci of fused (64.5) and carnation (71). The XR section is shown in figure 29. Here we note the right and

terminal segment and the segment to the left of it. There is a dark bump on the left end of this XR piece. I have been unable to determine whether this is a small part of the next adjacent segment of the X, which I have called the "clitellar" segment, or whether it is a small piece of the fourth chromosome. In figure 1 I have placed the break through the right end of the clitellar segment. Fused lies to the left of this break and carnation must lie to the right. It is interesting to note that the crossover value of carnation is 71 and its position in morphological units can not be farther to the left of the spindle fiber than six and a half, or perhaps it would be clearer to say it lies at least 69 units from the left end.

DISCUSSION

This study of the X chromosome confirms the two main conclusions presented in the first of this series of papers, namely, (a) that in salivary glands of old larvae homologous chromosomes undergo somatic synapsis during which the union of the two elements is so intimate that one apparent structure is formed, and (b) that each chromosome has a definite and characteristic morphology which enables one to identify the same element, or parts thereof, in cells of different individuals of a species.

The unpaired chromosomes, as is seen in the X of males, or in the autosomes of young larvae, are segmental in character. In some cases we can see the prophase split and presumably all elements have undergone this process. After the union of homologues in old larvae all trace of the single elements is lost but the dual structure (probably really tetrapartite) exhibits the same morphological differentiations as unpaired chromosomes. So far as I have been able to determine, the union of homologues does not involve much if any twisting of the single chromosomes. It is true that one sees some twisting in incompletely synapsed chromosomes but this has been considered as probably due to the technique employed. In triploid larvae, the three homologues unite in the same intimate way as in diploid cells, but the morphological differentiations are somewhat less pronounced, due perhaps to the thickness of the tripartite element. If one of the chromosomes carries an inversion, such as the delta 49 X, it has been observed that all three elements unite, beginning at the point of spindle fiber attachment and continuing up the point of inversion, and from this point the aberrant element remains single while the other two homologues are paired in the usual way.

All these facts point to the particulate and very specific attraction between the parts of homologous chromosomes. Presumably, the same force operating in salivary gland cells is also responsible for the union of homologues in meiosis, and while we cannot see the actual segregation of normal and aberrant elements in the salivary gland, we can at least determine

how the chromosomes synapse and from this infer, with greater probability, how the same elements would behave in meiosis.

It is interesting to note that the union of homologues takes place in the same intimate way in both male and female larvae. This fact may be interpreted as supporting the conclusion of LEAGUE (1929) regarding the normality of the meiotic phenomena in spermatogenesis and casts doubt on those studies in which the authors attempt to show that no synapsis occurs in the male.

Why homologous chromosomes should unite in somatic tissue is unknown. It is conceivable that it may have an adaptive value, for if the genes are little chemical factories it might be better to have the same type of centers acting under one roof, so to speak, rather than have them scattered. No doubt a good deal of light will be shed on this whole subject through a study of the ontogenetic history of these chromosomes, and it is best, perhaps, not to attempt to interpret the imperfectly known facts at present.

The segments of the X chromosome have definite proportional lengths and each shows a characteristic and often a very striking pattern of lines and bands. So far, I have noted no change in the pattern when the normal position of the segment is changed, as in some one of the various types of dislocations. There are three segments of the X, however, which may show oneof two patterns, these are the lozenge, the furrowed and the right hand terminal segments. Apparently, the source of this variation is due to the way the lines are distributed on a segment (and not to quantitative differences of the lines) so that in one case there may be a crowding towards one end forming a conspicuous band, while in another larvae, the lines are more scattered. But a given type of pattern is often (always?) constant for the individual and in the end may have a genetic basis. This is one of the many questions awaiting investigation.

