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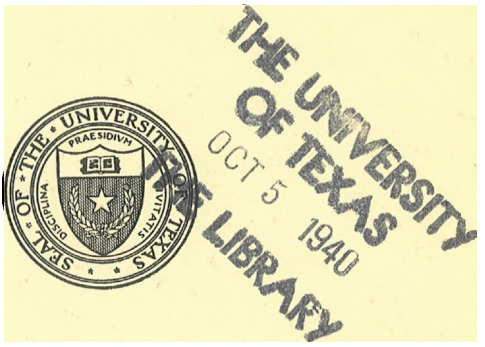
STUDIES IN THE GENETICS OF DROSOPHILA

Directed by

J. T. PATTERSON
Professor of Zoology
The University of Texas



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The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

Sam Houston

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man. It is the only dictator that freemen acknowledge, and the only security which freemen desire.

Mirabeau B. Lamar

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PREFACE

The articles appearing in this publication are based on experimental work carried out in our genetics laboratory during the past few years by the writer and his co-workers. All of the problems dealt with in these articles were formulated in this laboratory, although the work on some of them was completed after certain of the authors had accepted employment elsewhere.

The first ten articles represent a continuation of a line of investigation begun in 1932. The object of this investigation was to study the effects of aneuploidy and chromosomal aberrations on the phenotype, viability and fecundity of *Drosophila*. This knowledge is considered a necessary basis for an attack on the general problem of speciation, with which the last two articles are concerned.

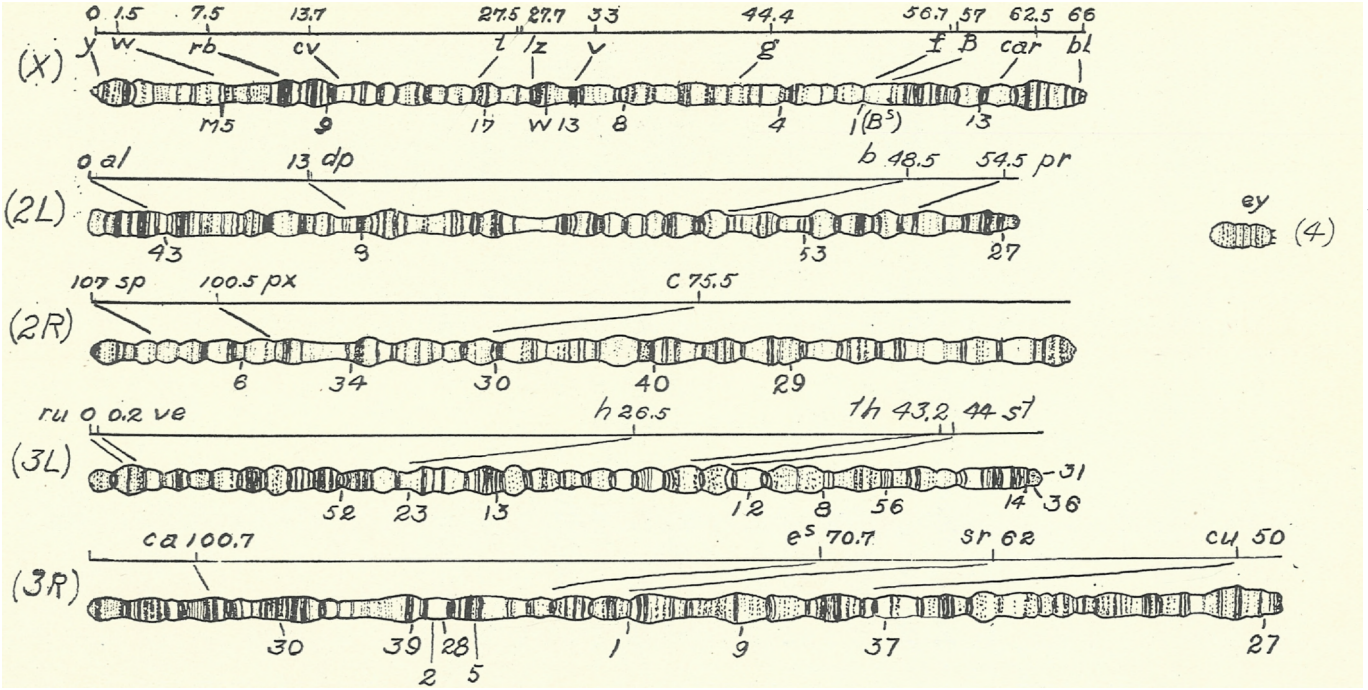
The writer wishes to express his appreciation for the assistance given by Professor Wilson S. Stone and Dr. A. B. Griffen. Professor Stone has devoted much time to a study and analysis of the experimental data and in giving help to several of the authors in connection with preparation of their manuscripts. Dr. Griffen has done the cytological work for those articles which required a direct study of the chromosomes. The writer wishes to direct attention to his fine drawings of the salivary gland chromosomes of *Drosophila virilis* (Article XI). His introduction of a flexible system for designating the bands of these giant chromosomes represents a forward step in cytogenetic analysis.

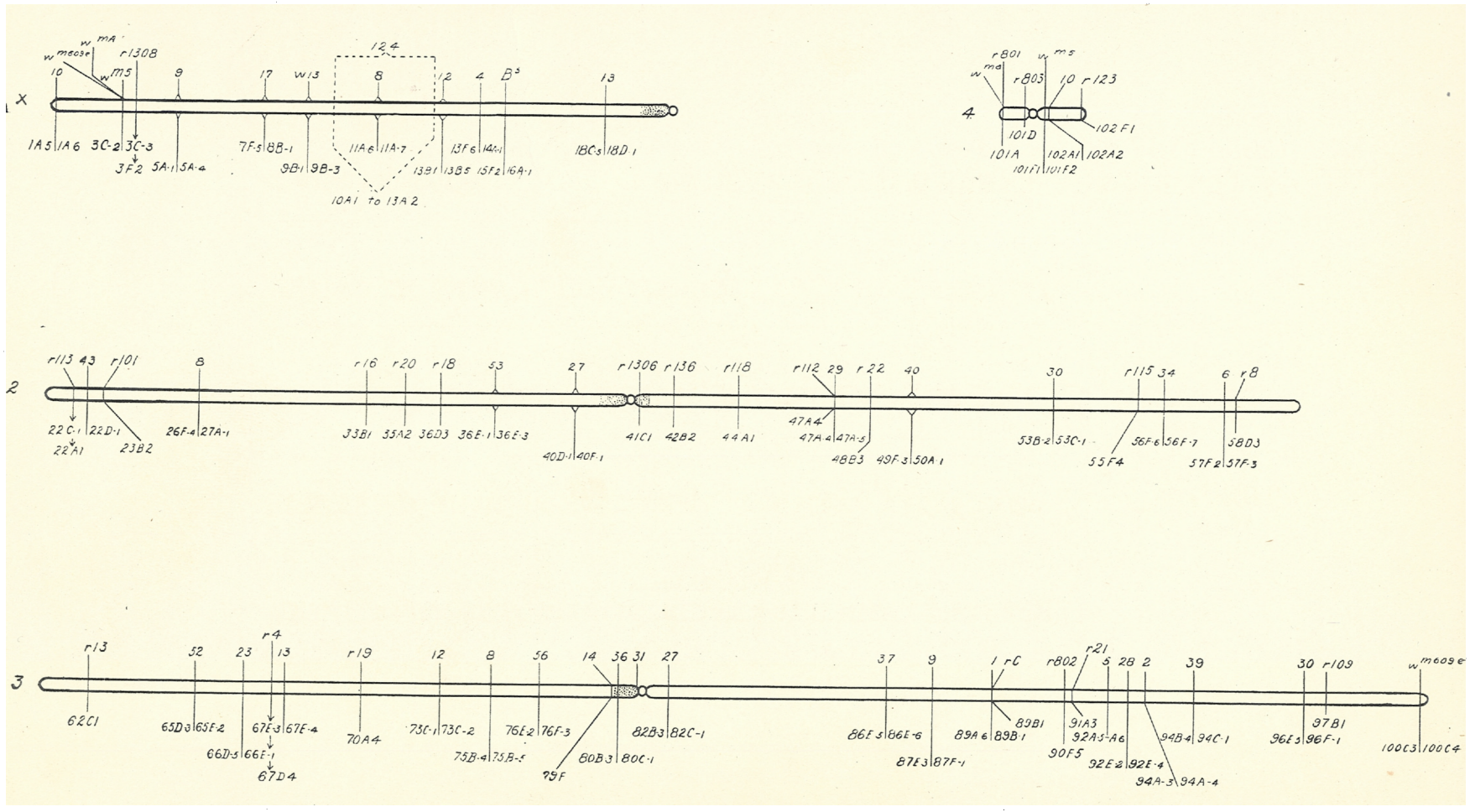
We are grateful to The University Research Institute, which, under the direction of Dean A. P. Brogan, has supplied funds for collecting material and for meeting the expenses of this publication.

J. T. PATTERSON.

Austin, Texas
May 20, 1940

Frontispiece. This shows a modified copy of Painter's map of the salivary gland chromosomes of *D. melanogaster*. A number of the rearrangements, as well as the positions of a number of gene loci relative to these rearrangements, are indicated. The diagram shows the location of the various rearrangements used in these studies in terms of Bridges' map (1935) of the salivary gland chromosomes. The locus of each translocation is indicated at its position between two numbered bands, except the reversals of w^{m5}. These are indicated as r8, etc., and only the band now placed next to the *white* locus, 3C2, is given.


