THE MORPHOLOGY OF THE THIRD CHROMOSOME IN THE SALIVARY GLAND OF DROSOPHILA MELANOGASTER AND A NEW CYTOLOGICAL MAP OF THIS ELEMENT

THEOPHILUS S. PAINTER
University of Texas, Austin, Texas
Received July 31, 1934

The present article is the second of a series of three papers dealing with the morphology of the chromosomes of *Drosophila melanogaster* as these appear in the salivary glands of larvae, and to the presentation of a new type of chromosome map. As in my paper on the X chromosome, which appeared recently in this journal, the brief descriptions and the numerous figures have been presented primarily with the view of assisting other investigators in the ready identification of the various landmarks along the two arms of the third chromosome, because it is clear that study of salivary gland material is going to play an important part in the future genetic studies on Drosophila. The cytological map we are able to present now is little more than a skeleton, in spite of the fact that some 33 translocations have been analyzed. The reason for this is two-fold. In the first place, a great many of the breaks are bunched between rather narrow genetic limits. Thus out of a total of 36, 17 fall between the genetic limits of scarlet and pink. In the second place, the markers used in the original experiment were rather widely scattered and neither the time nor, in some instances, have the necessary stocks been immediately available for the finer analyses needed to determine more exactly the genetic limits of these breaks. Miss META SUCHE is making these determinations and by the time this article goes to the printer we should be able to add new data.[1] In the meantime, the morphological positions of the breaks will be indicated. No doubt many other investigators have breaks in the third chromosome, the genetic limits of which have been relatively exactly determined, and it is believed that they will have no difficulty in placing these cytologically with the aid of the topographical maps presented in this study. In this way within a few years we should have a large number of gene loci placed within narrow morphological limits.

Aside from the usefulness of the topographical maps for the study of all types of chromosome rearrangements and the problems associated with this branch of cytogenetics, perhaps the points of greatest general interest center around the question of chromosome structure. For the present study shows very clearly that the third chromosome is differentiated

[1] I have substituted for the original figures 1 and 29 new chromosome maps presenting information concerning the third chromosome as of February 14, 1935. In most instances, the position of the gene loci indicated may be taken as approximately correct and is based on additional evidence not considered in this paper.

linearly into two types of areas, which are quite different morphologically. The greater part of both arms shows what we shall term, for the moment, a banded form. This corresponds, apparently, to the "euchromatic" area of HEITZ (1932) and probably carries most, if not all, of the active genetic material. In the region of the spindle fiber, however, there is a considerable amount of material which does not show a banded organization. In oogonial metaphase chromosomes this represents not only the primary constriction, or achromatic bridge, and the spindle fiber attachment zone but an appreciable amount of chromatin lying to either side of the achromatic bridge. This probably consists of inert chromatin, similar to that which the X chromosome carries, and which shows heteropycnosis (HEITZ) at the interphase in somatic mitoses. In the salivary gland, this area lies within, or better, is a part of, the "chromocenter" (HEITZ), or what I termed chromatic coagulum in my first papers. This inert chromatin HEITZ has termed "heterochromatin," a name which will be adopted here.

The translocations described were obtained by my colleagues and associates Dr. PATTERSON and WILSON STONE and the Misses SARAH BEDICHEK and META SUCHE, from a series of rayings carried out in the spring of 1933. A brief summary of their findings has recently appeared (PATTERSON et al 1934). The genetic analyses of the third to fourth translocation have been carried out by Miss SUCHE. At this place I wish to express to my colleagues my obligation and appreciation for the use of this material.

Two points concerning the behavior of the chromosomes in salivary glands must be borne in mind. The first is that in old larvae homologous chromosomes undergo somatic synapsis. The drawings of normal chromosomes in practically all cases are made from paired elements, the pattern of the segments being essentially the same before and after synapsis. The second point is that the two arms of the third chromosome appear as separate elements, the connection between the two being masked by the chromocenter to which they are attached along with the other elements.

In earlier papers (PAINTER 1934a, 1934b) the aceto-carmine technique has been described in sufficient detail. Here we will confine our remarks to some of the factors which alter the images we get in well-stained preparations.

STAINING

One of the most vexing details is the preparation of good aceto-carmine stain. One may prepare two batches of this fixative stain, in seemingly just the same way, and find that one works much better than the other, for some unknown reason. Granted good aceto-carmine, the following points may be noted: (1) there is some variation in the degree the stain will take in different sets of larvae; (2) in lightly stained preparations we

get a greater contrast in the patterns of lines and bands but many of the finer details will not be easily visible; (3) in old slides, or deeply stained preparations, the lines and bands of the faintly staining "achromatic" areas become much more prominent and, in general, there is much less contrast.

DISTORTIONS

These are due, presumably, to the mechanical effects of crushing nuclei in order to separate the chromosomes. It must be remembered that all elements are anchored to the chromocenter which lies on one side of the nucleus (PAINTER 1933a) and when the nuclear wall is broken, this connection is usually retained. The length of time of fixation prior to crushing may enter here. Distortions express themselves in a number of ways: (1) the breaking of chromosomes or arms, (2) twisting and bending at sharp angles, (3) local stretching when stress is thrown on a short area, an effect most often seen in the segments close to the chromocenter, (4) mashing and (5) telescoping or crowding together of adjoining segments. This last is always to be reckoned with because it results in the close apposition of a number of bands forming broad deeply staining areas, not usually seen. In general, areas with heavy chromatic bands are less subject to distortion than relatively achromatic segments.

Throughout this series of papers we speak of the chromosomes as being made up of segments, but a word of explanation should be inserted here. The shallow constrictions which bound the segments are sometimes very conspicuous and constant but in other areas they may be more variable in expression. At the outset the investigator will do well to pay more attention to the pattern of lines and bands.

In making the topographical charts of the left and right arms the same procedure has been followed as in my study of the X chromosome. Each segment is based on a camera lucida drawing of some chromosome, the whole chart being made by putting these typical segments together. The main emphasis has been placed on the pattern of the lines or bands, as these appear in a moderately stained preparation, together with the typical length and breadth of the segment, as I have observed it. In a study involving as much detail as the present one, the author can not hope to have escaped making some errors in the emphasis placed on the lines of some segments and it is to be expected that as limited areas are subject to intensive study the typical pattern may be altered somewhat, especially with regard to the finer details. However, since no segments on the type chart have been inked in without viewing at the same time a good preparation of the area involved under the microscope, the reader may be sure that something was seen at the point indicated even though its relative size or degree of staining may be incorrectly represented.

In the study on the X chromosome individual segments were designated by the names of the genes they were thought to contain. In the case of the third chromosome this information is so fragmentary that it has seemed desirable to adopt a temporary designation for landmarks. The genetic crossover map is represented above the topographic map and it is convenient to refer the various landmarks to the scale of the former. It must be understood that we do not mean to imply that a segment which lies under 70, for example, on the crossover scale necessarily carries the gene for sooty.

The point of spindle fiber attachment has been commonly assumed to be close to 48 crossover units from the left-hand end. So on the crossover scale the left arm shows 48 units and the right 57. We have thus disregarded the size of the spindle fiber region and the inert material to either side of it although in the oogonial metaphase this represents possibly 1/8 to 1/10 of the total volume of the entire chromosome. The crossover value of various genes has been taken from the Drosophila Information Service Number 1, March, 1934.

THE NORMAL TOPOGRAPHY OF THE LEFT ARM

Figure 1 shows the typical morphology of the left arm of the third chromosome. Figure 2 is a camera lucida drawing of this arm as it appeared in one of my preparations. With the aid of the numbers (from the crossover scale) placed along the latter, the reader will have no difficulty in identifying the various segments.

The most easily identified landmark of this arm is the right-hand terminal segment lying at the right in figure 1. This, the spindle fiber end, is very blunt and is usually more or less covered by a part of the deeply staining granules of the chromocenter to which this arm is anchored. A detailed description of this latter region will be given later in this paper. At the right-hand tip there are three heavily stained bands, one or more of which may be concealed by the chromocenter. To the left (roughly below 46 on the crossover scale) is a clear area traversed by faintly staining bands. It is this clear area with its faint bands lying between heavily stained sectors which usually catches the eye. Next comes a set of three broad deeply staining bands, the two outside ones being especially heavy while the middle one is lighter. Then comes a double and then a narrower band followed by two faintly staining bands. The segment is bounded at the left (below 44) by three narrow but sharply staining bands. A comparison of the chart with the terminal segments of figures 2 to 5, will show these features, as well as a number of the translocation figures (for example, figures 15, 21, 25, 26 and 28).