It is needless to say that the ease with which any given chromosome or part can be identified and followed depends on the distinctive nature of its pattern. In this respect the present study has been favored because the X is highly differentiated morphologically. But from almost daily contact and with some detailed study of the autosomes, I feel sure that they will be as easily mapped as the X. If we can thus analyze the chromosomes of a species qualitatively, and follow the fate of a segment in different individuals of a species, there is no obvious reason why the same method of study may not be applied to individuals of related species and, ultimately, perhaps, to widely divergent forms. At the present time, Mr. BREWSTER, one of my students, is making a comparative study of the chromosomes of a number of species of Drosophila. In *D. similans* the topographical features of the chromosomes appear to be identical with those of *D.*

melanogaster, so far as these have been worked out. The writer has been able to identify all of the outstanding landmarks of the X in *D. similans*, in Mr. BREWSTER'S preparations and the left arm of the second is similar in both forms. These initial observations give great promise for the value of this method, in comparative chromosome studies.

It will not be necessary to discuss, at length, the new type of chromosome map herewith presented, nor to point out its obvious practical applications. In contrast to the first cytological maps, such as those of DOBZHANSKY or of the writer, which were based on metaphase plates and were purely quantitative and hence of limited genetic value, the new picture of the X is essentially qualitative. By a study of chromosomal rearrangements of known genetic make-up it has been possible to show that certain gene loci must lie somewhere in a definite segment. With this knowledge, we can study new chromosome rearrangements and determine cytologically what has happened to a certain segment and its contained gene loci. Naturally, as we determine the morphological position of more genes the value of the new map will increase and the day may not be far distant when Drosophila geneticists, interested in chromosomal aberrations, may study the genetics of these breaks by a simple cytological examination of the material. Already, at this laboratory, we are using this method to check up translocations between the X and the fourth chromosome. Once such a translocation is isolated by my colleagues, it is examined cytologically and the point or points of breakage determined with regard to the 21 known gene loci. In this way, in usually half an hour or so, we can determine cytologically what it might take a month or two to find out by the genetic method and, perhaps what is more to the point, we can quickly select breaks which cover a region in which we are especially interested. Maps of the second and third chromosomes are now being prepared.

As already noted, except for the left-hand end of the X, the morphological positions of gene loci, in general, seem to correspond to the crossover values of the genetic map. It should be realized, however, that in most instances we have only been able to place genes within general limits of a segment or more, and that we know nothing, at present, regarding the local distribution within these limits. It is hoped that the studies of Mr. MACKENSEN will throw light upon this subject.

From a theoretical standpoint, the outstanding feature of the present study is the conclusion that neither the inert region of the X, nor of the Y chromosome, except as noted, show as recognizable structures in salivary gland cells. Here we will consider the evidence upon which these conclusions are based.

In metaphase plates of oogonial or of nerve cells, the X chromosome is

considerably longer, on the average, than either arm of the second or third chromosome. In salivary gland cells the arms of these V-shaped autosomes show as separate elements so we might expect the X to be a great deal longer than any other body in the nucleus. This is not the case, however. On the contrary, measurements of a few especially favorable cells showed that the X was materially shorter than either arms of the second or third chromosome. This was the first hint I had that, as compared with oogonial metaphase plates, the X of the salivary gland cells was shorter than we should expect.