0 1.5 7.5 13.7 27.5 27.7 33 44.4 56.7 57 62.5 66
(X)
y w rb cv l lz v g f B car bl
M5 9 17 W13 8 4 1(B5) 13
0 al 13 dp b 48.5 54.5 pr
(2L)
43 9 53 27
ey (4)
107 sp 100.5 px C 75.5
(2R)
6 34 30 40 29
ru 0 0.2 ve h 26.5 th 43.2 44 st
(3L)
31
52 23 13 12 8 56 14 36
ca 100.7 es 70.7 sr 62 cu 50
(3R)
30 39 28 1 9 37 27
2 5


X
w mecse
w ma
w ms
r1308
10
9
17
w13
8
12
4
B3
13
124
1A5 1A6 3C-2 3C-3
3F2
5A-1 5A-4
7F-5 8B-1
9B-1 9B-3
11A6 11A-7
13B1 13B5 15F2 16A-1
10A1 to 13A2
18C-5 18D-1
4
w ma
r801
w ms
r803
10
r123
102F1
101D
101A
102A1
102A2
101F1 101F2
2
r113 43 r101
8
r16 r20 r18 53 27 r1306 r136 r118 r112 29 r22 40 30 r115 34 6 r8
22C-1 22A1 22D-1 23B2
26F-4 27A-1
33B1 35A2 36D3 36E-1 36E-3
40D-1 40F-1
41C1 42B2
44A1
47A4 47A-4 47A-5 48B3 49F-3 50A-1
53B-2 53C-1 55F4 56F-6 56F-7 57F2 57F-3 58D3
3
r13
52
23
r4
13
r19
12
8
56
14
36
31
27
37
9
1 rC
r802
r21
5
28
2
39
30
r109
w m609e
62C1
65D-3 65E-2 66D-5 66E-1 67D4 67E-3 67E-4
70A4
73C-1 73C-2 75B-4 75B-5 79F
76E-2 76F-3 80B-3 80C-1
82B-3 82C-1
86E-5 86E-6 87E-3 87F-1
89A6 89B-1 89B1
90F5
91A3 92A-5 92A-6 92E-2 92E-4 94A-3 94A-4
94B-4 94C-1
96E-3 96F-1 97B1
100C-3 100C4

I. THE RELATION BETWEEN CHIASMA FORMATION AND DISJUNCTION

META SUCHE BROWN*

In earlier studies of translocation in Drosophila the analyses of crossing over and related phenomena have been based entirely on observed crossover data. Following Bridges and Anderson's first proof in 1925, the repeated demonstration of chromatid crossing over made it apparent that the observed values do not always represent the complete picture of chromatid exchange. Especially is this true in nondisjunctional gametes where the ratios of recovered strands differ from those in regular gametes. With the development of formulae by Weinstein (1932, 1936) and others (Mather 1933, Beadle and Emerson 1935) by which the chiasma configurations of chromosomes may be deduced from observed crossover data, a new approach is opened to the study of chromosome behavior.

In this paper the object has been to interpret the relation between crossing over and disjunction in both disjunctional and nondisjunctional gametes on the basis of chiasma frequency and distribution, where chiasmata represent loci of chromatid exchange. To study these relations six translocations involving the third and fourth chromosomes of *Drosophila melanogaster* with their several points of breakage strategically located were studied in heterozygous and homozygous condition. Crossing over and disjunction were studied both separately and in combination in each. By means of the general formula of Weinstein (1936) and modifications thereof adapted for exceptional gametes (see Appendix), all crossover data have been converted into the theoretical tetrad configurations. This conversion of crossover data into chiasmata will reveal not alone the formation of chiasmata in the recovered strands, but the frequency of such formation in all potential gametes.

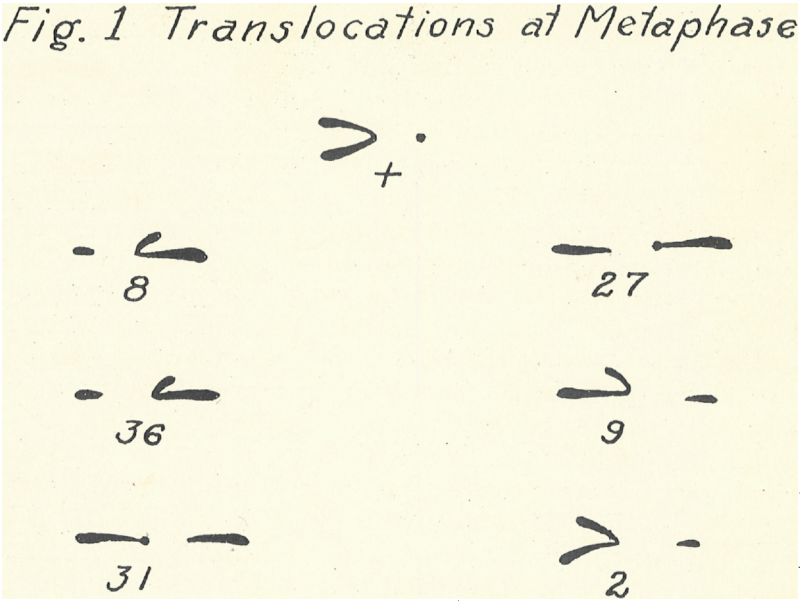
MATERIAL AND METHODS

The translocations used in the present study were selected from among thirty-seven cases of linkage between the third and fourth chromosomes produced by irradiation of normal males (Patterson *et al.* 1934). Position of break with respect to gene loci, cytological point of breakage in relation to the centromere, survival in homozygous condition and nonsurvival of aneuploid forms were factors considered in the selection of cases to be studied in detail. These translocations, all of which were mutual, have been incompletely described in two earlier papers (Patterson *et al.* 1934, Painter 1935). The genetic and cytological location of the points of breakage in these translocations are shown on the frontispiece. A more complete description follows.

*Now Division of Agronomy, Texas Agricultural Experiment Station, College Station, Texas.

In number 8 the third chromosome is broken in the left arm just to the right of *scarlet,* at band 75 C in Bridges' terminology (1935). Approximately three-fourths of the euchromatic area of the left arm is translocated to the proximal half of the right arm of the fourth chromosome between 102 D 1 and 102 D 3. In 36 the third chromosome is broken between *scarlet* and *pink* to the left of the centromere, at 80 C, with all of the euchromatic region of the left arm and three or four bands of the heterochromatin transferred to the right arm of chromosome 4 at 102 E. Number 31, with the third chromosome broken genetically between *scarlet* and *pink,* is broken cytologically in the middle of the unmapped region 80 near the centromere. The whole left arm including most of the heterochromatin is translocated to the right arm of the fourth chromosome at 102 F. In 27 the third chromosome is again broken between *scarlet* and *pink* but in this case to the right of the centromere, at 82 B. All the euchromatic area of the right arm with the exception of nine bands is transferred to the left arm (Griffen and Stone, 1938) of the fourth chromosome very close to the centromere. In 9 the third chromosome is broken genetically between *curled* and *stripe,* and cytologically at 87 E, near the midpoint of the right arm. The distal fragment is attached near the tip of the right arm of the fourth chromosome at 102 F 3. In translocation 2 the third chromosome is broken between *sooty* and *rough* at 94 A. The distal fragment of the left arm of the third chromosome, approximately a third of the euchromatic area, has been translocated to the right arm of the fourth chromosome at 101 F.

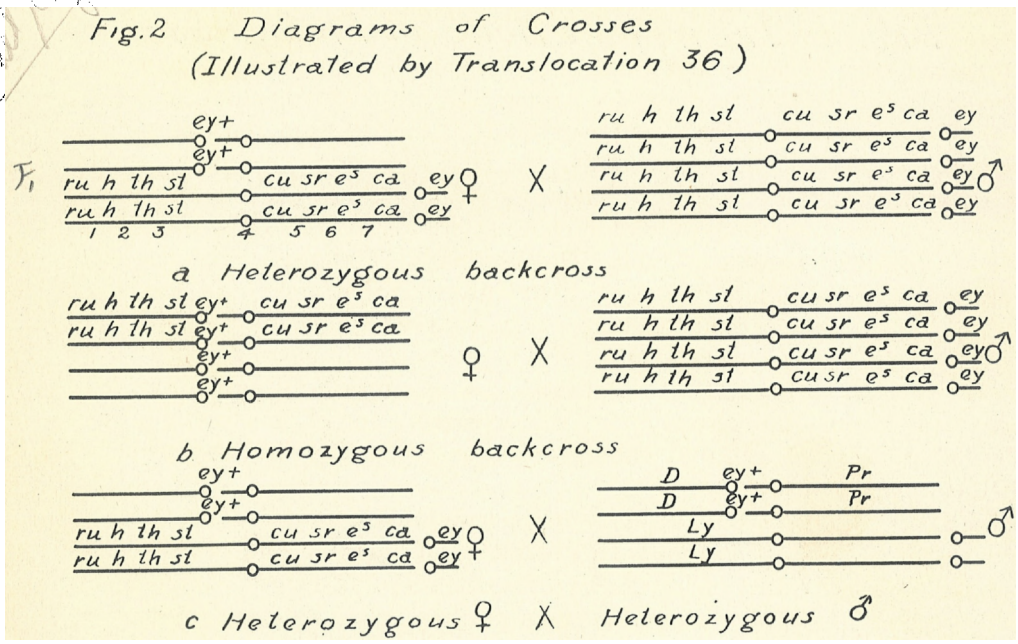
Oögonial and brain cell preparations of these translocations show the third chromosome broken into two fragments. In figure 1 are shown the intact third and fourth chromosomes of a normal fly and the altered configuration of each translocation. In 31 the arms of the third chromosome appear as rods, each with a part of the fourth chromosome attached. In 36 a rod, shorter than one arm of the normal third chromosome, and a J with a large hook are present. The rod is composed of the euchromatin of 3L plus part of 4R and 4L with the centromere of chromosome 4. The J is 3R, with most of the heterochromatin of 3L and part of 4R forming the hook. In 27 the rod is longer than in 36, and the J has a smaller hook. The rod is composed of 3L plus 4L; and the J, of 3R and 4R with the centromere of chromosome 4. In 8 the third chromosome is represented by a J with a prominent hook, longer than in 36, and a short rod. The rod is the distal end of 3L plus 4L and part of 4R with the centromere of chromosome 4. The J is composed of 3R and the heterochromatin of 3L plus part of 4R. In 9 the configuration is similar to that of 8. The J is composed of 3L with the proximal part of 3R and the tip of 4R forming the hook; the rod is the distal end of 3R plus part of 4R and 4L with the centromere of chromosome 4. In 2 there is an unequally armed V and a very short rod. The rod is the distal end of 3R missing from the short arm of the V, plus 4L and part of 4R with the fourth chromosome centromere.



To obtain females heterozygous for translocations for the study of crossing over, individual males of each translocation stock were crossed to ten homozygous *rucuca (ru h th st cu sr e^s ca) ey* or *Me ca/rucuca ey* females. When several males of a stock were crossed to *rucuca* the F_1 females of each male were kept separate. The F_1 females carrying the translocation and the *rucuca* genes were backcrossed, ten females to a bottle, to *rucuca ey* males within three days after emergence of the females. After six days the parents were discarded. The F_2 were counted and classified for a six day period after onset of hatching. Flies in which the distinction between *ey* and non-*ey* was doubtful were backcrossed to *rucuca ey* to insure correct classification.

For the homozygous crossover tests the *rucuca* genes were introduced into the translocations by crossing over. Flies bearing the required markers were selected in the course of the heterozygous crossover experiments for all translocations except 8. In this stock no crossovers between *st* and the point of breakage were recovered in the recorded data (Table 1), but by repeated backcrossing of heterozygous females the desired crossover was finally obtained. The following marked translocation stocks were established: for 8, 36, 31, and 27, homozygous *ru h th st* and *cu sr e^s ca;* for 9, homozygous *sr e^s ca* and *ru h th st cu/Me ca.* For 2, *ru h th st* females were crossed to *cu sr e^s ca* males. Dividing the markers in this manner between the two stocks eliminated the necessity for a completely unmarked third chromosome stock in which a possible contamination might not be detectable.