The next outstanding feature is a series of relatively achromatic seg-

ments lying in the region below about 30 to 38 on the crossover scale. Beginning with the narrow collar below 38, in figure 1, we have two more or less swollen segments bounded at the left by three heavy bands. A narrow heavily staining line separates these two segments which individually show very faintly staining lines. These two segments show a striking variation in diameter, though within an individual I am under the impression that the form is fairly constant. In some larvae they may be as much as three times the average diameter of the element, when, of course, they are very conspicuous, in other larvae they do not exceed the normal diameter by very much. Figures 1 and 2 show the usual size, in 3 and 5 the segments are relatively narrow while in figures 6a to c we have examples of enlarged segments. When I first began to study the salivary glands of larvae, enlarged segments were the rule but within recent months I have encountered fewer cases.

The next three segments are relatively achromatic and while not so variable as to width, seem to be structurally weak for the arm bends here more frequently, perhaps, than elsewhere and telescoping of segments is rather frequent.

The next outstanding landmark is a swollen segment lying at about 21 under the crossover scale. This is, ordinarily, the second most striking feature of this arm. Across the point of greatest diameter is a band of faintly staining lines. Figures 2 and 7 show this feature in typical form.

Lying to the left of 21, we have a series of segments, part of which showing heavily stained bands and others are more or less achromatic. None of these is outstanding nor easily picked up, and breaks in this region have to be carefully identified by beginning either at the left-hand end or else with the enlargement at 21. The left-hand end itself is not especially easily recognized until one becomes quite familiar with it. Figures 2, 8, and 9 show characteristic drawings. The bands of the terminal bulb stain lightly. This segment is constricted, at the right by two narrow but deeply staining bands, then come some rather broad diffuse bands followed by a heavily stained line. This description may be concluded with the remark that often the lines at the left end, which I have represented as diffuse and rather lightly staining, may be quite prominent in heavily stained slides.

THE NORMAL TOPOGRAPHY OF THE RIGHT ARM

This arm is the longest element in the nucleus and one of the easiest to identify by either the spindle fiber or the right-hand free end. In spite of this, it has proved to be the most difficult of all the elements for which to present a typical morphological map, due principally to the great length and the attendant distortions especially in the mid reaches. Added

EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida at table level. For salivary gland chromosomes a magnification of 900 diameters at the eye piece was used. Oogonial chromosomes were drawn either with a magnification of 2400 or 1800 diameters at the eye piece. The drawings have been reduced about a third in the plates. Throughout this paper I have used our local terminology for the III to IV translocations. Recently my colleagues have adopted a new set of symbols. Translocation VI 1, for example, is now written TA 3-4, 1 which means, a translocation found at Austin, Texas involving the third and fourth chromosome, No. 1. In similar manner VI 36 would be written TA 3-4, 36, and so on.

FIGURE 1.—This is a cytological map showing the typical morphology of the euchromatic portion of the left arm of the third chromosome. The scale above the figure is a crossover map. Below the figure the numbers refer to the various translocations studied. Lines shown from the crossover scale to the map show the limits of various gene loci.

FIGURE 2.—A drawing of the euchromatic portion of a left arm which was intact. The numbers placed along the drawing will assist the reader in identifying the various landmarks on the cytological map (figure 1).

FIGURES 3 to 5.—Presented to show the topography of the spindle fiber end of the euchromatic area.

FIGURES 6a to 6c.—Presented to show variations in the size of the achromatic enlargements lying between about 34 and 37 under the crossover scale.

FIGURE 7.—This is a figure of the spindle-like enlargement lying under 21 on the crossover scale.

FIGURES 8 and 9.—These are drawings presented to show the topography of the left-hand end of the left arm.

FIGURE 10.—This is a drawing of the left-hand end of the left arm of a larva heterozygous for translocation VI 34. The normal end of the synapsed homologs is shown to the left and below while the fourth chromosome, which has replaced the normal tip of one element, is shown above.

FIGURE 11.—This figure is from a larva heterozygous for the VI 22 translocation. The normal component lies to the left while the element carrying the tip of the fourth chromosome is shown to the right.

FIGURE 12.—This drawing was taken from a larva hyperploid for the VI 52 translocation, and shows the III L-IV component, not all details drawn.

FIGURE 13.—This is a drawing taken from a larva heterozygous for translocation VI 23.

FIGURE 14.—From a larva heterozygous for translocation IV 13.

FIGURE 15.—From a larva heterozygous for translocation VI 12. The aberrant element with its tip from a fourth chromosome lies below synapsed with the normal homolog above. The dotted lines to the right in the figure shows where another element crossed.

FIGURE 16.—From a larva heterozygous for translocation VI 8. The aberrant III L-IV element which received its spindle fiber attachment from a fourth chromosome is shown above to the left. The tip of the fourth which covers the broken end of the left arm lies to the right, and is somewhat mashed out.

FIGURES 17 and 18.—From individuals heterozygous for translocation VI 56. Figure 17 shows the salivary gland chromosomes while figure 18 is from a dividing oogonium.

FIGURES 19 and 20.—Showing two drawings taken from larvae heterozygous for translocation VI 14.

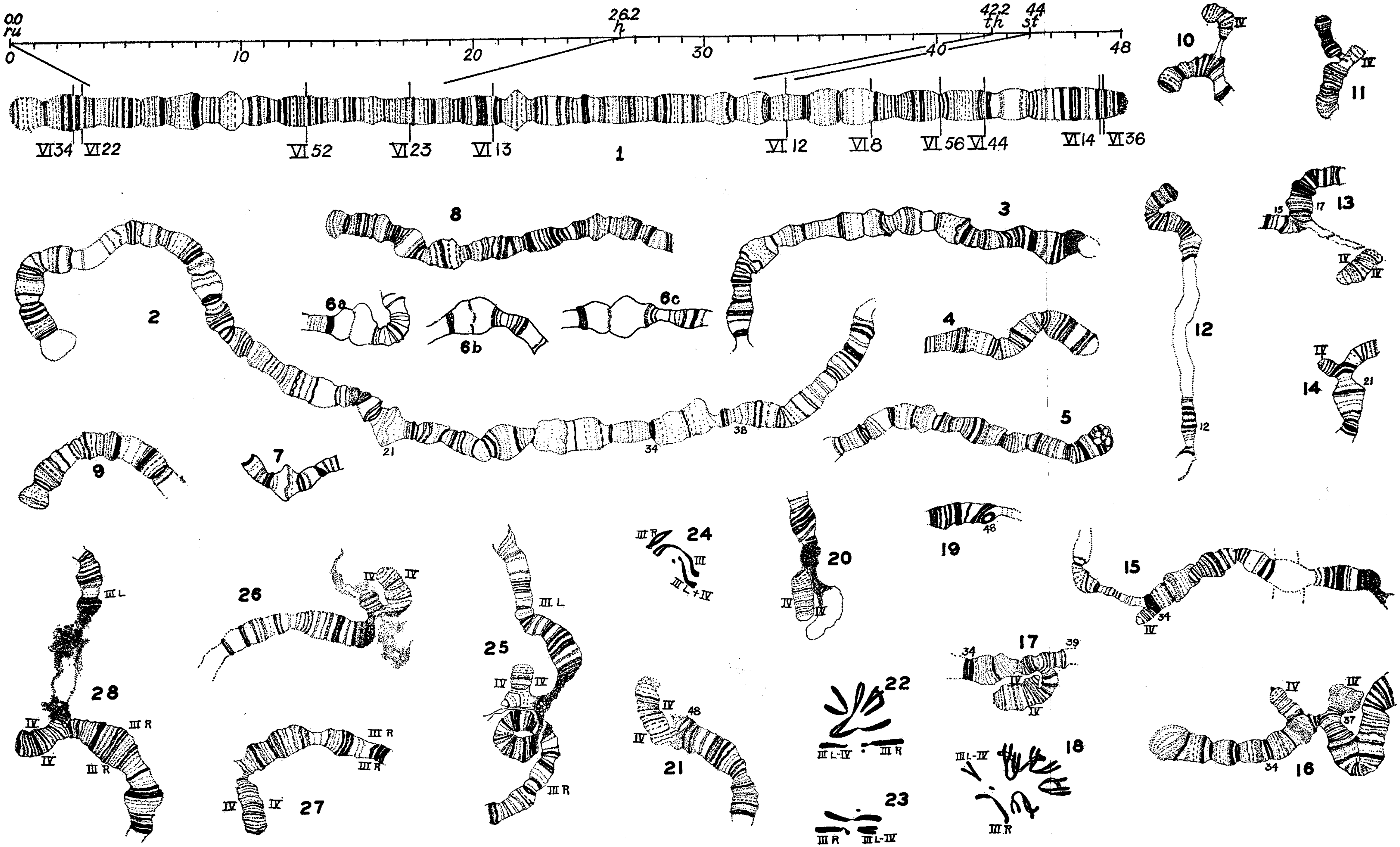
FIGURES 21 to 23.—From individuals heterozygous for translocation VI 36. Figure 21 is from the salivary gland, and figures 22 and 23 show the third chromosomes in dividing oogonium.

