A NEW METHOD FOR THE STUDY OF CHROMOSOME ABERRATIONS AND THE PLOTTING OF CHROMOSOME MAPS IN DROSOPHILA MELANOGASTER

THEOPHILUS S. PAINTER

University of Texas, Austin, Texas

For many years *Drosophila melanogaster* has been a classical object for genetic studies but, due to the small size of the chromosomes and to the fact that we have been able to make little headway in the study of these elements in meiosis, it has been necessary to turn to less favorable material genetically, in order to study some of the questions which have arisen with the progress of cytogenetics. The past fall the writer began a study of the salivary glands in larvae of this fly with the hope that it would be possible to recognize the same chromosome in any cell, and in this way to get more accurate measurements of translocated pieces and, in general, to gain some insight as to the nature of dislocations of chromatin. It has been found that the chromosomes in the salivary glands have a definite and, within limits to be described, a constant morphology which enables one to recognize the same element in any cell. The elements are so distinct and constant in their morphology that we can follow a translocated segment to its new site, or if we are dealing with a mutual translocation, can determine just how much of each chromosome is involved in the exchange. Needless to say, this places in our hands a method of studying translocations and of plotting cytological maps which is a vast improvement over the study of metaphase plates heretofore used. But what is of far greater importance is the discovery, wholly unexpected, that in the salivary glands of old larvae the chromosomes may undergo somatic synapsis. As a result, homologues pair line for line, band for band, and unite into one apparent element. If, however, one of the homologues has an inverted section, we get typical inversion figures, such as may be seen in the meiosis of corn, and by the bands and other landmarks we can tell, cytologically, just where the inversion occurred. If one chromosome has been deleted, it pairs with its homologue except at the region of the deficiency; here the normal chromosome buckles and by comparing the lines of the two elements we can tell just how much is missing from the deleted element. This gives us a new and relatively exact method for the study of inversions and deficiencies and enables us to make a direct attack upon many of the problems associated with these aberrations.

As new as the facts reported above may appear, the morphological observations upon which they are founded (unknown to the writer at the

time he reached the above conclusions) have been recorded in the litera-
ture for many years, for some of the other Diptera. He, therefore, can only
lay claim to having shown, by a study of inversions, the intimate char-
acter of the union of homologues and to the rather obvious application of
the findings to a study of present day problems in cytogenetics. The
most recent paper on this general subject is that of HEITZ and BAUER
(1933) dealing with *Bibio hortulanus*, in which they report that in the
Malpighian vessels the haploid number of chromosomes may be found
due to the union of homologues. They maintain that the different elements
in the nucleus can be identified in any cell by such differences in mor-
phology as length, method of terminalizations, association with plasmo-
somes, etc. There is nothing in this paper nor in the earlier literature re-
viewed by them, which would indicate that the association of homologues,
so often seen, was anything more than a close apposition of elements.

The purpose of this paper is to give some of the evidence upon which
the conclusions presented in the first paragraphs are founded, to point
out the usefulness of the material for cytogenetic studies, and to give
some of the outstanding conclusions which have been reached. It is planned
to publish detailed studies dealing in turn with the separate elements of
the complex, and with the general question of chromosome structure. A
paper dealing with the X chromosome is now in press in GENETICS.

At this place the writer wishes to express his thanks to his colleague,
Mr. WILSON STONE, whose intimate knowledge of the genetic make-up of
the stocks in this laboratory has been an invaluable aid in making this
study.

The method employed for the preparation of slides is simply to dissect
out the salivary glands of old larvae about ready to pupate, place these on
a slide, add aceto-carmine, cover with a cover glass and stain for a few
minutes. Then crush the glands by pressing on the cover glass with a nee-
dle, remove the excess of stain with filter paper, seal with vaseline and
study under blue-green light. The slides show up best a day or two after
preparation.