The second line of evidence was given by Mr. STONE's "forked-Bar" break (X-IX-4). In metaphase plates, the break separates the X into two pieces of about the same volume. In salivary gland cells, however, the gray segment carrying forked is roughly four times the length and volume of the XR piece which carries Bar. From this we see that the XR element is only one-fourth the size we would expect it to be, judging from oogonial metaphase plates. Or, in other words, about three-eighths of the X chromosome is missing in salivary glands. On the other hand, the size of the forked (XL) segment which is roughly 59 units (in a total of 75.5) from the left-hand end, corresponds well with the crossover value of forked (62), and likewise, the remaining 13.5 units of the crossover map fits well with the 16.5 morphological units of the Bar or XR element. How can the small size of the XR piece be explained? There are two rather obvious possibilities: (1) That the size of the metaphase chromosome is determined by the distribution of the chromatic material and, since the right-hand end of the X is heavily chromatic, as compared to the left-hand end, this causes the Bar segment to appear unduly large in metaphase plates. (2) That the inert region of the X is not represented at all or only in part, in the active salivary gland. There are other possible explanations which we need not consider here. A consideration of the two possibilities just outlined led to a study of deletions, which at metaphase are very large, due to the presence of inert material, but which genetically carry only a small fraction of the genetic map. Deletion 14 was the only stock available for study, but it has proved critical for the questions before us. Genetically, deletion 14 carries less than one crossover unit from the left-hand end, and at the right end only the normal allele of bobbed is known to be present. In metaphase plates of oogonia, however, this deleted X is roughly between a third and a fourth of a normal X in volume. The inert region is what gives the deleted X its large size. In salivary glands, deleted X 14 proved to be very small, altogether not much more than a twentieth of the normal X. As a study of the morphology showed, this size is due almost wholly to the contribution made from the left end, which, of course, is easily recognized. The inert region and the area carrying the normal allele of bobbed consists

of a small nipple-like projection. This makes it abundantly clear that the inert material is not represented, to any appreciable extent, at least, in the salivary gland cells.

The above conclusion regarding the absence of the inert area from the X of salivary glands is in agreement with the finding regarding the Y chromosome, the two lines of evidence giving support to the theory that a part of the X chromosome is homologous to the Y, as WILSON postulated long ago (1911).

The evidence concerning the Y chromosome may be summarized as follows: First, both male and female larvae show the same number of chromosomes, the only difference being that the X of the male is much narrower and perhaps because of this, much less deeply staining than the X of females. From this initial observation it was clear that the Y certainly is not represented by a separate recognizable element. Either it is synapsed with some chromosome, presumably the X, or else it is not present in the salivary glands of males or is represented only by a small unrecognized piece (or pieces) in the nucleus. If the X and Y are synapsed, we should expect that the latter would be very narrow and non-chromatic since the X of males is much thinner and less deeply staining than the paired X's of females.

The first test made for the Y was a study of delta 49 males. The delta 49 X carries an inversion, the morphological position of which we have described in the foregoing pages. When females heterozygous for a normal and a delta 49 X are studied, we get typical inversion figures (see figure 10). If now the Y is synapsed with the X in the region of the inversion, a study of delta 49 males should reveal it. However, although the inverted region was easily identified in the male, the outline of the delta 49 X was perfectly regular at these points, and there was no suggestion of the presence of an achromatic element. This made it clear that as far to the left as furrowed (43.4) there was no trace of the Y chromosome.

To study the right-hand end of the X, males carrying both components of the "forked-Bar" translocation were examined. If the Y is synapsed with the X in the region of Bar (62.2) and to the right of this locus, it should be associated with the XR element. In general, the evidence was negative, that is, no trace of a Y was seen, but in one case I found what I have interpreted as the Y chromosome. This is shown in figure 28. Here the XR element is represented and its right-hand end tip seems to be made up of two components. The normal XR is below and lying on top of this, but distinct from it, except at the point of spindle fiber attachment, is a second structure which has four bands which are apposed to similar bands on the last segment of the XR piece. The outline of the second element is extremely faint and is soon lost in the general coagulum of the nucleus. This

faint element is, I believe, a part of the Y chromosome which, for some reason, has not completely united with the X, and as a result, can be seen. There is some additional evidence which gives strength to this conclusion. That is, it has been shown that the normal allele of bobbed must lie on or about one of the bands adjacent to the spindle-fiber area of the X, and, genetically we know that bobbed is carried by the Y, and this is just the area shown by the Y in figure 28.