FIGURES 24 and 25.—From individuals heterozygous for the VI 45 translocation. The condition of the third chromosome, as these appear in dividing oogonia, is shown in figure 24. Figure 25 was taken from the salivary gland.

FIGURE 26.—From a larva heterozygous for translocation VI 31.

FIGURE 27.—From a larva heterozygous for translocation VI 4.

FIGURE 28.—From a larva heterozygous for translocation VI 27.


0.0
ru
26.2
h
42.2
th
44
st
0
10
20
30
40
48
VI34
VI22
VI52
VI23
VI13
1
VI12
VI8
VI56
VI44
VI14
VI36
2
3
4
5
6a
6b
6c
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
III L
III R
IV
III L-IV
III L+IV
21
34
38
48
12
15
17
37
39

factors are a tendency of certain regions to telescope or stretch and a marked similarity of the patterns of several segments which lie close together.

We will begin with the spindle fiber end which lies to the left in figure 29 (plate 2). A camera lucida drawing of an entire arm is shown in figure 30. The numbers placed along this figure refer to the crossover scale of figure 29.

The right arm tapers rather sharply at the end which is attached to the chromocenter and then broadens out into a series of three or four ill-defined and relatively achromatic segments. Then it narrows again at a point below about 52, on the crossover scale. This club-like achromatic end is one of the outstanding morphological features of this arm and since it often pulls free from the chromocenter, it is frequently seen. In some preparations the paired and synapsed homologs show a bifurcated tip (figures 31–33); at other times there are two ring-like vesicles at the end, as in figure 30. Where the latter were first observed, I was struck with their similarity to the "insertionslücke" which BELAR (1929) figured for Chorthippus and I was inclined to interpret them as the points of spindle fiber insertion. As it turns out, however, these structures are simply the stumps of the protoplasmic strands (viewed head on) which run into the chromocenter and serve, directly or indirectly, to connect the two arms together (see p. 315).

The next outstanding feature is an enlarged spindle-shaped segment lying between 62 and 63. This is a very useful landmark and various drawings of it are shown in figures 30, 31, 34 and 40. From this segment on to about 71 we have a series of heavy bands bounding more or less achromatic segments which seem to be structurally weak as they often stretch a good deal and thus make the appearance of this area quite variable. Here the investigator should follow the pattern of the bands very closely. Beginning about 71 we have two segments which are also apt to stretch or telescope and often show little sign of the constriction figured for the typical arm. These are followed to the right (75–77) by a narrow area which leads up to a segment with a very striking pattern. The features which catch the eyes first are the heavy bands lying below 77 and a trifle past 79. This segment tapers to a constriction just below 80 on the scale.

The next two segments are also very characteristic, the sequence of the heavy bands of the segment lying below 82–83 being outstanding. Note the sequence of two broad, one narrow and then one broad heavily stained band. Sometimes, as in figure 30, these bands appear to bound short achromatic segments.

Beginning between 87 and 88 we again have a section showing two

broad, one narrow and one broad band, and care must be taken not to confuse this area with that lying below 83. Between these heavily banded segments is a relatively achromatic section which, for some reason, seems to be little subject to distortion, and its pattern can generally be observed.

Below 90 to 94 is a lightly stained area which is very useful as a landmark because the narrow bands are in series of three. From 94 to the right we have a number of ill-defined segments with many heavy bands. The details of the pattern are shown in figure 29. Note especially the pattern on either side of the VI 30 break. To the left of this break we have three thin but sharply staining lines, and to the right four conspicuous bands with the sequence of one broad, one narrow and two heavy bands.

The outstanding landmark of the free end of the right arm is between 103 and 104 and consists of four conspicuous bands in the order, beginning at the left, of one broad, one narrow and then two broad lines.

TRANSLOCATIONS

Four general types of translocations have been found during the course of this study. (a) In the majority of cases we have mutual exchanges between the euchromatic or banded areas of one or the other arm of the

---

FIGURE 29.—A cytological map of the euchromatic area of the right arm of the third chromosome showing the typical morphology as this appears in salivary glands. The crossover scale is above and the translocations studied are indicated below the figure.

FIGURE 30.—A drawing of the euchromatic area of a right arm which was intact.

FIGURES 31 to 33.—Three drawings of the spindle fiber end of the right arm.

FIGURE 34.—Presented to show, in more detail, the character of the enlargement which lies under about 62–63 of the scale of figure 29.

FIGURES 35 to 37.—These are drawings to show the morphology of the right-hand tip of the right arm.

FIGURE 38.—From a larva heterozygous for translocation VI 37.

FIGURE 39.—This drawing is from a larva heterozygous for translocation VI 9, with a few details omitted. The fourth chromosomes are mashed out and appear unduly broad.

FIGURE 40.—From a larva heterozygous for translocation VI 1. This figure is discussed in detail in the body of this paper (see p. 309).

FIGURE 41.—From a larva heterozygous for translocation VI 20.

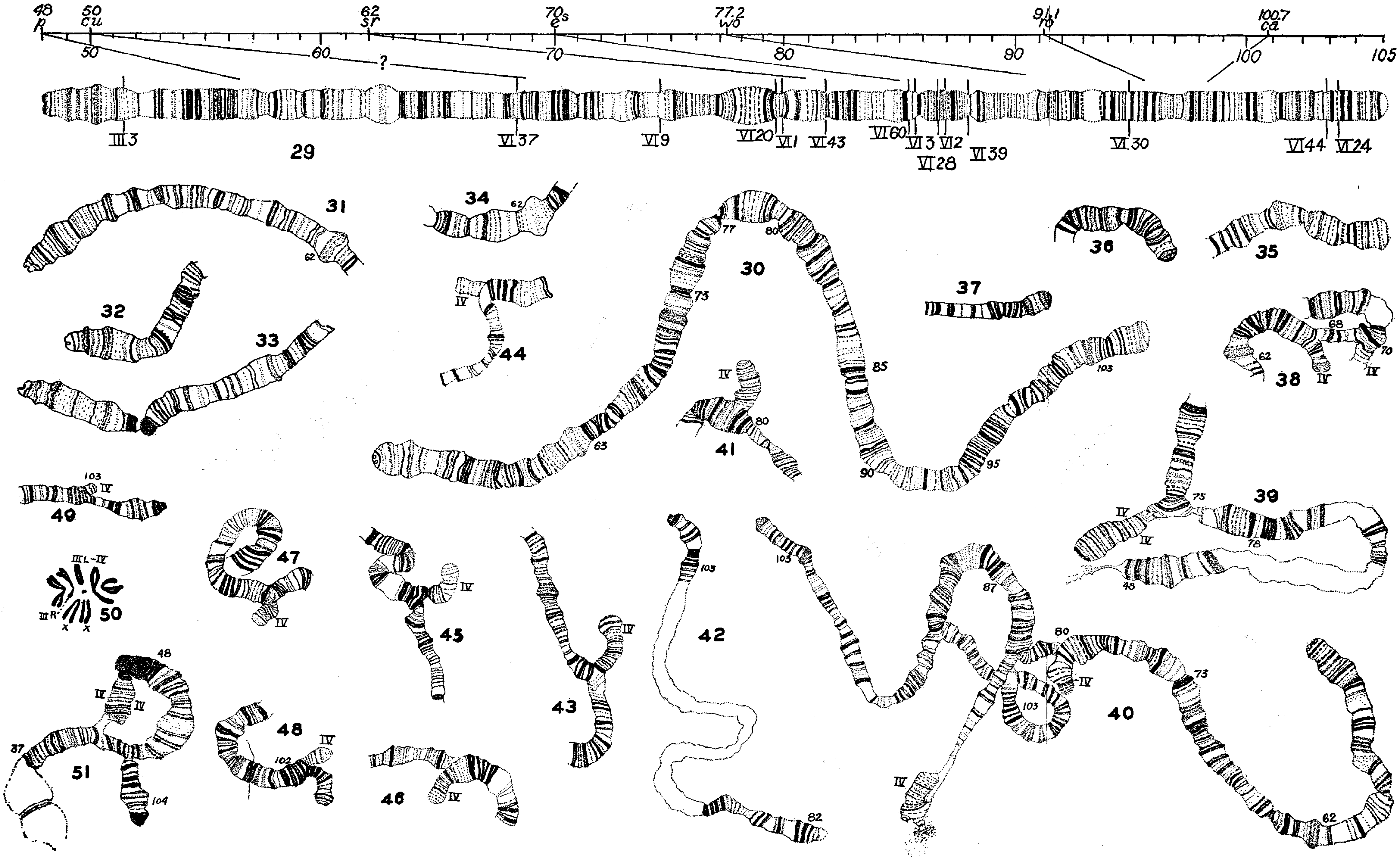
FIGURE 42.—This drawing was taken from a larva hyperploid for translocation VI 43. Most details have been omitted from the III R–IV segment.