When one has prepared a slide from an old female larva of normal stock,
under low power a number of nuclei will usually be seen which are con-
spicuous because the contained elements are large and deeply staining;
these should be selected for study. In figure 1 we have a drawing of such
a cell in which the nuclear wall has been broken and the contained ele-
ments spread apart so that we can follow each from one end to the other.
There are six conspicuous elements in this nucleus. One is small, roughly
two and a half times as long as its diameter; this represents the two fourth
chromosomes so closely apposed that we get no hint of its compound
nature. Of the five long elements, one represents the two X chromosomes,

which like the fourth chromosomes, are so closely joined that they appear as one. The four remaining elements are the left and right arms of the second and third chromosomes, respectively. All elements are labelled in figure 1. The identification of the several elements was made possible by a study of appropriate translocations and inversions, as will be described, in part, in the following pages.

All six of the elements are normally embedded at the spindle fiber end in a chromatic (with Feulgen's stain) coagulum or mesh work. In most preparations there is no indication of which arms belong together (in the cases of the second and third chromosomes) but occasionally, in crushing the nucleus, the elements are separated from the mesh work and in this event it is not unusual for two to remain attached together. In this way I came to associate what have proved to be the two arms of the second and third chromosomes, respectively, but I could not be certain of the identification until I had analyzed such stocks as Curly, which has inversions in either arm of the second, and various translocations involving both second, third and the fourth chromosomes. With the help of these stocks it has been possible not only to determine which arms belong together, but which are the so-called genetic right and left arms respectively.

A close study of any element such as II L in figure 1, that is, the genetic left arm of the second chromosome, shows the more or less achromatic matrix or substratum which has the general proportions of an ordinary earth worm. Lying either on the surface or just under the surface of this matrix we observe what appear to be chromatic bands (often described as discs). The cylindrical matrix shows a fairly uniform diameter throughout its length, but certain elements show local differentiations which within limits may be constant morphological features. In the case of the left arm of the second chromosome there are four such "vesicular areas." The matrix, at least after a few minutes fixation in aceto-carmine, is quite tough so that it may be stretched to two or three times its normal length with a consequent decrease in its diameter. The bands which strike one's eye, on the superficial examination of any preparation, are really made up of a number of lines (frequently two). These lines may be classified, in general, as thick and densely chromatic, thin and sharply chromatic, diffuse or fuzzy, discontinuously chromatic giving a series of dots or dashes, and finally, very thin in diameter and more or less faintly staining. One or more of these different types of lines go to make up a "band" in a relatively contracted area of any chromosome. It is exceedingly important to realize this fact because, when the matrix is stretched or otherwise distorted, the lines of a prominent band may be so far separated that the existence of a "band" is no longer obvious.

As we study the same chromosome in different preparations, in normal

stocks, we may get superficially quite different images. This may be due to differences in fixation and in the degree of staining, but principally to the degree to which the chromosome or arm is contracted, or stretched, or mashed. A freshly stained preparation shows the matrix as achromatic and the lines of the bands as lightly stained. But as the preparation gets older the lines stain darker red and the matrix, especially close to the lines, may be tinted with the dye. In old preparations one must study the lines of a "band" with care because when the matrix is stained, we may see a band, on superficial examination, which really doesn't exist. At the outset, one must be careful not to be misled by the presence of broken chromosomes, for experience has shown that certain elements are liable to break at certain definite weak points.

Attention should be called to the fact that there is a tendency, on the part of the observer, to focus on the matrix at the point of its greatest diameter. Since the matrix is appreciably thick, we do not see the lines on its upper surface clearly, and there is a shadow of the lines on the lower side. The net effect is a hazy band, an effect which is heightened by the somewhat oblique course which the lines often show. In focusing sharply on either the upper or lower surface of the matrix the true situation will be revealed and the vague band will disappear.

With the above points in mind we may now study several of the individual chromosomes or arms. The most easily identified element in the nucleus is the genetic left arm of the second chromosome. This is labelled in figure 1. Drawings of this same element, taken from other cells, are given in figures 2 to 7, the latter being the inversion figure obtained when a female heterozygous for Curly is studied. Using figure 2 as typical, and beginning at the distal end (to the left in figure 2) we may conveniently divide this arm into several sectors which have very definite boundaries. Sector a begins with the terminal bulb and runs through the heavy band bounding the first vesicular area. Sector b begins with the just named vesicular area and runs through the heavy band bounding the second vesicular area. Likewise sector c begins with this vesicular area and runs to the midpoint of a very prominent constriction. Sector d comprises the rest of this chromosome arm. All of these areas are indicated on the drawing with appropriate labels.   ·