In the above cross the results may be modified by the inclusion among the F_1 of females resulting from nondisjunction of the unmarked arm.



However, no equational exceptions bearing the recessive mutants of either parent in homozygous condition were recovered. In a subsequent test of females of 31 homozygous for the translocation and for *ru h th st,* crossed to heterozygous 31 *D Pr/Ly* males, no cases of nondisjunction of either arm of the third chromosome were detected among 2,378 F_1 flies. Among a sample count of 869 F_1 flies from a cross of homozygous 8 females by heterozygous 8 *D Pr/Me ca* males only seven cases of nondisjunction of 3L were recovered, in spite of more than fifty per cent of nondisjunction of the translocated arm in males heterozygous for a translocation and another chromosomal abnormality. Furthermore, the hatch from homozygous females is reduced below normal in only one case (Table 7). Hence it can be assumed that nondisjunction of an arm of the third chromosome, where it occurs at all in the homozygous translocation, occurs too infrequently to effect the crossover count. Certainly its occurrence in both sexes at the same time is negligible. In 9 the homozygous female heterozygous for all *rucuca* genes proved too inviable to make an adequate crossover count possible. In 2, as between *st* and *cu* in the above cases, random segregation occurred between e^s and *ca;* hence a separate test for crossing over in the translocated fragment was made by crossing females with *ca* in one fragment and *Pr* in the other to homozygous *rucuca* males.

To obtain a measure of the amount of nondisjunction occurring in the presence of a translocation, egg counts were made for the following crosses for each translocation; homozygous unmarked translocation males and females crossed to normal (wild type) females and males respectively; and males and females heterozygous for the translocation and for normal backcrossed to normal females and males respectively. The control was

a cross between normal males and females from the same inbred normal stock from which the translocations were produced and to which the translocations were crossed in all subsequent tests involving the normal chromosome. All four crosses of a translocation were made simultaneously. For 31, an egg and hatch count was made for males and females heterozygous for the translocation and for the *Dcx* inversion crossed to normal, in order to test the effect of an inversion on disjunction in the two sexes. From fifty to one hundred test flies were mated individually to wild in vials containing spoons of food. The spoons were changed and the eggs counted at approximately twelve hour intervals. Spoons with less than twenty eggs were discarded, except in crosses where all females laid poorly, when sixteen was the minimum number saved. Recorded eggs were placed in food vials to await hatching. Spoons containing more than fifty eggs were divided into two vials to avoid overcrowding of developing larvae. The offspring were counted and recorded as to number and sex when all flies had emerged.

The egg counts reveal the maximum, combined amount of all types of nondisjunction which occur. To determine if possible the relative proportion of nondisjunction of the two fragments of the third chromosome, heterozygous females were mated to specially marked heterozygous males. In this test only the third chromosome was studied; the disjoining behavior of the fourth chromosome could not be followed satisfactorily. The markers *Dichaete* (*D*) and *Prickly* (*Pr*) had been crossed into the left and right arms, respectively, of the translocated chromosomes. From a cross of these *D Pr* males by *Lyra* (*Ly*) females, F_1 *D Pr/Ly* males were selected and mated with females heterozygous for the *rucuca* genes and the translocation (Figure 1). These females were obtained as for the heterozygous crossover count by crossing individual translocation males to *rucuca ey* females. The combination of dominant markers in the F_2 will reveal from what type of meiosis the gametes were recovered. Flies from disjunctional gametes will be *D Pr* and *Ly;* from nondisjunction of 3L will be *D Ly* and *Pr;* and from nondisjunction of 3R, *Pr Ly* and *D*, where aneuploid forms do not survive. Exceptional gametes, being hypo- or hyperploid for the nondisjoining fragment of the third chromosome, can give rise to viable zygotes only when united with complementary gametes of the same type of nondisjunction. Hence the number of exceptional offspring recovered will be limited by the frequency with which complementary gametes of the same type of nondisjunction are produced simultaneously in both sexes. Crossing over which had taken place in the F_1 was revealed by backcrossing F_2 males individually to *rucuca* females. For 8, 31 and 2 both disjunctional and nondisjunctional flies were backcrossed; for 36, 27 and 9, nondisjunctional flies only.

Figure 2 illustrates all types of crosses. All experiments were carried out at a temperature ranging between 21° and 24° C.

Recombination data given in Table 1 were converted into chiasmata (Table 2) by means of the formulae given in the appendix. The two

arms of the third chromosome were considered independent in all calculations presented here. In heterozygous and nondisjunctional gametes of 8, 36, 31 and 27 the locus of the fourth chromosome as marked by *eyeless* was considered the dividing point between the two arms. A possible source of error is introduced by this scheme since any crossing over between *eyeless* and the centromere of the third chromosome is thereby shifted to the opposite arm. On the assumption that the closeness of this area to the point of breakage and to the centromere makes crossing over here a rare occurrence, the error may be considered small. In the control, heterozygous 9, heterozygous and homozygous 2 and in the disjunctional gametes of all translocations where the break was not completely marked with *eyeless,* observed crossovers in region 4, between *st* and *cu,* were arbitrarily divided equally between the two arms. As Mather (1936) points out, if the observed doubles involving region 4 are more often two singles across the centromere, the calculation based on an arbitrary equal division of such doubles may result in a frequency of double chiasma tetrads slightly higher than actually occurs. Cases of rare double crossovers involving region 4 and either adjacent region, and the extra crossover in the event of an odd number of observed double crossover flies, were considered as two singles, one in each arm. Since the number of double crossovers within the arm is not necessarily equal on the right and on the left of the centromere, the number of single chiasmata as obtained by subtracting a certain proportion of the corresponding doubles from the observed singles will not coincide in 4l and 4r. In homozygous 8, 36, 31 and 27, where all fourth chromosomes were attached to sections of the third chromosomes and carried the normal allele of *eyeless,* crossovers in the *st-cu* region could not be detected.

Calculations were made to determine the rates of nondisjunction of the left and right fragments of the third chromosome in males and in females. The sum of the rates of nondisjunction of 3L and 3R as determined from egg hatch counts, and the relative rate of nondisjunction of 3L and of 3R as recovered in the cross heterozygous males by heterozygous females were used in these calculations. A sample calculation is given in the appendix.

Results

In Table 2 are given the calculated chiasma frequencies per region for all crosses of each translocation using the experimental data of Table 1. At the right of the table the total single, double and triple chiasma tetrads, the total crossover configurations and the total chiasmata per arm, with the ratios of singles to doubles, are given in summarized form. In Table 3 are listed the total chiasmata per region, calculated from singles and multiples.

In comparing heterozygous translocations with the control in regard to chiasma formation we find, in the left arm, a reduction in single

chiasmata in regions near the break in 8; and in the right arm, a similar reduction in 27, 9 and 2. The decrease is most marked in 9 and 2. In 36 and 31 no reduction is apparent. However, when the total chiasmata per region are compared, as in Table 3, 36 also shows decreased crossing over in regions near the break, because double chiasmata involving these regions are reduced. In 31 the distribution of chiasmata is shifted from the end of the arm toward the centromere. In 8 and 36 lesser shifts in distribution of single chiasmata toward the end of the chromosome can be explained as compensatory increases accompanying reduction near the break. When the total single chiasmata in the translocated arm are compared, (summary of Table 2) we note that they are reduced in three cases, those broken well in the arm, 8, 9 and 2; and increased to a slight degree in the three cases broken at or near the centromere. The double chiasma tetrads are decreased in all cases in the arm involved in the translocation. The reduction is most marked in 2, with gradually increasing frequencies in 9, 8, 36, 27 and 31 in the order listed.

Comparing the total no-chiasma tetrads and the total chiasmata per arm are still other ways of determining to what extent a chromosome break affects chiasma formation. In the left arm, 31 shows little or no change, and 36 but a slight increase in no-chiasma tetrads. In the right arm no-chiasma tetrads are increased in all in the order 27, 9 and 2, the frequency being greatest in 2. Comparison of the total chiasmata per arm avoids the confusion caused by regional shifts in chiasma distribution and by any changes in the single: double ratios. Translocation 2 has the lowest frequency with progressively increasing values in 9, 8, 27, 36 and 31. It is apparent therefore that the reduction in chiasma formation in the broken arm of heterozygous translocations is inversely proportional to the length of the fragment translocated.

In the unbroken arm in heterozygous translocations the distribution of chiasmata is altered. Single chiasmata are increased in some regions, reduced in others, so that the total single chiasmata are equal to the control in three cases and reduced in the remaining three. Double chiasmata, too, are shifted, often reduced. The total double chiasma tetrads are reduced in three cases, as are the total chiasmata per arm. This reduction is found in 36, 31 and 27, the translocations in which the break is near the centromere and where there is little or no reduction in chiasma frequency in the translocated arm. In 8 and 2 the single, double and total chiasmata are comparable to the control. In 9 the slight decrease in single chiasmata is more than outweighed by an increase in doubles, the total configurations and total chiasmata both exceeding the control. This increase might be regarded as compensation for the decrease in chiasma formation in the right arm. However, no increase is found in the unbroken arm in 8 or 2; so compensation across the centromere cannot be universal. Table 3 shows clearly in which regions chiasma formation is increased or reduced, and to what extent the per cent of no-chiasma tetrads is changed.

From the experiments designed to obtain progeny resulting from exceptional gametes an excess of disjunctional offspring was recovered. In three translocations such disjunctional males were backcrossed to determine the amount and distribution of crossing over which had occurred in the heterozygous parent. Under identical conditions these crossover values should coincide with those obtained in the ordinary heterozygous cross. However, comparing the heterozygous and disjunctional values for 8, 31 and 2, we see from Table 2 and Table 3 that the single chiasmata and the total chiasmata vary from region to region. This shift is most marked in 31. In all three cases, however, when the sums of the single and double chiasmata as well as the total chiasmata from all crossover tetrads are compared the values for the two crosses of any one translocation very nearly coincide. Therefore these slight shifts in chiasmata distribution between the two crosses may be considered random variation, and only more pronounced differences are considered significant.