FIGURES 43 to 46.—Various breaks in the right arm taken from larva heterozygous for the translocation. Figure 43 is from translocation VI 60, figure 44 is from VI 28, figure 45 is from VI 2 and figure 46 is from VI 39.

FIGURE 47.—From a larva heterozygous for translocation VI 30.

FIGURE 48.—From a larva heterozygous for translocation VI 24.

FIGURES 49 to 51.—These figures are taken from larvae heterozygous for translocation VI 44. Figure 49 shows the condition of the right end of the right arm. Figure 50 is an oogonial metaphase, while figure 51 shows the normal and aberrant left arms. The case is described in detail on p. 314.


48 p
50 cu
62 sr
70s e
77.2 wo
91.1 ro
100.7 ca
50
60
70
80
90
100
105
?
III 3
VI 37
VI 9
VI 20
VI 1
VI 43
VI 60
VI 3
VI 2
VI 28
VI 39
VI 30
VI 44
VI 24
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
IV
III L–IV
III R
X
X

third chromosome and a fourth chromosome. As a result the distal (to the spindle fiber) part of the arm receives its spindle fiber attachment from the fourth with more or less of the euchromatin of the fourth, and the proximal part of the arm has its stump covered by the free end of the fourth. In oogonial metaphase plates, the distal part of the arm with its spindle fiber from the fourth appears as a rod-shaped chromosome. Such cases were studied by the salivary gland method in larvae heterozygous for the aberration. (b) There are a number of cases, however, in which one or the other arm of the third chromosome is replaced apparently by an intact fourth chromosome forming a j-shaped element. That is to say, in some way the spindle fiber end of the fourth becomes attached to the spindle fiber end of one arm, while the displaced arm acts as a free chromosome. In such cases the salivary glands show, as a rule, the normal configuration of all elements being attached separately to the chromocenter, but in oogonial metaphases the j-shaped compound chromosome and the displaced rod-like arm are readily identified. For this reason, most of the translocations falling between the loci of scarlet and pink were first studied in oogonia of female flies heterozygous for the exchange. (c) A few cases have been encountered in which a piece of one arm of the third becomes attached to the spindle fiber end of a fourth which is, so far as I can see, morphologically intact. In such cases, in salivary glands, we find the piece of the arm attached apparently to the fourth chromosome. (d) There is one case in which the tip of the right arm became translocated to the stump of the left arm, the distal end of the latter being translocated to a fourth chromosome. Such cases are easily analyzed by the salivary gland method but in oogonial divisions are unintelligible.

Due to somatic synapsis, in heterozygous larvae homologous parts tend to unite, just as they do at meiosis, and by comparing the patterns of the normal and the aberrant elements, one can tell very exactly just where the latter is broken. As I shall discuss in detail elsewhere, somatic synapsis seems to be a progressive process, and in any given cell it may not have been entirely completed. Figure 40 of plate 2 is presented to show the nature of the material with which we are dealing. This represents the right arm of the third chromosome in a larva heterozygous for a mutual exchange with a fourth chromosome, the break falling between the loci for the genes curled and stripe. This is the VI 1 break shown near the middle of the right arm in figure 29. Beginning at the spindle fiber end, which lies to the right in figure 40, the reader will have no difficulty in identifying the outstanding landmarks of this arm. The normal and the aberrant elements, beginning at the spindle fiber end of the euchromatic area, have united completely up through the conspicuous segment which lies at about 80. Here the two diverge and the lower element ends, showing

the pattern of about three-fourths of the fourth chromosome. The break in the arm originally was obviously just to the right of the two very heavy bands which lie close to 80 in figure 29. The normal and the spindle fiber end of the broken fourth chromosomes show in the lower part of figure 40 to the left. Here it will be noted that the two are synapsed for about a fourth of the length of the normal fourth (which lies on the left) and then the element at the right joins the normal right arm with which it synapses for some distance. At about 90 on the chromosome map (figure 29) the two elements diverge again, that is, they failed to synapse although the parts are homologous.

This is a fair illustration of the type of preparation from which the position of breaks have been determined in the following sections. In most of the other translocation figures only short sectors of the normal and aberrant arms have been drawn. In a few cases, as noted, the III L–IV component (or III R–IV) is shown by itself.

Translocations in the euchromatic area of the left arm

The topographical chromosome map (figure 1) shows that eleven breaks, accompanied by translocations, have occurred in the banded or euchromatic portion of the left arm. These will be considered in turn, beginning at the extreme left.

VI 34 and VI 22. Both of these translocations broke the left arm in the subterminal segment, as figure 1 shows. Figure 10 is from a larva heterozygous for VI 34 and may be of interest because it shows that all of the banded area of the fourth chromosome is attached to the proximal portion of the left arm. That is to say, the exchange between the fourth and the left arm broke the fourth beyond the banded portion of the latter but still distal to its spindle fiber attachment zone. Such cases have been frequently observed. In figure 11, showing the VI 22 translocation, the normal element lies to the left while the aberrant arm with its cap of fourth chromosome material is on the right. The two elements are synapsed below the point of breakage. Genetically both the VI 22 and VI 34 are to the left of roughoid (0.0) so that this gene must lie to the right of the points of breakage, as figure 1 shows.

VI 52. Figure 12 was taken from a larva which was hyperploid for this aberration, the duplicated piece being the left-hand tip with its spindle attachment from the fourth chromosome. The left arm is broken at about 12, as shown by figure 1. Genetically the left arm is broken between roughoid (0.0) and hairy (26.2) and thus the locus for roughoid must lie somewhere between the VI 22 and the VI 52 break.

VI 23. Figure 13 shows the normal homolog above and running to the left, while the aberrant element is drawn out to the right because its

fourth component has synapsed with the normal fourth. Here again it is to be noted that the fourth chromosome was broken between the euchromatic portion and its area of spindle fiber attachment. Genetically, the breeding data indicate that this break is very close to hairy so we can place the locus of this gene close to the line marking the VI 23 break in figure 1.

VI 13. Figure 14 shows that the left arm was broken close to 20 on the crossover scale of figure 1. Genetically, this break is between the gene loci of hairy (26.2) and thread (42.2); thus hairy must lie to the left and thread to the right of it.

VI 12. Figure 15 shows that the left arm is broken just to the left of the three heavy bands shown between 33 and 34 of figure 1. Genetically this break is between thread (42.2) and scarlet (44).

VI 8. This translocation also falls between the genetic limits of scarlet and pink. The point at which the left arm was broken is shown by figures 16 and 1.

VI 56. Genetically, the preliminary tests indicate that this break came between scarlet and pink. In salivary gland material, as figure 17 shows, the left arm is broken a trifle past 40 on the crossover scale of figure 1. The oogonial metaphase plates of females heterozygous for this translocation are of extreme interest. Figure 18 is almost a prophase stage taken from an attached-X female. The normal and broken third chromosomes are shown below and to the left in the figure. Note, that the III L-IV element is much shorter than the normal arm, and, in turn, that the broken third is much prolonged beyond the primary or spindle fiber constriction, in other words, the truncated left arm shows a length of about a fourth of the total length of the arm. If the genetic analysis proves to be correct, this means that the locus for scarlet must lie much farther away from the spindle fiber, in oogonial chromosomes, than its crossover value would indicate. Incidentally, the truncated left arm shows the secondary constriction described by DOBZHANSKY and others.

VI 44. This is a complicated case which will be described along with breaks in the right arm. On figure 1, the point at which the left arm was broken is shown between 41 and 42. The (limited) genetic data indicates that this break is between scarlet and pink.