Returning to sector a, and beginning with the terminal bulb, we note towards its base, first a sharply staining line followed by two thin lines, and second a very heavy line (usually appearing as double) labelled a_1. (Ultimately, it is hoped to replace this nomenclature by the symbols of the genes which lie at these points along the chromosome as has been done in the case of the X chromosome.) This is followed by a band less heavy (a_2) and then a very heavy band, a_3 comprising at least 3 heavy

lines which is as thick as a_1 and a_2 put together. About three diameters of the matrix farther along we have a very heavily banded area (a_4), often showing four heavy lines, and finally the limiting band of the area (a_5). Figures 3 to 6 illustrate the appearance of this terminal sector taken from
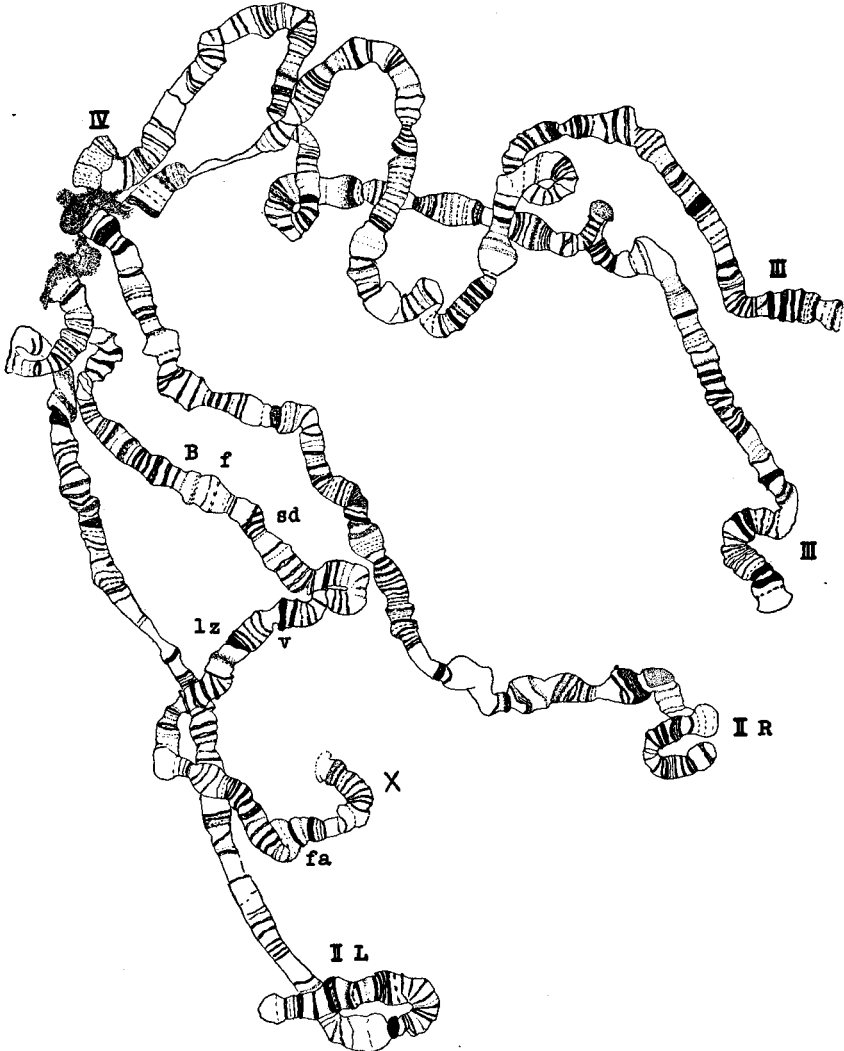

FIGURE 1.—This camera lucida drawing, taken from an aceto-carmine preparation, shows the chromosomes found in a salivary gland after the nuclear wall had been ruptured by pressing on the cover glass. In this case, somatic synapsis has been completed. The various elements are labelled, II L being the genetic left arm of the second chromosome.

other preparations. In a similar manner, the various other areas of this chromosome arm may be identified. Figure 7 is the figure obtained when a female larva heterozygous for Curly is studied. It has long been known

genetically that this arm is inverted in Curly. Again we can follow the regional differentiations with ease. The inversion has obviously occurred between bands a_3 and a_4 on the distal side of the arm and since the elements are paired throughout sectors b, c, and a part of d, it is clear that the other limit of the inverted area is about a third of the length of sector d. As a matter of fact, a close study of a number of figures has shown that the proximal limit of the inversion is between two specific banded areas. Needless to say, if we knew genetically the limiting gene loci of this inversion we could plot the position of such loci with great exactness.