Exceptional gametes showing nondisjunction of the unbroken arm were not obtained in numbers sufficient for adequate crossover tests. In translocation 8 in a total of 67 flies nondisjunctional for 3R, including 58 *D* and 9 *Pr Ly*, 30 were non-crossovers in 3R, 25 were singles and 12 were doubles. In translocation 31, in a total of 24 flies nondisjunctional for 3R, including 18 *D* and 6 *Pr Ly*, 18 were non-crossovers and 6 were single crossovers in 3R. Of 4 flies nondisjunctional for 3R in translocation 36,3 were non-crossovers, and one a single crossover in region 7. In translocation 27, 2 *D Ly* flies nondisjunctional for 3L showed no recombination in that arm. In translocation 9, of 4 flies nondisjunctional for 3L, 1 *D Ly* and 3 *Pr*, 2 were non-crossovers and 2 were single crossovers in 3L. Calculations made from the limited data obtained in translocations 8 and 31 are given Table 4. These and all following calculations from data for nondisjunctional tests include only gametes in which chiasma formation can be followed in two strands; i.e., gametes where two chromatids are recovered from the female. *D Ly* or *Pr Ly* flies are not included as only one possible crossover strand among the four of a tetrad is recovered. It is evident from Table 4 that chiasma formation in translocations 8 and 31 is greatly reduced in the intact arm when it fails to disjoin. In the disjoining arm, even though broken, chiasma formation is not below normal. In 8L the frequency of no-chiasma tetrads is decreased below heterozygous and control values, but the data are too limited to lend much significance to the comparison.

More extensive data are available for nondisjunction of the translocated arm or fragment. Table 2 shows that in the nondisjoining, i.e., the broken arm, of exceptional gametes, the single chiasmata are reduced in every region in each case, not only as compared to the control, but also as compared to the corresponding heterozygous disjunctional cross (Table 2). The double chiasmata are likewise reduced. The same relations are clearly evident from Table 3 and the summary of Table 2. Of

the translocations involving the left arm 8 shows the greatest reduction and 31 the least. The extent of the reduction is most obvious when the percentages of no-chiasma tetrads are compared. These are 57.09, 60.30 and 87.70, respectively, for 31, 36 and 8, with the comparatively low frequency of 13.46 per cent in the control. In 27, morphologically comparable to 36 to the left of the centromere except that no heterochromatin is involved, the chiasmata are reduced throughout the right arm, both in regard to single chiasmata (Table 2) and total chiasmata per region (Table 3). The non-crossover tetrads are four times as frequent as in the heterozygous test and twelve times as frequent as in the control. The reduction in total crossover configurations and total chiasmata in 27 are approximately of the same proportion as in the left arm of 36. In 9, as seen from Table 2, the single and double chiasmata are reduced in all regions except 4r as compared to the control. In comparison with the heterozygous values a further reduction is found in regions 5 and 6, within the limits of the nondisjoining fragment. The increase in region 4r, in the disjoining fragment, may be correlated with the decrease in chiasma formation found in regions to the right. However, this increase is not apparent in Table 3. The arbitrary manner in which the chiasmata in region 4r were calculated diminishes the significance of a shift in this region. The reduction in total per cent of chiasmata is greater than in 27, or in 36 and 8 in the left arm. In 2, as in 9, the single chiasmata are reduced in all regions of the right arm except 4r. Compared to the heterozygous (disjunctional) cross, the reduction is greatest in region 7, the region of the break. This fact invites a closer analysis of chiasma formation and disjunction in the two fragments of the right arm in 9 and 2.

In Table 5 are given the percentages of chiasmata as calculated separately for each fragment of the broken arm in disjunctional and nondisjunctional gametes of 9 and 2. For comparison the control was calculated separately for each translocation, with crossovers in the corresponding region, 5 or 7, divided between the left and right sections of the right arm in the proportion 1:2, to correspond roughly with the per cent of crossing over on the two sides of the break in the given region and to the position of the break on the salivary gland map with respect to the markers limiting the region in question. Examination of the table shows that in 9, there is a reduction in total crossover tetrads and total chiasmata in both fragments of the right arm in the disjunctional gametes. In exceptional gametes, chiasma formation in the left fragment of 3R is reduced but slightly below the heterozygous value, whereas in the right or nondispoining fragment the chiasmata are reduced from 60 per cent to 17 per cent. The non-crossover tetrads in the right fragment are twice as great as those in 9 heterozygous, and seven times as great as those in the control. It is evident from Table 5 that not only is the reduction in crossing over in disjunctional and nondisjunctional gametes confined to the arm in which the break occurred, but that in exceptional

gametes the further reduction is found primarily in the nondisjoining fragment.

In 2 the relations are comparable to those in 9. In the left fragment of the right arm the reduction in chiasma formation in disjunctional gametes is considerable, but there is little further reduction in the same regions in the nondisjunctional gametes. In the nondisjoining fragment, on the other hand, chiasma formation is practically eliminated. Therefore from 2 also we may conclude that the disjoining or nondisjoining of the translocated fragment of the arm has a negligible effect on crossing over in the other fragment.

In the disjoining arm of gametes showing nondisjunction of the translocated arm or fragment, chiasma formation in given regions may be reduced or increased without great change in total frequency. In 8 singles are shifted and reduced, but the doubles are increased with a resulting increase in total chiasmata. The total no-chiasma tetrads are slightly increased. In 36 and 31 the total singles are increased; the total doubles and the total chiasmata are slightly reduced. The frequencies of singles and doubles are such that the total no-chiasma tetrads are normal in 36 and slightly reduced in 31. In 27 the distribution of singles and doubles is shifted, but the total singles, total doubles and total no-chiasma tetrads are normal in frequency, as they are in translocation 2. In 9 the total singles and total doubles are increased. Hence in the intact, disjoining arm of nondisjunctional gametes, chiasma frequency is normal or slightly decreased when the break is near the centromere. When the break is within the arm, chiasma frequency in the intact arm is normal or increased.

In homozygous translocations, since there is a complete physical separation of the two arms, or the two fragments, of the third chromosome, random segregation occurs in the region of the break. The fourth chromosome carries the normal allele of *eyeless* and the centromere of the third chromosome is unmarked. Consequently no chiasma formation between *st* and *cu* in 8, 36, 31 and 27 can be detected. To allow for the loss of region 4 in the crossover data of the above translocations, and of region 7 in translocation 2, modified controls are introduced. In these controls single crossovers in regions 41 and 4r, or 7, are treated as noncrossovers; and double crossovers involving these regions, as singles. In the following description data from homozygous translocations are compared to the regular and to the modified controls.

In 8 and 36 there is a reduction in single chiasmata in the left arm in the regions near the break. The greater reduction in 8 is consistent with the interpretation that decreased distance from the centromere decreases chiasma formation (Beadle 1932 a), since corresponding regions are moved closer to the centromere in 8 than in 36. The fact that the total chiasmata are reduced below the heterozygous value, however, suggests that changing the centromere in both homologues has an added effect.

Length of the chromosome cannot be a factor, since in 2R1 (see below) a decrease in chromosome length is not accompanied by a reduction in chiasma frequency. In 36, the total single chiasmata are equal to the control, but the doubles again are reduced. It is probable that the absence of crossover data for region 4, because of random segregation in this region, is at least partly responsible for the decrease in doubles in homozygous translocations. When the homozygous values are compared to the values in the modified control, where doubles involving region 4 are considered singles in other region concerned, the decrease in doubles in homozygous translocations is less marked (Table 2).

In the right arm of the translocations involving the left arm, chiasma formation is subject to considerable shifts from region to region, and from singles to doubles, but the total chiasmata are comparable to the control in 8 and 36, and but slightly reduced in 31.

In the right arm of 27 there is slight decrease in region 5, with an increase in the adjoining region. As there is no heterochromatin in the right arm, the decrease in chromosome length is negligible. Apparently the marked regions are too far to the right of the centromere to be greatly affected by the slight change in centromere position. The total single chiasmata are normal, but the double chiasmata are reduced, making the total chiasmata somewhat lower than in either the regular or the modified control. In the left, unbroken, arm the single chiasmata are normal, but the doubles again are reduced; consequently the total chiasmata are slightly lower than normal.

The effect of a break in the middle of the right arm, as in 9, unfortunately could not be studied in the homozygous condition.

In the proximal fragment of the right arm of homozygous 2 there is an increase in single chiasmata in all regions except 7, which is unmarked. In the distal, translocated, fragment, chiasmata formation cannot be detected in this test. A general increase in 2R1 is likewise apparent when the total chiasmata per region are compared, as in Table 3. An increase in total doubles as well as total singles, in comparison with the modified control, indicates that the change in chiasma formation is significant.

In the left arm of 2 the single chiasmata are approximately normal, but the double chiasmata are increased; hence the total chiasmata exceed the control and the non-crossovers are reduced. There has been no decrease in chiasma formation in 2R1; but in view of the decrease in 2Rr, the translocated fragment, the increase in chiasma formation in 2L may be indirectly related to the break as discussed below.

In the cross of *Pr/ca* females to *rucua* males, designed to test crossing over in the translocated fragment of 3R in homozygous 2, two *Pr ca* females were recovered among 5,004 F_2 offspring. The expression of recessive *ca* eliminates the possibility that these are hyperploid for the Pr bearing fragment, hence the two flies must be cases of crossing over within the translocated section of 3L. Since the distance between the two genes involves 10.7 crossover units, it is obvious that recombination has been

greatly reduced below the normal value. The proximity of the centromere of the fourth chromosome undoubtedly is involved in the reduction in chiasmata frequency.

Breaking the third chromosome into two fragments has a marked effect on the distribution of chiasmata. In the nontranslocated arm there is a shift toward the centromere of single chiasmata in 8, 31, and 9 when heterozygous (disjunctional); in 8 and 2 homozygous; and in 8, 36, 31, and 2 when the translocated arm nondisjoins. The shift is toward the middle of the arm in 36 heterozygous, in 36 and 31 homozygous, and in 27 and 9 when the translocated fragment nondisjoins. A shift toward the end is found only in 27 heterozygous and homozygous, and in 2 disjunctional. In the broken arm, where chiasmata are usually reduced near the break, there is often a shift in single chiasmata to regions away from the break, i.e., toward the distal end of the arm in 8, 36, and 27 heterozygous and homozygous, and 31 homozygous. In 9 heterozygous the shift is in both directions from the break in the middle of the arm. In 31 heterozygous single chiasmata are shifted toward the centromere, as they are in many instances in the nontranslocated arm. Double chiasmata in the intact arm are shifted to nonadjacent regions (e.g. 5–7) in 8 and 36 heterozygous, 8, 36, and 31 homozygous and in 8 when 3L nondisjoins. In homozygous 2 and in heterozygous 9 and 2 when the translocated fragment disjoins or nondisjoins, double chiasmata are shifted toward the free end of 3L.