Translocations VI 36 and VI 14 are of extreme interest. Genetically they broke the third chromosome between scarlet and pink, but morphologically the break comes right at the border line between the banded or euchromatic area of the left arm and the region of the chromocenter. A study of oogonial metaphases should show us, therefore, how much of a contribution to the left arm is made by chromocenter material. Figures 19 and 20 show the right-hand tip of the synapsed normal and aberrant

arms of VI 14. In figure 19, it will be observed that only two of the three heavy bands are shared in common, the normal component, which is below, shows three. This means that the left arm broke between the second and third band with which the euchromatic area terminates. Figure 20 shows the connection with the fourth chromosome at the end distal to the spindle fiber. Figure 21 shows, likewise, that in VI 36 the left arm is broken between the second and third of the heavy terminal bands and that one component of the synapsed arm is attached to the distal end of a fourth chromosome which, in turn, is synapsed with a normal fourth. Figures 22 and 23 are oogonial metaphase plates of flies heterozygous for the VI 36 translocation. It will be noted at once that there is a considerable amount of chromatin beyond the spindle fiber constriction of the broken third. This chromatin represents something like a sixth or an eighth of the total length of the left arm and must be derived almost wholly from the chromocenter region. Oogonial division of VI 14, which I have not figured, shows essentially the same conditions as VI 36.

Translocations occurring within the chromocenter region

The exchanges occurring in the region of the chromocenter are of two general types. In the first type, one arm of the third is simply replaced by a fourth chromosome. As far as I can determine, all elements are morphologically intact and the new association of one arm of the third with a fourth is at the spindle fiber ends of the two elements. As a result, in oogonia we find the broken third represented by a rod-shaped arm of normal length, and a j-shaped element, the short arm of the j being formed by the fourth chromosome. Two cases of this sort are illustrated by the figures, altogether seven cases of this sort have been studied.

VI 45. Figure 24 shows the condition of the third chromosomes in a female heterozygous for this exchange. The several elements are labelled. It will be noted that the fourth attached to one arm of the third (in this case the left) is the same size as the normal fourth. In salivary glands (figure 25) we find the paired right and left arms synapsed up to the point at which they enter the chromocenter. The two fourth chromosomes which lie between these ends are synapsed for a greater part of their length but they diverge at the spindle fiber ends, one of which enters the chromocenter close to where the left end is attached. Morphologically, the two fourths and the synapsed arms of the third are entirely intact. It is clear, therefore, that the association between the left arm and the fourth has been established within the chromocenter. Figure 26 shows the condition of the salivary gland in translocation VI 31. Here again we see the two synapsed left arms, which are intact connected by a strand to a mass of chromocenter material to which one of the two paired fourths

is attached. Oogonial plates of other translocations of this sort are shown in text figure B (p. 319).

There are two translocations occurring in the region of the chromocenter, for which the genetic data indicates that the break is close to pink, or is on the opposite side of the spindle fiber attachment zone from the cases previously considered. Figure 27 shows the salivary gland condition of larvae heterozygous for VI 4. Here it will be noted that the euchromatic area of one of the two (paired) right arms is attached to the spindle fiber end of one of the (paired) fourths. In oogonial plates of flies heterozygous for this translocation (see VI 4, text figure B) we find an unexpected situation. The figure is taken from a female of the attached-X stock. The broken third chromosome is represented by two j-shaped elements. One of these is obviously formed by the union of a normal-sized fourth with the right arm, while the left arm has a considerable amount of chromatin lying beyond the spindle fiber zone. This can only be interpreted in one way. The association between the right arm and the fourth is just at the edge of the euchromatic zone of the former, and the knob of chromatin attached to the left arm represents, simply, the contribution which the chromocenter makes to the right arm in metaphase plates. Translocation VI 27, as figure 28 shows, is another example of this same sort, and oogonial plates show essentially the same condition found for VI 4.

Translocation in the euchromatic area of the right arm

The genetic data from translocations VI 31 and VI 27 described above indicated that they broke the third to the left but relatively close to pink (48). The salivary gland material indicates that the exchange was on the edge of the euchromatic area of the right arm, hence the locus for pink must be farther to the right. The only other break close to the spindle fiber region of the right arm is one known as X-IV, III, 3. It is a complicated case involving an apparent fusion of the X and a fourth chromosome and the association of a short piece of the right arm of the third with them. This case is dealt with at length in a joint paper with Mr. WILSON STONE (now in press). The point which concerns us here is that the fragment of the right arm of the third broke off a trifle past 51 on figure 29. When this piece (from 48 to 51) was tested genetically by Mr. STONE, it was found not to cover pink, so the locus of the latter must lie to the right of 51 on figure 29.

VI 37. This is a complex case which has not been worked out in all respects, but the genetic evidence indicates that the right arm broke between pink (48) and curled (50). Figure 38, taken from a heterozygous larva, shows that the right arm is broken close to 68 on figure 29. The locus for pink therefore lies somewhere between 51 and 68, on the topographic map (figure 29) and curled must lie to the right of 68.

VI 9. Genetically this break falls between the loci of curled (50) and stripe (62). Morphologically as figure 39 shows the break is somewhat beyond 74 on figure 29. In this case (figure 39) the paired fourths are mashed out and hence appear unduly broad.

VI 20 and VI 1, both broke the right arm close to 80. Figure 40 is a drawing from VI 1, already described, and figure 41 is taken from VI 20. Both of these breaks are between curled and stripe.

VI 43. As this is written the genetic evidence indicates that the locus of stripe (62) is very close to this break. Morphologically (figure 42 taken from a female hyperploid for the III R-IV piece) the break occurred close to 81 on figure 29.

We now have five different translocations which are genetically all beyond sooty (70) which broke the right arm in nearly the same place (between 85 to 88+ on figure 29). VI 60 is shown in figure 43. VI 28 is shown in figure 44. VI 2 in figure 45 and VI 39 in figure 46. No figure is given for VI 3. The locus for sooty must lie to the left of about 85 on figure 29.

VI 30. This translocation falls genetically between the loci for white ocelli (77.2 and rough 91.1). Figure 47 shows that this arm is broken close to 95, on the topographical map.

VI 24. This translocation broke the right arm beyond the locus for claret (100.7). Figure 48 places the morphological position of this break a little beyond 103 on the topographic map (figure 29).

VI 44. This is a complex translocation which will be dealt with in some detail. When the case was first studied cytologically, the genetic evidence was limited to the fact that crossing over was reduced in the left arm. When the salivary gland nuclei were examined almost the first cell examined showed the condition given in figure 49. Obviously, the right arm is broken close to 103 on the topographic map. For the time being no further cytological analysis was made, but later oogonial divisions of heterozygous flies were examined and revealed the condition shown in figure 50. It appeared as if the right arm was broken close to the spindle fiber region and that the III R-IV piece was about three-fourths the length of the normal right arm. The salivary glands were again studied and figure 51 tells the story. Here we see attached to a segment of the left arm the portion of the right arm missing in figure 49. At the same time, the remaining portion of the left arm (distal to the break) is translocated to a fourth chromosome. This condition explains, of course, why crossing over is reduced in the left arm. Recent genetic tests by Miss SUCHE indicate that the break in the left arm is between scarlet and pink. This case is of great interest not merely because of its complications and the demonstrated value of the salivary gland method, but it should put us

on guard against hasty conclusions drawn from oogonial chromosome studies.

THE CHROMOCENTER REGION

In the nuclei of salivary gland cells there is an amorphous mass of chromatic material to which all chromosomes or arms are attached. This mass is termed a chromocenter by HEITZ (1929) and according to this author is formed by the fusion of the heteropycnotic chromatin carried by some of the chromosomes. HEITZ (1933) has shown for *D. melanogaster* that during the interphase of somatic mitoses the Y chromosome (when present), the proximal half of the X, and small sectors of the second and third chromosomes adjacent to the spindle fiber region, do not become diffuse following the telophase but remain condensed and deeply staining (heteropycnotic). It is this "heterochromatin" which unites to form a "sammelchromocentren" or chromocenter for short.

In aceto-carmine preparations the chromocenter appears to be made up of coarsely granular and fibrous material in which there are many apparent vacuolated spaces. It stains deeply and seems to be one continuous mass.

In any given nucleus the position occupied by the spindle fiber ends of the chromosomes or arms seems to be largely the chance result of how these happened to lie with relation to the plane of the cover glass when the nucleus was mashed out and for this reason contiguity is of little significance. There is, however, some evidence which points to a definite orientation of the chromosomes about the chromocenter. If the material of the chromocenter is more or less broken up, in mashing the nucleus, it often happens that the two arms of the third chromosome will be found attached to the same piece. More frequently, however, one or the other arms will be found attached to the same bit of substrat to which the fourth chromosomes are anchored. This condition is extremely common and suggests that the points where the two arms of the third are attached to the chromocenter is close to where the fourths normally are inserted. The fourths being relatively very short are less apt to be displaced than the longer elements.