Observations similar to those made on the forked-Bar break have been made on a number of the aberrations in which the XR piece was short and with the same result, that the Y could not be found, in a recognizable form. It is safe to conclude therefore that the Y chromosome does not show up in salivary gland cells, like the other chromosomes, and except for the part which has been found in association with the terminal segment of the X and possibly some other fragments not yet identified, it is not present in a recognizable form. In this respect, the Y and the inert region of the X are similar in behavior and thus we have additional support for the theory (discussed at length by MULLER and PAINTER 1932) that the inert region of the X and the Y chromosome are, or were originally, in part at least, homologous.

There are two possible ways of accounting for the behavior of the inert area of the X and of the greater part of the Y chromosome. The first is, that, being genetically inert and hence of no use in ontogeny, the non-active regions have been discarded by diminution or some similar process, as development goes forward. The other explanation is that in the highly specialized salivary gland cells, only the active chromatin of the nucleus, or better, the active genetic material and its associated chromatin, appear in the nucleus as the annulate-like bodies we call chromosomes, and the inert material retains an uncondensed form and contributes, perhaps, to the chromatic coagulum, or reticulum found in all nuclei.[2]

[2] Since this article has been in press an extremely important and interesting paper has appeared by HEITZ (1933) dealing with somatic heteropycnosis in *D. melanogaster* and other Diptera. For a number of years HEITZ has been studying heteropycnosis in plants. He finds that definite sections of chromosomes tend to remain condensed in late telophase and early prophase forming "chromocenters." These chromocenters are made up of "heterochromatin" which HEITZ (1932) thinks is genetically passive or inactive material. The active genetic material, which is called "euchromatin" becomes diffuse at the interphase. With these concepts before him, HEITZ has studied the somatic chromosomes of a number of species of Drosophila, more especially *D. melanogaster*. In the latter species he finds that about half of the X chromosome is heteropycnotic in the interphase, and ties in this observation with the work of Dr. MULLER and myself on the inactive region of the X. In addition, HEITZ shows that both the second and third chromosomes have inactive material in the region of the spindle fiber.

In the salivary gland of *D. melanogaster* the inactive regions of the X and the large autosomes fuse to form what HEITZ calls "sammelchromocentren" or a compound chromocenter which is obviously the same structure which I have described as the "chromatic coagulum." Since this paper was sent to press I have devoted a great deal of time to a study of this compound chromo-

At the present time, the evidence before us does not allow us to decide between these two explanations. It is known, of course, that in Miastor there is an elimination of chromatin, from some somatic cells at least, in development, and while, so far as I know, this process has not been described for any of the Drosophila species it may well occur here. Were the inert chromatin of the X or the Y physically eliminated from the nuclei of the salivary glands, then we can understand why the only trace of the Y found is the short active area which carries the gene for bobbed (and possibly other small areas as yet unidentified) and why the X is relatively too short. At the same time we would have a simple genetic explanation to account for the enigmatic process of diminution, which has been described here and there in the ontogenetic history of widely separated forms. This problem is one of the most interesting which have resulted from this type of cytogenetic study and its solution obviously lies in a study of somatic mitoses of various types of tissue in developing larvae. If metaphase plates of very young salivary gland cells show the X and Y as we see them in germinal tissue, then the alternate view, that the inert areas are present in some unrecognized form, would be indicated. The latter view finds some support in the genetic evidence we have for the X. The crossover map of the X and the morphological picture we get from a study of salivary glands, correspond very closely. Whatever the nature or function of the inert region may be, the sex chromosome at meiosis behaves as if it did not exist, so far as crossing over is concerned, a fact which could mean that at this critical period the inert region was not an organic part of the X chromosome.

From the present study, we may conclude that, in metaphase plates about three-eighths of the X chromosome is made up of inert material.