To such shifts in chiasma distribution can be attributed much of the change in coincidence values seen in Table 6, since interference must relate to chiasma frequencies. The relation between single and multiple chiasmata is determined by the interference properties of the chromatids. In the absence of a correlation, positive or negative, between chiasma formation in another region, certain relations must hold. If there are x per cent of tetrads with a chiasma in region a (and nowhere or anywhere else) and y per cent of tetrads with a chiasma in region b (and nowhere or anywhere else) on the basis of chance we expect x per cent of y per cent, or xy per cent, of the tetrads to involve regions a and b at the same time, irrespective of any chiasmata occurring elsewhere. If there is a negative correlation, i.e., interference, we expect to get less than xy per cent. The relation of actual/expected, calculated as above, gives us a measure of coincidence. The coincidence values given in Table 6 were calculated as above from the converted data in Table 2.

In the control, coincidence never reaches one within the limits of either arm. In 3–4 translocations, interference may be increased or decreased with change in chromosome configuration. With one exception, when the break is in 3L, interference is decreased in the nonadjacent regions 5–7. Correlated with this change is a frequency increase in interference in regions 5–7 and 6–7. In translocations 9 and 2 interference is decreased in regions 1–2, but in 27, with the break near the centromere, interference relations are more nearly like those in translocations 8, 36, and 31. In homozygous translocations, except for changes due to shifts in chiasma

distribution, interference is increased only where there is a change in centromere position, i.e., in 8L. In heterozygous translocations interference is usually high in the broken arm in disjunctional gametes. Exceptions found in 2 either involve few crossover flies or crossovers on both sides of the break. In nondisjunctional gametes interference is apparently low in the translocated arm in regions far removed from the break, but in each exceptional case the number of flies on which the calculations are based is small. Hence the significance of these high coincidence values is doubtful.

Because not all regions, and hence not all chiasmata, are involved in the coincidence calculations, the single:double ratios are perhaps a better measure of interference relations. In this way the total interference in an arm can be measured. In the summary of Table 2 are given these ratios as calculated by dividing the total single chiasma tetrads of an arm by the total double chiasma tetrads. Modified control values are given for comparison with homozygous translocations. Examination of the table shows that the ratios are fairly constant, and equal to the control, in the intact arm in heterozygous and homozygous crosses. Exceptions are found in 9 heterozygous, where the double chiasmata are increased, and in 27 heterozygous and homozygous. When the comparison is made on the basis of a modified control, the deviation in homozygous 27 loses its significance. In the disjoining arm of nondisjoining gametes the ratios for 27, 9, and 2 are normal. Those in 8, 36, and 31 deviate from the control. In 36 it is to be noted that the number of flies upon which the calculations are based is relatively low.

In the arm involved in the translocation the presence of the break and the change in centromere position result in altered single:double ratios. In the heterozygous crosses the single:double ratios are increased in all, in inverse proportion to the length of the fragment translocated. In 2 this increase is apparent both when calculations are made for the whole arm or for 2R1 separately (Table 13, see below). In 9 the frequency of recovered double exchange flies restricted to each fragment of the arm is too low to make separate calculations of any significance. In 36L, 31L, and 27R the ratios are doubled in spite of little or no change in chromosome length. However, in 31L the ratio is only slightly higher than in 27L, which is not translocated. In homozygous crosses there is an increase in single:double ratios only when there is a considerable change in centromere position, i.e., in 8. When the break is near the centromere, and in 2, the homozygous ratios very nearly coincide with the corrected values of the modified control. In these cases normal chiasma formation is apparent. In 2 the regions studied are in the proximal fragment of 3R and not in the translocated fragment. In the nondisjunctional gametes the increased single:double ratios correspond closely to those of the disjunctional gametes from heterozygous translocations. Although the absolute amount of chiasma formation is greatly reduced below the heterozygous value the singles and doubles are reduced in the same proportion as in the disjunctional gametes, the ratios remaining the same. The difference in

chiasma formation in heterozygous disjunctional and nondisjunctional gametes is therefore a matter of degree and not of kind.

In general the ratios of single:double chiasmata are approximately normal in all cases where chromatids of the same length are involved, unless the centromere position has been changed, e.g., 8 homozygous. A marked increase in the single:double ratio is found only in the translocated arm, and then only, with the exception noted above, in crosses where the break is heterozygous.

Examination of figure 3(F) (see Appendix) shows that eleven different strand combinations are found among the sixteen gametes resulting from double chiasma tetrads in which both exchanges occur in the nondisjoining fragment. Of these eleven, one can be formed by two strand crossing-over only, four by two or three strand, two by three strand only, and four by either three or four strand. Among 1110 exceptional progeny resulting from nondisjunction of 3L in 8 and 31, fifteen multiple crossover flies containing two crossovers in the left arm were recovered. These crossovers were classified according to the type of configuration from which they were produced as follows: from two strand, one case: from two or three strand, five: from three strand, three: and from three or four strand, six cases. In view of the small number of double crossovers the results are in excellent agreement with expectation on the basis of random two strand, three strand and four strand double exchange. There is no indication of any influence of the first chiasma in the formation of the second, i.e., no evidence of chromatid interference.

The per cent of nondisjunction as tested indirectly by counts of eggs and hatch is shown in Table 7. A comparison of the homozygous translocations with the control shows that the per cent of hatch of eggs from homozygous females is approximately normal in all cases except 8. This case is slightly below normal. That the reduction in egg hatch cannot be attributed wholly to nondisjunction is proved by an experiment with homozygous translocation females crossed to heterozygous translocation *D Pr/Me ca* males. In this test only seven cases of nondisjunction of the translocated arm, and none of the intact arm, were found among 869 F_1 flies. Since the homozygous males of this stock are semi-sterile, it is possible that the reduced hatch of the females is due to a similar, but lesser, sterility. In tests of homozygous males the hatch from eggs fertilized by males of 36 and 31 is comparable to the control, and also to the hatch from eggs of homozygous females. In 2 the value for males is slightly below that for females of the same translocation and the control. In 8, 27 and 9 no offspring were obtained from some males; from others the hatch ranged from 0.0 to 92.0 per cent. No completely fertile strain could be isolated, nor was the specific cause of sterility determined. Apparently, however, there is no correlation between the sterility and the point of breakage in the translocation. Hence it appears that disjunction is normal in both males and females homozygous for translocation, although sterility due to other factors may mask the data of egg hatch counts.

In tests of heterozygous males the hatch is approximately fifty per cent in all cases; or if the values are corrected to compare with a theoretical one hundred per cent in the control, the hatch is slightly greater than fifty per cent. There is a slight difference between translocations but no variation which can be correlated with the position of the break. On the assumption that the difference between the control and the experimental values is due to the inviability of the aneuploid zygotes, the results indicate that regardless of the point of breakage slightly less than fifty per cent of the gametes produced in heterozygous translocation males are nondisjunctional for part of the third chromosome. In tests of heterozygous females on the other hand the hatch varies with the position of the break. There is a correlation between the amount of nondisjunction as measured by inviable eggs and the length of the translocated fragment. Table 7 shows the lowest hatch, 52.2 per cent, in 2, and the highest hatch, 69.5 per cent, in 31. Other values are intermediate. The figures indicate that the amount of nondisjunction in heterozygous females is inversely proportional to the length of the translocated fragment, insofar as the per cent of eggs hatched is a measure of disjunction.

The effect of an inversion in males and in females was tested by substituting the *Dcx* inversion for the normal chromosome in heterozygous translocation 31. In these tests the hatch from 4670 eggs from test females was 1743 flies, or 37.38 per cent. This is slightly more than half the per cent of hatch from heterozygous females without the inversion. The hatch from 5422 eggs fertilized by test males was 2318 flies, or 42.75 per cent. This hatch is approximately four-fifths as great as the hatch from inversion free males. The results indicate that although nondisjunction is increased in both sexes when an inversion is present, the effect of the inversion is less marked in males than it is in females.

In Table 8 are given the results of an experiment in which heterozygous translocation females were crossed to heterozygous translocation males. The figures reveal, not the relative frequency of the different types of disjunction, but merely the relative numbers of zygotes recovered. Since viable zygotes are produced only when complementary gametes of the same type of nondisjunction unite, the recovered classes represent only a small fraction of the total nondisjunctional gametes formed. As seen in the table, nondisjunction of both fragments of the third chromosome occurs in all translocations. It is immediately evident, however, that zygotes resulting from nondisjunction of the translocated arm or fragment are recovered far more frequently than those resulting from nondisjunction of the arm with its own centromere. Comparing translocations, we find that the largest per cent of exceptional offspring is obtained in 2, from gametes in which the distal fragment of the right arm failed to disjoin. The next highest per cent, 18.12, found in 9, likewise results from nondisjunction of the distal fragment of the right arm. In 8, with the greater part of the left arm translocated, 11.97 per

cent exceptional offspring were recovered. In 36 and 27 we recover 6.69 per cent and 5.71 per cent, respectively, of zygotes resulting from nondisjunction of the translocated arm. In 31, nondisjunction of the left arm results in 7.75 per cent exceptional offspring, whereas nondisjunction of the right arm produced only 0.17 per cent zygotes. Excepting 31, in which the per cent of exceptional offspring is slightly greater than in 36 and 27, the per cent of zygotes recovered from exceptional gametes is inversely proportional to the length of the translocated fragment.

Zygotes from gametes in which nondisjunction of the arm retaining its original centromere occurred are less than one per cent in all cases. With the break in the right arm the percentages 0.04, 0.06 and 0.10 correspond nicely, in inverse order, to the length of the fragment involved. Similarly, the 0.09 per cent in 36 corresponds to the per cent of recovery in 27. With 0.17 per cent in 31, only the 0.76 per cent in 8 is out of order.

In Table 9 are given the number of equational exceptions recovered among the nondisjunctional flies of each translocation. The production of such equational exceptions is illustrated in Figure 3. These equationals were treated with the other nondisjunctional individuals in the analyses of the data. As Dobzhansky (1933) has pointed out, the number of equationals homozygous for a given gene depends upon the frequency of chiasma formation between that gene and the centromere.

In the course of disjunction and crossing over experiments of translocation 2 a number of flies hyperploid of the translocated fragment of 3R were found. Among 1149 *Pr Ly* males, supposedly heterozygous for *Ly* and for the translocation carrying *Pr*, crossed to *rucuca* females, one male proved to have two intact third chromosomes, bearing *Ly* and *rucuca*, respectively, and the *Pr* bearing fragment of 3R. In the same experiment, hyperploidy was detected among the offspring of four *Pr Ly* males by the presence of *Pr Ly* F_1 in addition to *Pr* and *Ly*. In the offspring of eleven of 1496 *D* males crossed to *rucuca* suppression of *ca* in a limited number of flies homozygous for *ca* and other *rucuca* genes was evidence of hyperploidy. In disjunctional flies hyperploids were detected either by the suppression of homozygous *ca* or the presence of *Pr* without *D* among the offspring of three males in a total of 990 *D Pr* crossed to *rucuca*. In other combinations hyperploids were not detectable.