When all the elements are anchored to the chromocenter there is no hint of any direct connection between the two arms of either the second or third chromosome. It is only when an arm is pulled away from the chromocenter that we have an opportunity to determine the nature of the connection. The image we get depends largely on the age of the preparation. In slides a day or two old the blunt end of the left arm almost invariably shows a good deal of the chromocenter material adhering to it (text figure A1) and this may be strung out for some distance. The connection must be a very intimate one, but one gains the impression that the chromocenter material is simply adhering to the outside of the tip,

just as would happen if a match stem were inserted into some sticky substance and then withdrawn (text figure A, also figures 15, 20, 25, 26 and many others). On the other hand, the connection between the right arm and the chromocenter must be a very loose one because it seems to pull free easily and usually none of the chromocenter material adheres to it.

If now, we examine a slide a few minutes after staining, in favorable cells where an element is pulled away a little from the chromocenter, we can observe arising from the ends of the paired homologs of all the elements, usually two thin and achromatic strands of protoplasm. After a few hours this connection usually dissolves or breaks, a fact which was

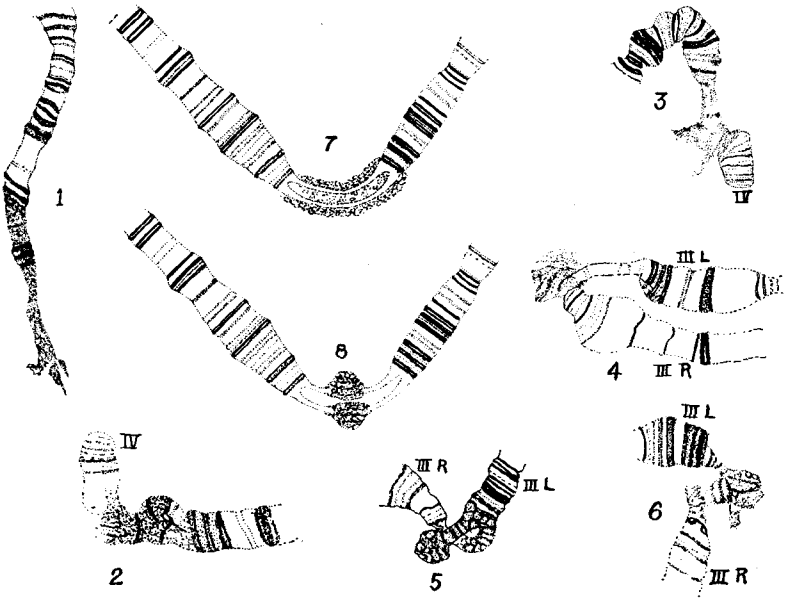


FIGURE A.—Figures A-1 and 2 show the close association between the euchromatic area of the left arm and the material of the chromocenter. Figure 3 shows the achromatic strands connecting the right arm to the chromocenter. Figures 4, 5 and 6 show how the left and right arms may be connected together. Figures 7 and 8 are diagrams to illustrate two possible ways of interpreting the nature of the connection between the two arms of the third chromosome.

accidentally discovered when an especially good illustration was left over night before drawing. Text figure A2 shows a typical case for the left arm and A3 a typical case for the right arm and it will be noted that the points of insertion are near the paired fourth chromosomes. In these and some of the following illustrations it is difficult to represent the achromatic character of these protoplasmic strands. They are usually so transparent as to show only with reduced lighting, but sometimes they show a faint suggestion of banding or they may be more or less covered by the granules of the chromocenter. In the latter event the granules seem to be adhering

to the outside of the strands. Text figure A4 is an interesting illustration. We see arising from a mound at the tip end of the left arm, a single transparent strand which runs to the base of the right arm, but which does not connect directly with it, so far as I can observe. At the end of the right arm (to the left) there is a mass of chromatic material which I interpret as the remains of the other strand which has broken (we are dealing with synapsed homologs) and some material from the chromocenter. Text figure A5 shows two processes, in this case apparently covered with chromatic granules, arising from the ends of the synapsed left arms and running to and connecting with the synapsed right arms. Text figure A6 shows the two arms connected to a common mass of deeply staining material. In addition to these figures, as we glance through the plates, we find further evidence. It is rather common to find lying between the attached arms an enlarged area which is chromatic. Figure 26 illustrates a case of this sort.

What has been described for the two arms of the third applies to all the other elements. Both the X's and the fourths are anchored to the chromocenter by narrow and usually transparent strands. From these observations it is clear that narrow and more or less transparent strands of protoplasm serve to anchor the banded portions of the elements to the chromocenter, and the conclusion seems warranted that these strands form, in part at least, the achromatic bridge between the arms seen in prophase and early metaphase stages. There is, however, associated with these strands some of the deeply staining chromocenter material. In text figures A7 and A8 two possible interpretations are shown diagrammatically. In A7, the two clear strands are shown connecting the two paired arms with a coating of chromocenter material on the outside. This interpretation would seem to fit well with text figures A1 and A4. In text figure A8, the chromocenter material is shown more or less localized or enclosed in a sac-like enlargement. This interpretation is suggested by text figures A2, A3, A5 and figure 26. In any event the main point to be stressed is that the banded or euchromatic area ends sharply and that the chromosome or arm is organized differently in the region adjacent to the spindle fiber. The character of the spindle fiber attachment zone, which lies within the chromocenter, is not known, at the present time.

DISCUSSION

Cytological and crossover maps

My study of the X chromosome showed a rather close correspondence between the morphological position of gene loci, on the salivary chromosome, and the crossover maps except at the extreme left-hand end (where gene loci are much farther apart than their crossover value would suggest,

due perhaps to the terminalization of chiasmata). Thus a break falling between lozenge (33.7) and vermilion (39.7) was actually 34 morphological units from the left-hand end, and the same general relationship is shown by other breaks. When we examine the cytological maps of the two arms of the third (figures 1 and 29) we are somewhat handicapped by a lack of exact genetic data for many breaks, nevertheless certain facts stand out sharply.

The genetic map of the third chromosome is made up of 105 crossover units, and as the spindle fiber is close to 48, the left arm has 9 less units than the right. The total length of the euchromatic areas of the left and right arms, as these appear on my original drawings is 640 millimeters, and thus, on the average, about 6 millimeters of map length should represent one crossover unit. If the left arm carries 48 units it should be 288 millimeters long and it actually measures 282. The right arm, in turn, should be 342 millimeters long and is actually 358. On the assumption that the banded areas of the two arms carry all of the genetically active material, then the actual lengths of the two arms corresponds well with the crossover units each is supposed to contain.

The cytological map of the left arm (figure 1) shows, in general, that the morphological position of gene loci are much farther to the left than their crossover value would indicate. Thus hairy (26.2) is placed close to 19 morphological units from the left end; thread (42.2) lies to the left of 33 morphologically and scarlet (44) apparently lies to the left of 37 units from the left-hand end. In passing it should be pointed out that the VI 34 and VI 22 breaks are to the left of roughoid (0.0) the gene locus farthest to the left of known mutations in the third chromosome. If the distal ends of the third are like the free end of the X, then this material to the left of roughoid will scarcely amount to one crossover unit.

In a similar way we observe that the topographical map of the right arm shows a similar pushing of gene loci away from the spindle fiber. Thus stripe (62.2) must lie at least 80 morphological units from the left-hand end of the whole third, and sooty (70) to the left of 85. On the other hand a break (VI 44) which is beyond claret, comes 103 units from the left end.

The greatest discrepancy, as we should expect, is between the crossover values and the morphological positions of scarlet and pink. Genetically these are 4 crossover units apart, but physically they must be at least 22 morphological units apart. This represents something over 1/5 of the total length of the banded areas and does not take into account, at all, the region of the chromocenter. Of course, crossover units simply express the frequency with which crossing over occurs between gene loci and it is clear that in the region between scarlet and pink there is less crossing over per morphological unit (4 in 130 millimeters on my original drawings) as

compared to the curled-stripe area where 10 crossover units are represented by 75 millimeters of length on my drawings. This all amounts to saying, what we have long known, that crossing over is reduced in the region of the spindle fiber, and now through a study of appropriate breaks by the salivary gland method it should be possible to gain exact information on this genetic problem.