In comparing the figures of this left arm of the second chromosome in the drawings (figures 1, 2, 3, to 7) the reader will gain a fair idea of the constancy of the landmarks described as well as the variations in the superficial appearance of this element in different preparations. The sources of these variations has been discussed above.

In order to show the typical and undistorted structure of the X chromosome figure 8 (shown in two sections) has been prepared by combining a number of camera lucida drawings of this element. Thus each feature is an actual camera lucida sketch taken from a drawing which showed the given area in typical form. The approximate location of some of the gene loci already determined is shown by appropriate symbols. There are three features of the X which are useful in identifying this element. The first is the terminal bulb (to the left in the upper section of figure 8) taken together with the fact that the whole left hand half of the X shows no relatively deeply staining and broad banded areas. About the middle of the X in the region of v (vermilion) are two very conspicuous bands, which stain very deeply, the one to the left being about twice as broad as the one to the right. It may be noted that in my preparations there is some tendency for the X to break at the constriction to the right of the m (miniature) region. To the right of the B (Bar) region the X becomes heavily chromatic, the five deeply staining banded areas constituting, perhaps, the most conspicuous feature of the entire element. The spindle fiber end (to the right in the lower section) has a terminal bulb which carries a great deal of chromatin, but this area is subject to distortion due, presumably, to the fact that it is anchored here to the chromatic coagulum. By comparing figure 8 with the X of figure 1, the reader will be able to identify most of the prominent land marks. Parenthetically, it may be pointed out that the heavily chromatic part of the X lies well to the right of the Bar locus, and while, as this is written, I am not sure of the locus of carnation, we may say that this chromatic region is relatively poor in known active genes, if, indeed it carries any except the normal allelomorph of bobbed. Conversely, that region of the X most crowded with gene loci (the left

hand end) shows relatively very little chromatic material. These facts are obviously of great interest genetically as well as cytologically.

In figure 1, the characteristic appearance of the paired fourth chromosomes may be seen. It has a peculiar granular appearance unlike any of the other chromosomes and there are no heavy lines. Usually, one can identify three or four lines, a little more conspicuous than the rest, and the free end often appears a little denser than the spindle fiber area. The figures 9 to 11, three additional drawings of the paired fourth chromosomes are given. Two of these show, at the spindle fiber end one or two small, but very distinct, ring-like vesicles which probably represent the points of attachment of the chromosomes to the mitotic spindle. Similar structures are seen on the X and the second and third chromosome, there being typically two for each (paired) element.

The chromosome configurations shown in figures 12 and 13 are of especial interest since they illustrate both the *ClB* inversion of the X chromosome and a case of a mutual translocation between the X chromosome and the fourth. We will describe the latter first.

The stock involved is known in our laboratory as "Mottled 5," originally isolated by MULLER and studied genetically by BOLEN (1931). The genetic evidence in this case indicated that there had been a mutual translocation in which the gene for bent (located on the fourth) was linked with white eye and the other loci to the left of this locus on the X. Likewise, the other genes of the fourth were linked with the whole X chromosome to the right of white. Cytologically, the normal fourth and the XL-IV elements were not noticeably different in size in metaphase plates of oogonia. A study of the salivary gland cells is very illuminating as is shown in figure 12a and b, taken from the same nucleus. Figure 12a represents the left end of the paired X chromosomes. The fourth chromosome component lies to the left in this figure, the normal left hand end of the X to the right. Obviously, the original X broke near *fa* (facet) and part of the fourth chromosome took its place. Beyond this point the two X's are fused together. In figure 12b we have the normal fourth chromosome and XL-IV element. The reader will recognize the tip of the X lying to the right. The normal fourth and the XL-IV are joined, or, at least, lie close together at their spindle fiber ends. Both show one line in common labelled *b* (bent). The apparent spindle fiber attachment area of both elements is also seen. Since the volumes of the two elements (the normal fourth and the XL-IV) are not greatly different, it is clear why in metaphase plates of oogonia one could detect no difference in size between them. It should be noted that neither the IV-XR nor the XL-IV chromosomes show any scar at the point of break and translocation.