In the foregoing discussion, I have limited myself to only a few of the many points which will have suggested themselves to the reader. Foremost stand questions about the intimate structure of chromosomes, whether the chromatic bands lie on or within the matrix? What part carries the genes? What is the relation between the genes and the chromatic substance? and a host of similar questions. From the first, such questions have dominated

---

center and a full discussion of my findings are given in a paper now in press (PAINTER and STONE). From a study of breaks in the second and third chromosome I am convinced that HEITZ is correct in his conclusion that both these autosomes have inert areas in the region of the spindle fiber. On the other hand, in my opinion, the total volume of the chromatic coagulum or chromocenter in salivary glands is not large enough to account for all of the inactive material of oogonial or nerve cell metaphases. It is possible that the active euchromatin, representing the banded areas of all the chromosomes, is more greatly hypertrophied than the inert material, or that some of the inert chromatin is in solution and hence not easily detected by the technique used, or that some of it has been eliminated by diminution. Up to the present moment I have no critical evidence to decide between the various possibilities.

our interest in these studies, but as tempting as it may be to speculate upon them, at present it seems unprofitable to consider these points until we know more about the genesis and ontogenetic history of these peculiar chromosomes. This latter field has been explored somewhat, in other dipteran forms, by ALVERDES (1912) and by TANZER (1922). While these several accounts differ in details, it would appear that, in salivary glands, the chromosomes are differentiated beyond the point reached by cells which will subsequently undergo division. What is needed now is a very thorough study of this whole period, in the larva of *D. melanogaster*, or some closely related form, and with this background we can consider the questions of chromosome structure, with more assurance of being able to make definite progress in the general field. This line of work is being pushed at present here in Austin.

LITERATURE CITED

ALVERDES, F., 1912 Die Kerne in den Speicheldrusen der Chironomus-Larvae. Arch. f. Zellforsch. **9**: 168–204.

BOLEN, H. R., 1931 A mutual translocation involving the fourth and the X-chromosomes of Drosophila. Amer. Nat. **65**: 417–422.

DOBZHANSKY, T., 1930 Cytological map of the second chromosome of *Drosophila melanogaster*. Biol. Zbl. **50**: 571–685.

GLASS, H. B., 1933 A new allelomorphic compound presenting the phenotype of the wild *Drosophila melanogaster*. J. Genet. **27**: 233–241.

HEITZ, E., 1932 Die Herkunft der chromocentren. Planta **18**: 571–639.

1933 Die somatische Heteropycnose bei *Drosophila melanogaster* und ihre genetische Bedeutung. Zeit. f. Zellforsch. u. mikr. Anat. **20**: 237–287.

LEAGUE, B. B., 1930 The normality of the maturation divisions in the male of *Drosophila melanogaster*. Science **71**: 99.

MACKENSEN, OTTO, 1934 A cytological study of short deficiencies in the X chromosome of *Drosophila melanogaster*. Amer. Nat. **67**: 76.

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.

MULLER, H. J. and STONE, W. S., 1931 Analysis of several induced gene-rearrangements involving the X-chromosome of Drosophila. Anat. Rec. **47**: 393.

PAINTER, T. S., 1931a A cytological map of the X-chromosome of *Drosophila melanogaster*. Science **73**: 647–648.

1931b A cytological map of the X-chromosome of *Drosophila melanogaster*. Anat. Rec. **51**: 111.

1933a A new method for the study of chromosome rearrangements and the plotting of chromosome maps. Science **78**: 585–586.

1933b A method for the qualitative analysis of the chromosomes of *Drosophila melanogaster*. Anat. Record Supplement No. 4, **57**: 90.

1934 A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics **19**: 175–188.

PAINTER, T. S. and MULLER, H. J., 1929 Parallel cytology and genetics of individual translocations and deletions in Drosophila. J. Hered. **20**: 287–298.

TÄNZER, E., 1922 Die Zellkerne einiger Dipteren larven und ihre Entwicklung. Zeit. f. wiss. Zool. **119**: 114–153.

WILSON, E. B., 1911 Studies on chromosomes VII. J. Morph. **22**: 71–110.