The question now arises, why should there be a difference between the X and the third chromosome in the correspondence between cytological and crossover maps? It might be thought that it is the presence of two arms, as compared to the rod-like X. On the other hand, it must be

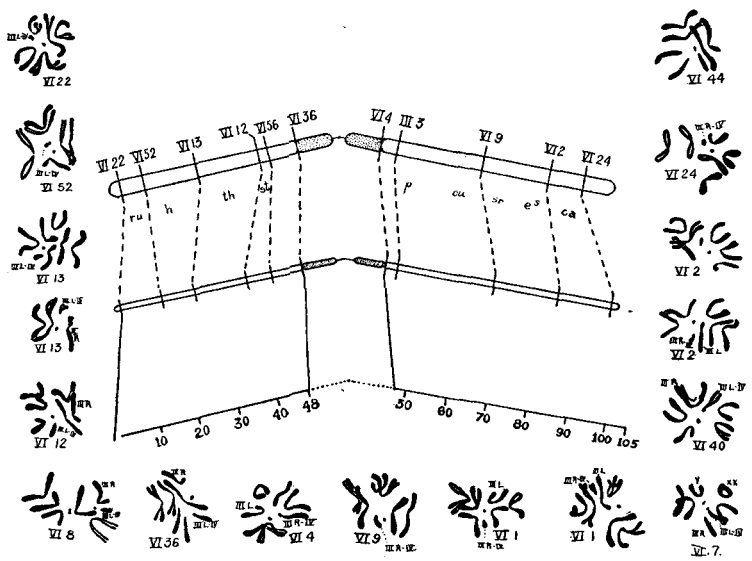


FIGURE B.—Around the periphery of this figure are a number of oogonial metaphase plates, taken from young adult flies which were heterozygous for the aberration indicated below the figures. In many instances the females were of the attached-X stock so that the two X's are joined and a Y chromosome is usually present. The upper central figure represents the third chromosome as it appears in oogonial metaphase plates. The middle central figure represents the salivary gland map while the lower is the crossover map.

remembered that nearly half of the X, next to the spindle fiber is made up of inert material so that physically the active gene-bearing material is far removed from the spindle fiber attachment zone. In the case of the third chromosome, the amount of inert chromatin is relatively small and it is possible that it is not sufficient to cancel the inhibiting effect of the spindle fibers. There are several other possible explanations of course.

A study was made of oogonial plates of a number of aberrations in which the points of breakage are well separated in order to compare the relative sizes of broken chromosomes in metaphase plates and salivary glands and the results are presented in text figure B, in the form of an old style

cytological map. At the outset it is well to emphasize, as both DOBZHANSKY and I have done in the past, the inherent difficulties in estimating the size of fragments relative to the chromosomes as a whole. If KAUFMANN (1934) is right, however, in his contention that there is a "differential contraction or extension of different segments" (p. 140) of chromosomes, even though we could draw and measure the length of pieces accurately, it would be of limited value because one would not know whether he was dealing with an extended or contracted phase of accordion-like chromosomes. In making estimates of the size of chromosome segments I have used linear dimensions in the main, and less value has been given to thickness.

In making a schematic outline of the third chromosome I have represented the two arms as uniform in diameter with only the primary constriction showing. Secondary constrictions have been reported for this element. According to DOBZHANSKY (1930) there are two, one in each arm not far removed from the primary. HEITZ (1933), however, maintains that only one arm has a real constriction, the other being formed presumably by the crossing of sister strands (see MULLER and PAINTER). And KAUFMANN (1934), who seems to be more constriction minded than the others, describes no less than five or six secondary constrictions for the whole third, if I interpret his figures and the text properly. In oogonial metaphase plates these secondary constrictions do not usually appear, and since the experts in these matters do not agree as to their position in the relatively large nerve cells, it seems wise to represent the chromosome much as it ordinarily appears. In any event, the main value of constrictions is their use as markers and in view of the development of the salivary gland chromosome technique where there are numerous physical markers and an abundance of constrictions which any one can see, it is quite clear that the number of secondary constrictions in prophase or metaphase chromosomes is now a matter of secondary importance and interest.

In text figure B the two arms of the third are shown as uniform in diameter because in salivary glands they exhibit this condition, and I quite agree with KAUFMANN (1934) that the tapered form seen in metaphase plates is largely the result of the prophase split and separation of sister strands. The right arm is represented as 10 percent longer than the left because there is actually this difference in the salivary gland and the crossover maps. Most investigators, in the past, have represented the two arms as equal in length, and in the contracted metaphase chromosome, a 10 percent difference would scarcely be apparent. It is interesting to note, however, that KAUFMANN (1934) shows the right arm somewhat longer than the left in prophase stages of giant nerve cells, where the chromosomes are relatively long. Below the metaphase chromosome is a greatly

reduced scheme of the salivary gland maps, with the inert material (stippled) lying about the spindle fiber. Below this the crossover map is so placed that the crossover units fall within the euchromatic areas of the salivary gland maps. The positions of selected breaks are shown and there are camera lucida drawings of oogonia upon which the diagrams are, in part, based.

One outstanding feature of text figure B is the wide separation of gene loci for scarlet and pink on the metaphase chromosome. The proximal (to the spindle fiber) limit of scarlet is given by translocation VI 56, and the inner limit of pink is determined by the X-IV, III 3 break. No metaphase figures of the truncated right arm of the latter are available (all figures show it united with the fourth) but it involves 22 millimeters of the euchromatic area of the right arm, on my drawings, so I have estimated its size by measuring the amount of euchromatic material between the inert area of the left arm and the VI 56 break (which is 8 millimeters on the metaphase chromosome) and dividing this by half. Once the morphological positions for the loci of scarlet and pink are determined, there is a fair correspondence from these points running distally between the position of genes on metaphase and salivary chromosome maps. Unfortunately, many of our breaks have not been localized genetically and until this is done we are unable to plot many points on the crossover map.

That there is a discrepancy between cytological and crossover maps was first pointed out by Dr. MULLER and myself (1929) and one case cited was a break involving the third chromosome. Later the same year DOBZHANSKY (1929 and 1930) published a cytological map of the third chromosome based on 5 translocations and in these studies he calls attention to the wide separation of scarlet and of curled from the spindle fiber area. One of DOBZHANSKY'S translocations (named "e") broke the left arm apparently, just where VI 36 and VI 14 did, and the position which DOBZHANSKY gave to scarlet corresponds very closely to the position I have arrived at, on the basis of the VI 56 break. The same correspondence is found in the general position of curled. I have placed pink farther away from the spindle fiber than DOBZHANSKY'S maps suggest. Of course, these discrepancies are largely due to the fact that there is a very appreciable amount of the inert material in both arms of the third chromosome next to the spindle fiber, as HEITZ (1933) has already pointed out, and we turn now to a consideration of the character of the chromatin which lies outside of the euchromatic area.

The presence of genetically inert material in chromosomes was first demonstrated at this laboratory by the combined cytological and genetic work of Dr. H. J. MULLER and myself (PAINTER 1930, MULLER and PAINTER 1932) for the X chromosome of *D. melanogaster* and has since

been confirmed by a number of investigators. In my paper dealing with the X chromosome of the salivary gland, which appeared recently in this journal, it was shown that the active genetic region of the X is represented by the banded or euchromatic part and that the inert material is represented, in whole or in part, by the material of the chromocenter. The present work shows that the third chromosome is differentiated likewise into euchromatic areas and a chromocenter region and we interpret the latter just as we did in the case of the X.

Another line of evidence which indicates a similarity of behavior of the inert region of the X and the material adjacent to the spindle fiber in the third chromosome is the work of HEITZ, dealing with heteropycnosis, more especially his most recent work on *D. melanogaster*. Here he shows that in somatic mitoses the half of the X next to the spindle fiber area tends to remain condensed in late telaphases, the interphase and early prophases, when the more distal half is diffuse. The former is, of course, the genetically inert region and HEITZ cites the work of Dr. MULLER and myself as supporting the conclusion, which he had reached before our work had come to his attention, that heteropycnotic areas of chromosomes, in general, represent genetically inactive regions. This conclusion was not based on any direct genetic evidence but seems to have been an inference drawn from the passive behavior of the heteropycnotic areas, as these appear in plants.

HEITZ has shown that in addition to half of the X, and all of the Y when present, there are heteropycnotic areas adjacent to the spindle fiber region in both the second and third chromosomes. These observations have just been confirmed by KAUFMANN and also by one of my own graduate students (unpublished data). These heteropycnotic areas correspond to the area of the third chromosome which does not show the banded form.

In addition to his excellent work on *D. melanogaster*, HEITZ has studied heteropycnosis in several other species of Drosophila all of which show evidence of heteropycnotic areas for part or all of the elements. *D. virilis* shows the greatest amount of inert material, nearly a half of each chromosome except the fourths, and as a result there is an enormous chromocenter in this species. *D. hydei* shows the least, the inert material being confined to the sex chromosomes. Previous to his work on Diptera, HEITZ had made a series of studies on heteropycnosis in plants (1929, 1931) and had shown that the phenomena was quite common, especially in the mosses and that the areas may fall anywhere along the chromosome, that is, it is not necessarily associated with the spindle fiber region.