Figure 13 shows the points on the X chromosomes where the *ClB* inversion took place. This figure was taken from a female larva heterozygous for the "Mottled 5" X which is normal in genic sequence except for
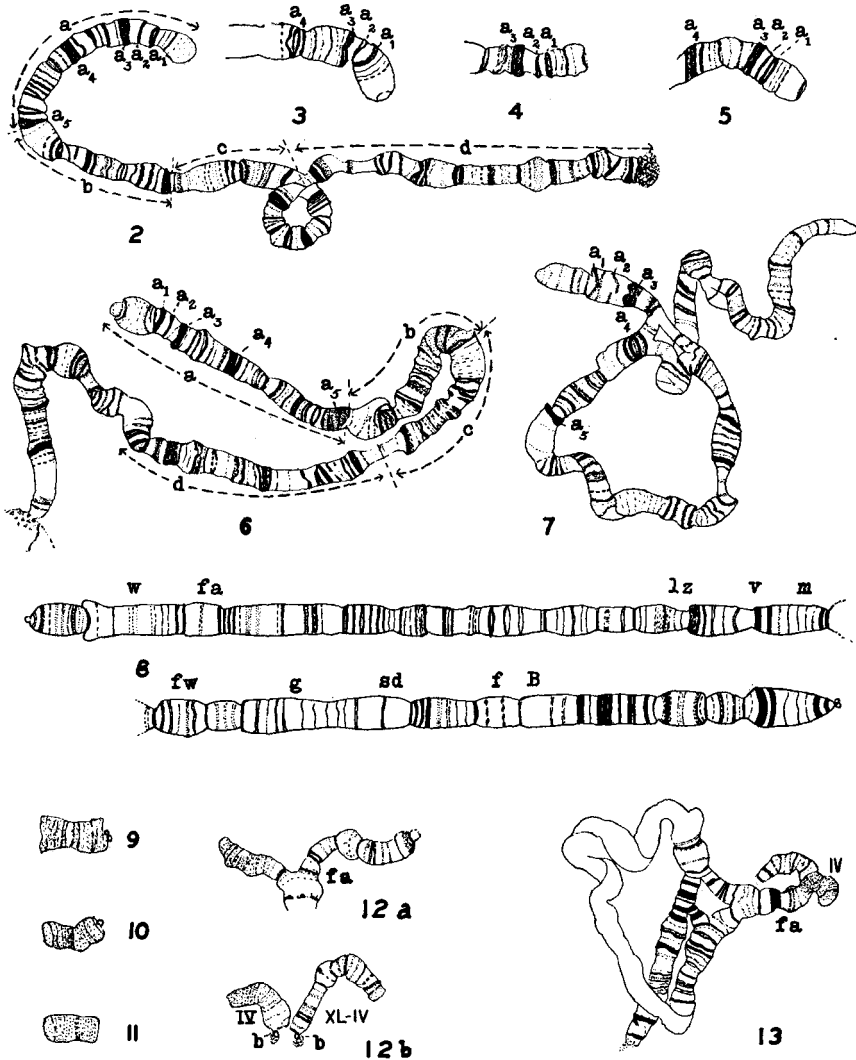
FIGURES 2 TO 13.—The typical morphology of the left arm of the second chromosome is shown in figures 2 and 6. Figures 3 to 5 show the terminal segment taken from other cells. Figure 7 is the inversion figure found in the left arm of the second when the larva is heterozygous for Curly. Figure 8, made by combining camera lucida drawings, shows the typical structure of the X chromosome. The approximate location of a number of gene loci, as determined by a study of breaks, is indicated by the conventional symbols. Figure 9 and 11, illustrate the morphology of the paired fourth chromosomes. Figure 12a and b, taken from the same cell, illustrate a mutual translocation between the X and the fourth chromosome. Figure 13 is the inversion obtained, when a larva heterozygous for *ClB* is examined. In this case, the normal X carries a piece of the fourth chromosome on its left hand end.

the part involved in the translocation and a *ClB* chromosome. The *ClB* inversion occurred at the right hand end between the second and the fourth heavy bands of the series found in the proximal sixth of the X as described above, and we can see the fourth and fifth lines joined to the left hand end of the X at a point which, morphologically, is about three matrical diameters to the right of the lines separating white and facet. Except for the short distance where the arms diverge, the two elements are paired.