Discussion

Chiasmata have long received the attention of cytologists and from the beginning their relation to crossing over has been stressed. Although the early conclusions reached by Janssens, Belling, Sax and Darlington did not coincide in regard to the exact role of chiasmata in recombination their work served to give geneticists a concrete basis for the discussion and interpretation of genetic phenomena. Cytological and genetic studies concur in establishing the early prophase of the first meiotic division as the time and place of crossing over. On the hypothesis that the chiasmata demonstrated cytologically at diakinesis and metaphase are the result of

crossing over (Darlington 1937) it is possible to correlate cytological and genetic data. Yet the exact relation between the three closely associated phenomena of meiosis, synapsis, crossing over and disjunction, has not been conclusively demonstrated.

The association of reduced crossing over with nondisjunction of whole chromosomes (Bridges 1916, Anderson 1929, 1931, Gowen 1933) first suggested a causal relationship between the two phenomena. Translocation studies (Anderson 1929, Dobzhansky 1930, 1931b, 1933, Dobzhansky and Sturtevant 1931, Beadle 1932b, Glass 1933, 1935) added abundant evidence of a negative correlation between crossing over and nondisjunction. Critical experiments designed to test the nature of this relationship have led to the conclusion that the relation is indirect. Dobzhansky (1933) concludes that the determining factor in both phenomena is synapsis, which is in turn conditioned by the force of attraction exerted between homologous loci previous to crossing over. On his theory competition between the loci of two non-homologous chromosomes involved in a translocation leads to less frequent or less intimate pairing, followed by reduced crossing over and frequent nondisjunction. Yet the amount of crossing over and the amount of nondisjunction are our only genetic means of measuring the intimacy of synapsis. Glass (1935), accepting close prophase pairing as an essential prerequisite to crossing over, nevertheless finds such a relation between crossing over and disjunction too direct. Glass postulated the determination of an axis of segregation, conditioned by forces exerted between homologous loci, but only secondarily related to crossing over. Beadle (1932b) concludes that "normal disjunction is dependent upon post diplotene association of chromosomes which is dependent on chiasma formation, which, in turn, is related (either as cause or effect) to crossing over." Pipkin (1940) found chiasma formation to be only an accessory agency in determining the disjunction rate in females heterozygous for a 2–3 translocation.

Since disjunction is a question of segregation at the first meiotic division, cytological examination of this division when translocations are present has thrown light on the disjoining behavior of chromosomes. In forms which can be studied cytologically, the formation of rings, chains, chains plus univalents or bivalents has been demonstrated (McClintock 1930, Beadle 1932b, Sax and Anderson 1933, Burnham 1934). The evidence indicates that the association of chromosomes in rings or chains is determined by the formation of chiasmata which hold chromosomes together. In the absence of chiasmata homologous chromosomes are not associated and the univalents pass at random to the poles. When bivalents are formed, partly homologous, partly non-homologous, the members of the different pairs are distributed independently of each other and disjunctional and nondisjunctional gametes are produced with equal frequency. When rings or chains are formed, disjunction or nondisjunction occurs depending on whether alternate or adjacent centromeres pass to

the same pole. The frequency of nondisjunction varies with the chromosome configuration, the number of chromosomes involved in translocation, the type of chiasmata and the organism.

Sax and Anderson (1933) found in *Tradescantia edwardsiana* that nondisjunction is more frequent in rings of four or more chromosomes than in chains, and found further that nondisjunction is increased in configurations with subterminal chiasmata. This same effect of interstitial chiasmata is found in *Pisum* (Sansome 1932). From a review of data from *Rhoeo, Oenothera, Datura, Zea, Pisum,* and *Campanula,* they conclude that rings of isobrachial chromosomes or rings with terminalized chiasmata disjoin more regularly, and that rings of heterobrachial chromosomes or rings with interstitial or subterminal chiasmata undergo nondisjunction more often. They cite, for example, nondisjunction in excess of fifty per cent in rings of heterobrachial chromosomes *(Rhoeo, T. reflexa),* nondisjunction typically fifty per cent in rings of four chromosomes *(Zea, Pisum, Tradescantia)* and nondisjunction of less than fifty per cent in configurations of isobrachial chromosomes with terminalized chiasmata *(Campanula, Oenothera).*

If the above conditions prevail in *Drosophila,* it is probable that in this form chiasmata terminalize. Otherwise chiasmata near the break, i.e., interstitial chiasmata, would be recovered more frequently in nondisjunctional gametes. In the absence of terminalization, the homologues would stay together and in such cases as 36, 31 and 27, where no change in length of euchromatin has occurred, chiasmata would show grouping near the break.

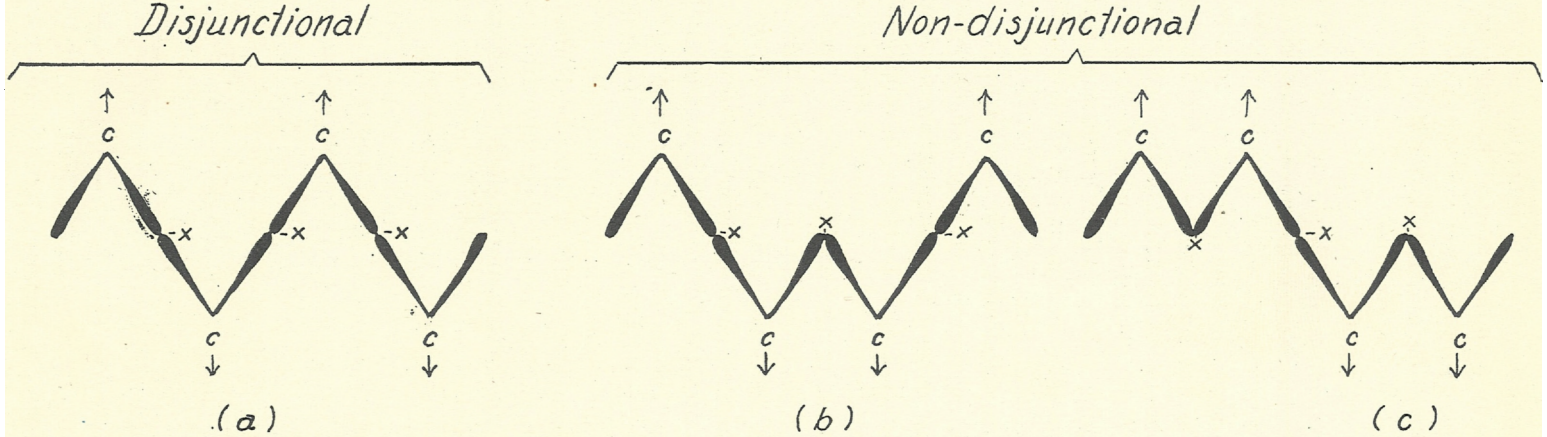
From the genetic data available in *Drosophila* translocations it is possible to construct the probable configurations at diakinesis and metaphase. In mutual 2–3 translocations the synapsed configuration is probably a cross. When all four arms have at least one chiasma, a ring of four chromosomes is formed; when three arms have chiasmata, a chain of four. When chiasmata are present in two adjacent arms, a chain of three chromosomes and a univalent result; and when chiasmata are present in alternate arms, two bivalents may be formed. Glass (1935) discusses disjunction in heteroaxial 2–3 translocations in relation to pachytene configuration without correlating crossover data, hence the metaphase configurations conditioned by chiasmata cannot be determined. Examination of Dobzhansky's data for a 2–3 translocation shows that in the disjoining arms of nondisjunctional gametes, crossing over was normal, hence approximately one chisma per arm was formed. In the nondisjoining arms crossing over was reduced by half; hence a chiasma was present roughly fifty per cent of the time. Dobzhansky's data cannot be analyzed directly to determine the chiasma configurations. In Pipkin's (1940) analysis of her data, most often a chiasma was present in each arm giving a ring of four, and next in frequency was a chain of four resulting from a chiasma in three of the arms. By comparison and analogy to this case, we infer that most of Dobzhansky's configurations

were chains of four chromosomes. On the basis of crossover frequency it appears that any arm is equally likely to be open at the ends. Thus four types of chains, equal in frequency, are possible. Each chain configuration results in disjunction and nondisjunction with equal frequency. Most probably only one type of nondisjunction, that resulting from the random distribution of the open arms of the chain, occurs in any one type of chain. The chiasma frequency of one per arm in the disjoining arms in all nondisjunctional gametes, and of approximately five-tenths per arm in the nondisjoining arms, is obtainable only on this assumption. If nondisjunction of all types occurred in each chain configuration, and disjunction occurred in equal frequency with the sum of the two types of nondisjunction (as random distribution of two pairs, two by two, would produce), crossing over in the disjoining arms of nondisjunctional gametes would be reduced, and would be equal in frequency in all types of disjunction and nondisjunction. Dobzhansky's data are not in agreement with this second assumption, although cytologically this phenomenon has been demonstrated. Unfortunately most cytologists do not differentiate between the two types of segregation of adjacent chromosomes in a chain of four, and do not give figures of the comparative frequency of the two types of nondisjunction. Gairdner and Darlington (1931) do make this distinction and report an equal frequency of the two types of nondisjunction from chains of four in *Campanula*.

Burnham (1932) and Brink and Cooper (1932) have reported cases of interchange in maize which gave a T configuration in synapsis rather than a +. In these cases the interchange involved unequal segments, one being very small; Brink and Cooper in fact spoke of their case as a nonmutual translocation. In both cases only about 25 per cent pollen sterility resulted as the aneuploid class deficient for the very small sector survived. Both Brink and Cooper and Burnham report only one type of nondisjunction configuration. In Burnham's data of 379 metaphase 1 configurations, 180 were disjunctional and 199 were nondisjunctional of one type. Figure 4 illustrates the possible types of separation 2 by 2 from a chain of four chromosomes. Types a and b are those found in maize and postulated for Dobzhansky's case in *D. melanogaster*. See also Pipkin (1940).

In Dobzhansky's and Pipkin's equiaxial 2–3 translocation the configurations are similar but the two types of nondisjunction giving an equal number of chromosomes in each gamete differ in frequency in the two translocations. In Glass' heteroaxial 2–3 translocations forming a cross with one short and three long arms the two two-chromosome types of nondisjunctional gametes are unequal in frequency, disjunction of the longer arms occurring more frequently. In 3–4 translocations the pachytene configuration is probably a modified T with the two adjacent arms formed by the fourth chromosomes very short. Furthermore, since the length of the translocated fragment varies with the position of the break, the two long arms differ in length in different translocations. At metaphase,

Fig. 4 Segregation of a Chain of Four Chromosomes at Anaphase



(a) and (b) show random separation of the ends with two adjacent pairs of centromeres separating. In (c), only one pair of adjacent centromeres separate.

c = centromere

x = chiasma

unless the fourth chromosome regularly forms a chiasma, the configuration is most likely a chain. In the equiaxial translocations of Dobzhansky and Pipkin apparently the rates of nondisjunction are the same in both sexes. In the 3–4 translocations, disjunction is not necessarily the same in all cases nor in both sexes.