Regarding the nature and function of the inert chromatin we are at present in the dark. One point seems clear and that is the phenomena is not confined to or necessarily connected with the sex chromosomes and

that speculations (see for example, p. 350 of MULLER and PAINTER) which have this premise as a basis are ill-founded. In the salivary glands the inert region of the chromosomes is differently organized from the euchromatic part. There appears to be a core of transparent (matrical?) material to which the chromocenter material adheres in an irregular fashion and not in the orderly way the chromatin of the euchromatic areas is laid down. This picture seems to suggest that we are dealing either with undifferentiated chromatin or with excess material of some sort, but there are other possibilities. At a later time it is planned to consider the question of chromosome structure in detail. As this is written, it seems probable that the salivary gland chromosomes may be regarded as a chromonema lying straight. There are local deposits of chromatin along this (chromomeres); there has been a great hypertrophy of the matrix which has stretched the chromomeres laterally until they show as bands in what is essentially a tetrapartite structure (because each of the synapsed homologs is probably split before somatic synapsis).

Distribution of Breaks

PATTERSON and his associates isolated 36 translocations between the third and fourth chromosomes and 32 of these have been studied by the salivary gland method. The cases not analyzed fall within the genetic limits of scarlet and pink. When we consider the distribution of these breaks (figures 1 and 29) we find that 11 came within the euchromatic area of the left arm, 15 in the corresponding area of the right arm and 7 within the chromocenter. The breaks in the left arm are well distributed, though there is some crowding close to the chromocenter end (figure 1). In the right arm, by contrast there is a paucity of breaks until we reach the distal two thirds and here there is a marked tendency for the breaks to be bunched. No less than five, for example, fall within 3 morphological units in the region of 90 (figure 29).

If we consider the breaks from the genetic point of view we find altogether 17 out of the 36 cases come between the loci of scarlet and pink. This tendency for the breaks to occur in the region of the spindle fiber is not confined to the third. Nearly half of the translocations between the second and fourth chromosomes fall between purple and curved (PATTERSON et al 1934) and in a paper now in press Mr. STONE and I have shown that out of 14 translocations between the X and fourth, which are viable in the male, half fall within the chromocenter.

It will be apparent, at once, from the evidence discussed in the foregoing section, that while nearly half (17 out of 36) of the breaks fall between scarlet and pink, a distance of 4 out of a total of 105 crossover units, nevertheless these gene loci are physically widely separated, not

only by euchromatic material, but the inert and spindle fiber region as well. I have estimated that in the oogonial chromosome the distance separating these loci is not far from a fifth or fourth of the total length of the entire chromosome. Even with this separation we could not expect so many breaks here unless there is some definite reason for it. Again, in the right arm from about 48 to 68 there is only one break (which is a very involved case) while in the same region on the opposite side there are 6 breaks for a shorter physical distance. On the other hand, the distal portions of the right arm show a marked bunching of breaks not seen in the left arm. There must be some physical basis for this tendency for breaks to fall within limited areas while other areas are free from them.

At first sight it might be thought that certain regions of the third chromosome are more prone to undergo breakage than others, but the real explanation, probably, is to be sought in the orientation of the chromosome at the time the sperm was irradiated. This brings us to a discussion of a very illuminating phase of the salivary gland chromosome work, and that is the evidence for nuclear organization.

In the salivary gland each chromosome or arm is anchored to a common mass of material called a chromocenter. The latter name is relatively new to most of us, but the orientation indicated is nothing more than the persistence of the telophase arrangement. It so happens that in *D. melanogaster* there are heteropycnotic areas adjacent to the spindle fiber region of the large chromosomes and the fusion of this material makes a conspicuous marker. All of the evidence which we have on translocations points to close physical contact or proximity as a condition of mutual exchange during or immediately following irradiation. If it were possible to irradiate salivary glands and then in subsequent divisions to follow the chromosomes we should expect exchanges to be most numerous in the region of the chromocenter, or close to it, because this is the region of the most intimate physical contact. There can be doubt that at the beginning of spermiogenesis, in *D. melanogaster*, the chromosomes have the telophase orientation, as a result of the second maturation division. Should this arrangement persist, or tend to persist, in the mature sperm head, then we should expect to find what we have found indeed, a predominance of exchanges in the region of closest physical contact. To explain the bunching of breaks (or its absence) in more distal regions of the chromosome we would have to assume that the element tended to coil around in the sperm head in a rather definite way, so that it was close to or far away from the fourth. This general explanation has been used by my colleagues to explain the bunching of the numerous translocations which they obtained (see PATTERSON et al).

In studying the phenomena of somatic synapsis in the salivary gland

it has been quite evident that the telophase orientation of chromosomes plays a considerable role in the ability of homologous parts to get together. This is most clearly seen in exchanges involving the fourth chromosome. When, as is shown in figure 40, the free end of the fourth chromosome is attached to the third far removed from the spindle fiber region, synapsis of this area with its normal homolog in heterozygous larvae is very rare, while the spindle fiber ends regularly undergo synapsis. In a similar manner a good many cases have been studied in which there was an exchange beyond the euchromatic region of the fourth so that a long segment of the right arm of the third received its spindle fiber from the fourth and morphologically nothing more. Now when we study larvae hyperploid for such a segment of the third, we find that it regularly is associated with the paired fourths and only occasionally will there be a synapsis with homologous region of the normal third chromosomes. I have interpreted this to mean, not that the attraction between the spindle fiber area is greater than elsewhere along the chromosome, but that normally the spindle fiber areas of the fourth chromosomes are anchored to the same general region of the chromocenter and this physical contiguity favors the union. Whether or not the segment attached to the spindle fiber region of the fourth synapses with an homologous region of the normal chromosome will depend largely on how close they happen to lie in any given nucleus, and how many other elements lie between them. If such factors operate in the salivary gland cells where synapsis is a slow and progressive process, it seems inevitable that in meiosis we would have the same effect only in more pronounced form in these cells where changes go on more rapidly. Thus, it is possible that the attraction between the ends of chromosomes, such as BELLING has often described, has a simple physical basis.

On the whole, the writer is inclined to the view that the idea of nuclear orientation will prove helpful in our interpretation and understanding of chromosome behavior in meiosis, especially in forms in which there are aberrant elements, and that in the future this factor should be given due consideration in theoretical discussions concerning synapsis and crossing over.

From a theoretical standpoint the exchanges made between the third and fourth chromosomes in the region of the chromocenter are of extreme interest. Here we have either the replacement of one arm by a fourth forming a j, or a part of one arm is attached to the spindle fiber end of the fourth also forming a j. The questions which concern us here deal with the nature of the spindle fiber region and since we are unable to see this area clearly in salivary glands, or at least have not recognized, up to the present, these areas in the chromocenter, no direct evidence can be presented on

this point. At the same time we can not overlook the very obvious question of what happens when one arm of the third is replaced by a fourth chromosome. Since the displaced arm persists, it must have a spindle fiber attachment zone of its own. But how about the other arm? Does the spindle fiber of the fourth do double duty or does the other arm have a spindle fiber zone of its own? There is much evidence in favor of the latter point of view. Various aspects of these questions are discussed in a joint paper with Mr. STONE where we take up, in detail, the apparent fusion of the X and fourth chromosome.

LITERATURE CITED

BELAR, K., 1929 Beitrage zur Kausalanalyse der Mitose. Arch. Entw. Mech. Org. **118:** 359–484.

DOBZHANSKY, TH., 1929 Genetical and cytological proof of translocations involving the third and fourth chromosomes of *Drosophila melanogaster*. Biol. Zbl. **49:** 408–419.

1930 Translocations involving the third and the fourth chromosomes of *Drosophila melanogaster*. Genetics **15:** 347–399.

HEITZ, E., 1928 Das Heterochromatin der Moose. Jb. Bot. **69:** 762–818.

1932 Die Herkunft der Chromocentren. Planta **18:** 571–636.

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

KAUFMANN, B. P., 1934 Somatic mitoses of *Drosophila melanogaster*. J. Morphol. **56:** 125–156.

MULLER, H. J. and PAINTER, T. S., 1929 The cytological expression of changes in gene alignment produced by X-rays in Drosophila. Amer. Nat. **63:** 193–200.

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*. Anat. Rec. **47:**

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 quantitative analysis of the chromosomes of *Drosophila melanogaster*. Anat. Rec. Sup. No. 4, **57:** 90.

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

1934b The morphology of the X chromosome in salivary glands of *Drosophila melanogaster* and a new type of chromosome map for this element. Genetics **19:** 448–469.

PATTERSON, J. T., STONE, WILSON, BEDICHEK, SARAH and SUCHE, META, 1934 The production of translocations in Drosophila. Amer. Nat. **68:** 359–369.