The description given for the left arm of the second, the X and the fourth will serve to illustrate the conditions in the salivary gland, and to bring out the value of the method for a study, especially of inversions, translocations, deletions and the plotting of chromosome maps. As this is written I have made no attempt to determine which are the genetic right and left arms of the third chromosome. One arm of the third, at the free terminal bud, often resembles the left hand end of the X, but the heavy bands immediately following, in the case of the third, should cause no one to be misled.

So far we have dealt with the elements of old female larvae. The condition encountered in the male is the same as for the female, except that the X chromosome does not stain deeply, and seems more apt to be distorted as if it were structurally much weaker. No sign of the Y is seen in the normal male, from which we might infer that it has fused with the X. This may, indeed, be the case. But in genetic setups where we should expect the Y free, I have been unable to identify it with certainty. In any event, it appears that special staining methods will be required to bring out the presence of this element.

<div align="center">DISCUSSION</div>

Of the large number of questions which are raised by the present work, the writer proposes to take up here only a few points, primarily those concerning the validity of the method as a means of studying inversions, (for which it is a method *par excellence*), translocations and the position of gene loci.

The first point to be raised is, are the structures found in the nuclei of salivary glands chromosomes, and is there sufficient constancy in the elements of the salivary gland to enable us to identify the same chromosome or chromosome arm in different preparations? The evidence described in the foregoing pages has been presented, primarily, to show that this is true. Each chromosome (or arm) has a characteristic morphology expressed, for the most part, in the way the bands, or better, the constituent lines of the bands, are arranged and, to a lesser extent, in differentiations of the matrix. The arrangement of the bands is different in all the elements. During the process of pairing, which occurs in late larval stages homologues unite line for line, band for band. When one knows the charac-

teristic form of the normal elements, a translocated piece can be followed to its new position, or, in cases of mutual translocations, we have been able to determine the points at which each chromosome was broken and the extent of the exchange. If one homologue carries an inverted section, we can determine, cytologically, with relatively great exactness the points at which the inversion occurred.

All the above facts have been brought out and illustrated in the foregoing pages, and it seems unnecessary to enter into a lengthy discussion at this place. It should be emphasized, however, that while there is sufficient constancy in the sequential relation of the lines and differentiation of the matrix, to allow us to do the things enumerated, we must realize that the elements in the nucleus are labile and while the matrix shows considerable resistance to stresses such as mashing or stretching, these processes necessarily distort the superficial appearance of the chromosomes. Bands which, in the normal condition of the chromosome, are very useful criteria for identification through distortion of the matrix, may be pushed together locally, or so far separated that the banded effect is lost. Again, a mashed area of the matrix may stretch or break the lines running around the matrix, thus entirely altering the appearance of the region. But all these distortions are easily understood, once the labile nature of the chromosomes is appreciated and will give the experienced investigator no trouble. When we speak of constancy of chromosome morphology, we mean constancy in the relative position of the lines which go to make up the bands .we ordinarily see.

The differentiations of the matrix are extremely varied. Many of the striking figures seen are undoubtedly artifacts and due to the technique employed. In other cases, however, vesicular areas are constantly seen at definite points along a given chromosome and these are of service in identification. In *D. melanogaster*, none of the elements show a constant association with the plasmosome, nor have I seen the elaborate terminalization figures described by HEITZ and BAUER. All of the elements have a more or less pronounced annulate or segmented form in that the matrix shows at irregular intervals slight local constrictions followed by bulges. These would seem to hint at the fundamentally compound nature of Drosophila chromosomes.