The other measurable difference between the sexes, and between different translocations, is crossing over. Hence it will be well to consider disjunction and the metaphase configuration in relation to chiasma formation. In the male, where no crossing over occurs, the total frequency of nondisjunction is the same in all cases. In the female, nondisjunction varies with the position of the break and with the frequency of crossing over. The variation is consistent in that disjunction increases above the male rate as the length of the fragment increases and as chiasma formation approaches the normal value. As the break is moved toward the centromere and fragments become equal in length, disjunction increases, rising above fifty per cent. This situation is comparable to that in *Oenothera* where the disjunction rate is consistently above fifty per cent.

From the egg and hatch counts of heterozygous 3–4 translocations crossed to normal, we can determine the total amount of nondisjunction which takes place, but we have no clue as to the relative frequency of the several possible types of disjunction. Judging by the comparative infrequency of nondisjunction of the non-translocated arm, as determined by recovered zygotes in the cross heterozygous males by heterozygous females, the approximate fifty per cent hatch from heterozygous males suggests that the translocated arm disjoins at random, while the non-translocated arm usually disjoins normally. However, in many translocations (Brink and Cooper 1931, Beadle 1932b, Dobzhansky 1933, Glass 1935) the total per cent of nondisjunction is approximately fifty per cent, regardless of the relative proportion of the different types. Furthermore, the data from 3–4 translocations indicate that the translocated arm does not segregate at random in the female. Disjunction in the female is related to the position of the break, and in such a way that the total nondisjunction is decreased below a frequency of fifty per cent as the length of the fragment increases, and as chiasma formation approaches the normal value.

A measure of the relative rates of nondisjunction of 3L and 3R can be obtained only from viable zygotes. However, we cannot determine the relative rates of the two types of nondisjunction directly, since the recovery of zygotes from nondisjunctional gametes is limited to the union of complementary gametes resulting from the same type of nondisjunction in both sexes. Neither can we determine from the zygote frequencies if the relative rates are the same in the two sexes. Therefore, since the total per cent of nondisjunction is not the same in both sexes in the above translocations, the frequency of gamete formation cannot be obtained by extracting the square root of the observed zygote frequencies, as Dobzhansky (1933) and Pipkin (1940) did.

As Dobzhansky (1930, 1931b) has pointed out, when both fragments of a 2–4 or 3–4 translocation undergo nondisjunction, six classes of gametes are produced. Of these two are disjunctional, and four represent the reciprocal classes of two types of nondisjunction.

In table 10 are given the relative frequencies of gametes resulting from the two types of nondisjunction as they can occur in males and in females heterozygous for a translocation. L♂ and L♀ represent the gametes resulting from nondisjunction of L in males and in females, respectively; and R♂ and R♀ the gametes resulting from nondisjunction of R in males and in females, respectively. To the right are given the relative frequencies of the two types of nondisjunction as they can be recovered among the zygotes resulting from a cross of heterozygous males by heterozygous females. Under these relative frequencies are indicated combinations of male and female gametes from which the combinations found in the zygotes can be obtained.

In translocations giving equal frequencies of zygotes resulting from the two kinds of nondisjunction, such as the equiaxial 2–3 translocation of Dobzhansky, the combinations 3, 5, and 7 can apply. Where the two types of nondisjunction are recovered with equal frequency in the zygotes, and where the frequency of both types of nondisjunction combined is equal in the two sexes, as determined from egg counts, the simplest assumption is that the two types of nondisjunctional gametes are produced with equal frequency in each sex, and that the frequencies of the two are the same in both sexes. However, by making suitable assumptions with regard to the values of L♂ and R♂ and L♀ and R♀ equal gamete and equal zygote frequencies can be obtained from unequal frequencies of L♂ and R♂ and L♀ and R♀ in the two sexes (see Appendix).

For 3–4 translocations, in which nondisjunction of the translocated arm is recovered more frequently, combinations 1, 2, 3, 4, and 7, for greater nondisjunction of L, and 3, 6, 7, 8, and 9, for greater nondisjunction of R, can apply. The absolute zygote frequencies depend upon the relative frequencies of gametes in the two sexes. It is not necessary that the proportions of the two kinds of nondisjunctional gametes be the same in the two sexes, or even that the same kind be more frequent in both male and female, although this situation seems more probable.

A calculation of gamete frequencies such as made by Pipkin (1940) for a 2–3 translocation can be made to determine if the rates of nondisjunction are the same in the two sexes. If by solving for x, y, and z in the ratio $4x^2 : 2y^2 : 2z^2$ . . . where x^2, y^2, and z^2 represent the frequencies of the disjunctional zygotes and two classes of nondisjunctional zygotes recovered in a cross of heterozygous males by heterozygous females, the sum of y and z as calculated for each sex agrees with the combined rates as determined from egg counts, the rates in the two sexes may be considered equal.

For any one translocation it is not difficult to select a scheme in which the nondisjunction rates not only can be estimated but satisfactorily explained in both sexes to fit that particular case. But in view of the

variation in configurations, in chiasma frequencies and in disjunction rates in the above 3–4 translocations, it is difficult to choose one scheme which will apply equally well in all cases. Although it would be logical to assume that the mechanism is the same in all cases, the relative rates of nondisjunction of L and R may well vary with each translocation. The rates of nondisjunction occurring in males of the above 3–4 translocations must be such that the combined frequency of nondisjunction of the two fragments is approximately equal in all cases regardless of the position of the break. In females, on the contrary, the rates of nondisjunction must show some correlation with the position of the break. In one sex at least the relative frequencies of the two types of nondisjunction must be such that nondisjunction of the translocated fragment is more frequent than nondisjunction of the nontranslocated fragment.

Values for the rates of nondisjunction of the left and right fragments of the third chromosome can be obtained by calculation, using the sum of the frequencies of nondisjunction of L and R as determined by the egg hatch counts of heterozygous translocations by normal, and the relative frequency of nondisjunction of L and of R as recovered in the cross heterozygous males by heterozygous females. Two calculations were made; the first, using the experimental egg hatch; the second, using the egg hatch as corrected on the assumption that the 88 per cent hatch in the control represents the expected normal hatch. Two sets of values are obtained from most calculations because a quadratic equation is involved. Examination of Table 11 shows that in each set one sex has one low and one high rate of nondisjunction, and the other sex two rates that vary in different translocations from very unequal to almost equal. Since the relative rates are reversed in males and females in the two sets of values, the choice between the two sets of values must be determined by the difference in disjunction in heterozygous males and heterozygous females. When heterozygous males are crossed to normal, the egg hatch is approximately the same in all translocations regardless of the point of breakage. When heterozygous females are crossed to normal the disjunction rates, as determined by egg hatch, vary with the position of the break. This difference suggests that the male sex is the sex in which the disjunction rates are uniform and approximately fifty per cent and the female sex the one in which the rates are variable. Equally convincing is the evidence from crossing over. In males crossing over is uniformly absent. In females chiasma formation varies with the length of the fragment translocated. Therefore it can be concluded that the correct set of values is the one in which the male rates of nondisjunction are uniform in all translocations, and the female rates vary with the translocation. In Table 11 the values are given. It will be noted that in many cases the nondisjunction rate of the nontranslocated arm exceeds that of the translocated arm or fragment. When correction is made for egg hatch, the rate of nondisjunction of the intact arm is decreased in some cases to an imaginary number. In the ideal set of data from the genetic viewpoint, the fraction $\frac{-b \pm \sqrt{b^2-4ac}}{2a}$

would be $\frac{-b \pm 0}{2a}$ and only one set of values, all positive, would be derived. Hence it appears that the correction made for egg hatch is too low in some cases and too high in others.

There is justification for taking the value where the two roots are equal as the closest approximation to the real value. This value can be found by changing the corrective factor for egg hatch to the number which satisfies this condition. This has been done and is shown in Table 11. The method is to be found in the appendix. These values are in complete accord with the hypothesis that in males uniformly the nondisjunction rate of the nontranslocated arm is low but that of the translocated arm is high while in females the rate in the translocated arm is dependent on the chiasma frequency while that of the nontranslocated arm is low. Among the possible sources of error in the calculations is the fact that the data used are from different types of crosses. In the egg counts, only unmarked translocations were used; in the cross heterozygous males by heterozygous females, the eight recessive genes of the *rucuca* stock and the three dominants, *D, Ly,* and *Pr,* were present. Furthermore, the translocations themselves, as well as these gene differences, undoubtedly had a differential effect on viability.

Hence the rates of nondisjunction given in Table 11 are not to be accepted as the actual values, but as approximations. The validity of the calculated rates can be checked in several ways. When the egg hatch is corrected, in several cases only one value for nondisjunction rates is obtained. These approach the ideal case. When uncorrected data are used, the disjunctional gametes from no-chiasma tetrads (Table 12), calculated as discussed below, exceed the nondisjunctional gametes. On the assumption that Nx tetrads contribute equally to disjunctional and nondisjunctional gametes, the calculated Nx gametic frequencies should be equal. When corrected egg hatch is used, these frequencies are more nearly equal, and when only one set of values is obtained, the gametic frequencies are equal. Hence the correction made is in the right direction, and the corresponding rates of nondisjunction should be more accurate. Furthermore, the ratios of disjunctional to nondisjunctional gametes from chiasma tetrads can be compared. When the correction factors given in Table 12 are used to compute the frequencies of nondisjunctional gametes from chiasma tetrads, the ratios obtained from data uncorrected for egg hatch closely parallel those obtained from data corrected only for egg hatch.

Therefore, one can conclude that although the nondisjunction rates for L and R in males and females given in Table 11 do not represent the exact values, the method of calculation does make it possible to postulate with a reasonable degree of certainty what the relations are. In males, the translocated fragment segregates almost at random, with a nondisjunction rate of slightly less than fifty per cent. The nontranslocated fragment usually disjoins, yet occasionally nondisjoins with a rate such that the sum of nondisjunction of L and R does not exceed fifty per cent.