The question may now be raised, how do we know that the chromosomes of the salivary gland give us any proper conception of the structure of a "normal" chromosome? The answer is that since we have no direct cytological evidence concerning the structure of Drosophila chromosomes except that afforded by the salivary glands, the only way we can test the validity of the concept is to see how it fits in with such indirect evidence as we have. So far as cytology is concerned, it may be said at once that the

structure of the chromosomes, as revealed in the salivary gland nuclei, is in accord with the modern concept of the intimate structure of chromosomes obtained from other organisms, more especially from plants. The ideas of a matrix, a chromonemata carrying the gene string, and of local deposits of chromatic material around the gene string, have all found ample expression in cytological literature in recent years. Indeed, the picture we get from the salivary gland chromosomes allows us to reconcile the apparent conflict between the chromonemata and the chromomere concept of chromosome structure. For, if it is granted that chromatin is deposited locally along the spiral gene string, then we have a structural basis for the chromomeres, as they are commonly described in cytology. Since the writer proposes to take up the whole matter of chromosome structure in a later paper this phase of the subject may be concluded here with one remark, namely, that it seems to me inherently improbable that the fundamental structure of a chromosome will be materially altered by physiological activity.

So far as the genetic evidence is concerned, the concept of the structure of the X chromosome obtained from a study of the salivary gland is in very close agreement with the crossover map concept. In fact, with the possible exception of the locus for the normal allelomorph for facet, there is a close numerical agreement between the linear position of a gene locus on a schematic X chromosome 75.5 units long, and its crossover position (new map). On such a chromosome chart, the right hand limit of lozenge, for example, is about 35 units from the left hand end. That is to say, a break which, determined genetically, was between lozenge and vermilion, morphologically broke the chromosome some 35 units from the left end. While we do not know exactly where the break occurred genetically, we can say that lozenge lies to the left of the point of breakage. The crossover position of lozenge on the new map is 33.7. In a similar manner, we may say that the right hand limit of miniature, on our chromosome chart, is about 41 units from the left hand end. (The crossover value of miniature is 42.2.) And finally, Bar lies between the morphological limits of 58 and 62 units from the left hand end. Its position genetically is 62.2. From this it will be seen that the agreement between the new cytological map and the crossover map is very close.

The writer fully realizes that the position of Bar on the X chromosome in salivary glands is very different from the position of the element in metaphase plates of oogonia or nerve cells, as described by PAINTER (1931), STERN (1931), DOBZHANSKY (1932) and MULLER and PAINTER (1932). In metaphase plates the locus of Bar is near the middle of the chromosome while in the salivary gland chromosome it occupies its normal position judged by its crossover value. The question arises, what becomes of the

inert area of the X chromosome? It certainly does not show, as we might expect it to do, from a study of metaphase plates. This question is tied up with the problem of the Y chromosome and a discussion of the various possibilities will be given in my paper dealing with the X chromosome.

It has been clearly shown in the foregoing pages that in the nuclei of female larvae (and of males as well), homologous elements may join in such intimate union as to appear as one body. That this is so is indicated by several lines of evidence. In the first place, in cells of younger larvae or less advanced cells of old larvae, we may see various stages of this union. Just when the elements begin to unite and where the process starts, are questions of interest which have not, as yet, been worked out. However, there is some evidence which suggests that the process begins at the spindle fiber end. Thus, in the case of the "Mottled 5," while the greater part of the fourth chromosome is attached to the left end of the X, nevertheless, the normal fourth tends to join with the XL-IV component, at the region of the spindle fiber although little more than the spindle fiber end of the IV element is left.

That the chromosomes of Drosophila should exhibit a tendency to lie together in the spireme stages is not surprising. The recent work of HEITZ and BAUER (1933) shows this to be the case in the nuclei of the Malpighian tubules in *Bibio hortulanus*, and it has long been known that in Diptera, in general, homologues tend to lie together in all types of metaphase plates. This fact, of itself, is an indication of an attraction between homologues, or else that some highly developed cellular mechanism is responsible. If the attraction is chemical in nature, a moment's consideration of the facts will lead us to the conclusion that it is particulate and not a general mass reaction, for not only do like chromosomes lie together, but like parts are usually apposed. The astonishing feature is, as the present study has shown, that the attraction is so strong that it brings about an apparent fusion, and so specific that like bands or areas join up even though the form of the entire chromosome must be distorted in order to bring this about, as in the inversion figures. This brings us to a second line of evidence for union, and that is the complex figure we obtain when one element of a pair carries an inverted section. Inversion figures and mutual translocation constitute, to my mind, the most convincing evidence of the intimate nature of the chromosome union in these gland cells.

From a theoretical cytological standpoint, this intimate somatic synapsis is one of the most interesting features of the present study. Heretofore, synapsis has been thought of as a unique feature of germ cells approaching maturation. The present study has shown that in salivary gland cells we have a process which is externally similar. This raises a host of questions of particular interest to cytologists and would seem to offer a new avenue

of approach to the old problem of the cause for the behavior of the chromosomes during meiosis. What is the nature of this attraction between homologues in the salivary gland? What happens to the chromosomes during pupation and in the glands of the adult? What is the purpose of the union, and when and where does it begin? Does other somatic tissue show this besides the gland cells? How extensive is the process in other insects? All these questions await investigation, and while it would be hazardous to forecast the answer it seems safe to suggest that we will have new light on the processes which lead up to the maturation divisions in germ cells.

It should be pointed out that in *D. melanogaster*, after the union of homologues, we have six elements in the cell and not the haploid number as described by HEITZ and BAUER for *Bibio hortulanus*. In principle, however, my observations agree with these authors. In *D. melanogaster*, the two arms of the large V-elements show no obvious connection, each being embedded separately in the nuclear meshwork. In reality, of course, there is a somatic synapsis of homologous elements, and the reason the haploid number does not appear, is due to this structural peculiarity of the V-shaped chromosomes.

If it should be found that in somatic cells, in general, in Drosophila, homologues are as closely associated at the interphase as in the salivary gland cells, we can easily understand the mechanics of mottling which often results when one chromosome of a pair carries a "weak spot," as a result of a translocation or other causes. (See recent paper by PATTERSON 1933 in which such cases are described and pertinent literature is cited.) For, when homologues begin to untangle in the prophase a "weak" chromosome might easily tear in two. Likewise, it is obvious that if there were crossing over between homologues during somatic synapsis, induced by irradiation (PATTERSON, 1929), or occurring sporadically in normal tissue, we would have the physical basis for true somatic segregation. In this connection it may be noted that HEITZ and BAUER report the intimate association of chromosomes in Bibio in cells of the intestine, salivary glands and brain cells, as well as in the Malpighian bodies. However, whether this is simply a case of close apposition or not cannot be shown until inversions are studied.

Another point of interest, cytologically, is the fact that the spindle fiber ends of all the chromosomes (or arms for V-shaped elements) are apparently imbedded in a common mesh work. This seems to hint that there may be a very definite structural organization in the nucleus in the metabolic ("resting") phase. One wonders what relation this meshwork or coagulum bears to the spindle fiber mechanism. When the chromosomes (or arms) are torn free from the coagulum we often see at the tip of the spindle fiber end a small ring-like structure, two in paired chromosomes, and one

such alveolar body for single elements. These probably represent the point at which the chromosomes are attached to the mitotic spindle. Such structures have been widely reported in the past decade. If the interpretation given is correct, it would follow that *D. melanogaster* arose from a species of flies which carried 12 points of spindle fiber attachment, the two arms of the second or the third chromosomes, respectively, representing originally the rod-shaped chromosomes. The work of METZ (1916) indicates that 12 is the highest number of elements found in any species of this genus. Whether or not both arms of the second (or third) chromosome now possess separate attachment points I am not prepared to say, but this appears likely. The whole question of chromosome structure will be taken up later, at which time a full review of the current literature will be given.

LITERATURE CITED

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

DOBZHANSKY, Th., 1932 Cytological map of the X chromosome of *Drosophila melanogaster*. Biol. Zbl. 52: 493–509.

HEITZ, E. and BAUER, H., 1933 Beweise für die chromosomennatur der Kernschleifen in die Knäuelkernen von *Bibio Hortulanus* L. Z. f. Zellforsh. u. mikr. Anat. 17: 67–82.

METZ, C. W., 1916 Chromosome studies on the Diptera. J. Exp. Zool. 21: 213–280.

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.

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

PATTERSON, J. T., 1929 Somatic segregation produced by X-rays in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Washington 16: 109–111.

          1933 The mechanism of mosaic formation in Drosophila. Genetics 18: 32–52.

STERN, C., 1931 Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktorenaustausche. Biol. Zbl. 51: 547–587.