In females, in translocations like 2 where the break is near the free end of the chromosome and where the sum of nondisjunction of L and R in the females equals the rate in males, segregation of the translocated fragment is likewise almost at random, with little nondisjunction of the nontranslocated fragment. As the length of the translocated fragment increases in other translocations the frequency of chiasma formation increases. On the assumption that a chiasma insures association of chromosomes at metaphase, when a chiasma is formed in the translocated fragment a chain of three chromosomes (disregarding the fourth where there is no information on chiasma formation) will usually be formed, since the mean chiasma frequency in the nontranslocated fragment is approximately one. In the chain of three chromosomes formed by 3–4 translocations, the middle chromosome, the normal third, will segregate more often from both fragments. But as the frequency of chiasma formation in the translocated arm approaches that in the nontranslocated arm, segregation of the two fragments becomes more nearly the same as in chains of four chromosomes where the segregation of the types *a* & *b* of Figure 4 of alternate and adjacent arms is often at random. Therefore in a two to one separation of centromeres nondisjunction of the nontranslocated arm increases as nondisjunction of the translocated arm decreases below fifty per cent. Hence the nontranslocated arm may nondisjoin with a frequency approaching that of the translocated arm when the mean chiasma frequencies of the two are equal.

In translocations 36 and 27, the calculated rates of nondisjunction of L and R are nearly equal, but the derivation of two values, and the excess of disjunctional gametes from no-chiasma tetrads, indicate that the correction for egg hatch is not sufficient. In 31, nondisjunction of the nontranslocated fragment is much less frequent than that of the translocated fragment, but the presence of imaginary roots of numbers indicates that the correction may have been too great in spite of an approximate equality of disjunctional and nondisjunctional gametes from no-chiasma tetrads. The actual values for these translocations therefore probably lie somewhere between the calculated rates for 36 (or 27) and 31.

Using the frequencies of no-chiasma tetrads and of chiasma tetrads (100–Nx) among disjunctional and nondisjunctional zygotes, the frequencies of disjunctional and nondisjunctional gametes produced from each type of tetrad can be calculated. In Table 12 these frequencies are given, as calculated using the disjunction rate in the heterozygous female as determined by egg hatch, and the preferable rates of nondisjunction as obtained from Table 11. In the uncorrected data the disjunctional gametes from no-chiasma tetrads exceed the nondisjunctional gametes from no-chiasma tetrads. If the chromatids of no-chiasma tetrads are distributed at random, no-chiasma tetrads should lead to an equal frequency of Nx among disjunctional and nondisjunctional gametes. In any event the frequency of Nx in disjunctional gametes should not exceed the frequency in nondisjunctional gametes. When only one rate of nondisjunction is

obtained, or in cases where the rate of nondisjunction of the nontranslocated arm is decreased to a frequency equal to or less than that of the translocated arm, the disjunctional and nondisjunctional gametes from no-chiasma tetrads are approximately equal. The assumption is that those cases which still show an excess of disjunctional gametes have not been corrected sufficiently. In other words, the nondisjunction rate of the translocated arm is too low, and of the nontranslocated arm, too high. Hence a correction factor, given within parentheses in Table 12, is introduced. This correction factor is obtained by dividing the frequency of disjunctional gametes from Nx tetrads by the frequency of nondisjunctional gametes from Nx tetrads when the translocated arm nondisjoins. The same correction factor is used to correct the frequencies of gametes from both types of tetrads in the disjoining arm of nondisjunctional gametes. When the nontranslocated arm nondisjoins, as in 8 and 31, the correction factor serves to reduce the number of nondisjunctional gametes from the two types of tetrads, since the rate of nondisjunction of the intact arm varies inversely with the rate of nondisjunction of the translocated arm in the above calculations.

From the proportion in which disjunctional and nondisjunctional gametes are produced from tetrads in which one or more chiasmata are present, we can obtain a measure of how effective chiasmata are in directing disjunction. By dividing the number of disjunctional gametes from chiasma tetrads by nondisjunctional gametes from chiasma tetrads we obtain the ratios given in Table 12. These ratios vary between 4.7:1 and 21:1. However, the difference in disjunctional and nondisjunctional gametes from chiasma tetrads is subject to the same error as the difference in disjunctional and nondisjunctional gametes from Nx tetrads. Therefore the nondisjunctional gametes from chiasma tetrads can be corrected by using the same correction factor. The ratios obtained from data so treated vary between 3.6 and 12.6. When the egg hatch is corrected the difference between the two ratios for any one translocation is slight. Allowing for error introduced by the nondisjunction rates, which are not necessarily the actual values, we may draw the conclusion from the ratio of disjunctional to nondisjunctional gametes from chiasma tetrads that a chromosome arm in which at least one chiasma is formed disjoins normally five to ten times as frequently as it nondisjoins.

It is seen in Table 12 that the per cent of Nx gametes is very high in 9 Rl and 2 Rl. This is true in disjunctional as well as nondisjunctional gametes, since chiasma formation is reduced on both sides of the break in heterozygous translocations when the break is within the arm. In either case, since Rr is the translocated fragment, Rl disjoins normally, as it segregates with L and the third chromosome centromere. Apparently the presence or absence of chiasmata in Rl is of little importance in the disjunction of the entire fragment. The data indicate that nondisjunction of this fragment is infrequent in 9 as well as in 2, and length of the fragment is undoubtedly a factor. Yet it would appear that it is the chiasmata in L which determine disjunction of the nontranslocated fragment. The

additional length which Rl adds to L to make up the nontranslocated fragment acts to increase its rate of disjunction indirectly; namely, the longer Rl, the shorter Rr, and the fewer the chiasmata which will form in the translocated fragment. When chiasmata form in Rr and a chain of three chromosomes is formed, the possibility of nondisjunction of L (plus Rl) exists. When no chiasmata form in Rr, the configuration remains a bivalent, and L plus Rl disjoins normally from the third chromosome. We infer that the fourth chromosome is probably not involved to form a chain of four as disjunction here is not comparable to the results of Burnham (1932) with maize on the same basis.

In the disjunctional gametes of heterozygous translocations there is a decrease in total chiasma tetrads in the translocated arm in all cases except 31. The reduction cannot be explained by Dobzhansky's (1931a) hypothesis of competitive pairing, as the attraction between the genes of the fourth chromosome is scarcely sufficient to compete with the genes of the third. That competition from the fourth cannot be responsible is borne out by Beadle's (1933) study of haplo–4 translocations, in which the reduction in crossing over is equal to that in diplo–4 translocations. The suggestion of Dubinin *et al.* (1935) that the centromeres are attracted toward a fixed position in the nucleus during meiotic prophase furnishes a more tenable explanation of the reduction in crossing over in heterozygous translocations. In this hypothesis sections translocated to the fourth chromosome experience difficulty in synapsing because they are pulled toward the center away from their homologues. The lesser reduction in crossing over as the point of breakage approaches the centromere of the third chromosome is in agreement with this hypothesis. The reduction in chiasma formation in heterozygous 2 Rl likewise can be explained by the change in configuration, the intact arm being attracted toward both the proximal fragment and the distal fragment, now moved toward the center of the configuration by the centromere of the fourth chromosome.

In the nontranslocated arm the frequency of chiasma tetrads and of total chiasmata is reduced in translocations with the break near the centromere, i.e., 36, 31, and 27. On the assumption that no-chiasma chromosomes are distributed at random, an increase in Nx gametes in these translocations is to be expected. The fact that the increase is as great in the intact arm as in the translocated arm agrees with the hypothesis that in these translocations nondisjunction of the two arms is more nearly equal. In translocations with the break nearer the end of the chromosomes, where the nontranslocated fragment is long and nondisjoins less frequently, little or no decrease in chiasma formation should be apparent. The normal or increased chiasma frequencies in the nontranslocated arm in 8, 9, and 2 are in agreement with this hypothesis.

In gametes in which the translocated fragment fails to disjoin chiasma formation is apparently greatly reduced in the nondisjoining arm. No-chiasma gametes are frequent, and single and double chiasmata are rare. In translocation 2 practically all gametes nondisjunctional for the translocated fragment are from tetrads with no chiasmata within the limits

of the fragment. This is consistent with the hypothesis that in heterozygous 2 females the translocated fragment segregates almost at random as in the male. In 8 and 9, with longer fragments, more chiasmata are formed, and the no-chiasma gametes are less frequent. In 36 and 27 the Nx frequency is still lower, and in 31 only fifty per cent of the gametes are from Nx tetrads. Hence, chiasma frequency varies directly with the length of the fragment and its rate of disjunction.

However, an analysis of the single:double ratios in nondisjunctional gametes (Table 13) and a comparison of these with those in disjunctional gametes from heterozygous translocations, discloses the fact that among those nondisjunctional gametes resulting from chiasma tetrads, chiasma formation in relative frequency of single and double exchanges is no different than in disjunctional gametes. In Table 13 are given the actual frequencies of no-chiasma, single, double and triple chiasma configurations in comparison with the frequencies expected (X) on the basis of random distribution of chiasmata. These calculations were made by Haldane's (1931) method, using the Poisson expression $\frac{e^{-m} m^x}{\lfloor x}$, where m is the mean number of chiasmata in a given case. The deviation of actual from expected frequencies demonstrates first of all the phenomenon of interference. This is found in the normal third chromosome as well as in the translocations. It is apparent also from the difference between actual and expected single:double ratios. In the absence of interference the expected single:double ratios vary with the per cent frequency of chiasmata. With a mean chiasma frequency of one per arm, as in normal 3L, the expected ratio of singles to doubles is 2:1. As the mean chiasma frequency rises above one, as in 3R, the single:double ratio decreases. When the mean chiasma frequency is below one per arm, as in the heterozygous translocations, the single:double ratio rises above 2:1. Therefore, since the chiasma frequency is much less in nondisjunctional than in disjunctional gametes, the expected single:double ratio is much higher in the nondisjunctional gametes. However, in spite of the greater frequency of Nx among nondisjunctional gametes, as compared to the disjunctional gametes, the actual single:double ratios remain the same. Hence interference in chiasma tetrads in heterozygous translocations must be the same regardless of whether chromosomes are to disjoin or nondisjoin. The same type of meiosis contributes to disjunctional or nondisjunctional gametes. The apparent great difference between the two lies in the fact that each represents a selected group of gametes produced in heterozygous translocations. No-chiasma tetrads contribute equally to disjunctional and nondisjunctional gametes. Chiasma tetrads, on the other hand, usually produce disjunctional gametes in a ratio varying between 5:1 and 10:1 as shown above. It cannot be determined how many no-chiasma gametes may be derived from unsynapsed fragments, since Nx chromosomes may or may not have been synapsed; but when chiasmata are formed, disjunction usually takes place. Therefore the nondisjunctional gametes

comprise principally no-chiasma gametes with a limited number of gametes from chiasma tetrads. The disjunctional gametes are derived from an equal *number* of no-chiasma tetrads with an accumulation of gametes from chiasma tetrads. Therefore, even though the number of Nx gametes may be the same in disjunctional and nondisjunctional classes, the per cent frequency of Nx in the two groups will be determined by the frequency with which chiasmata are formed in heterozygous translocations.

A picture of the chiasma-disjunction relations in heterozygous translocations can therefore be obtained only when the data from disjunctional and nondisjunctional gametes are combined in the proportion in which these are formed in the female. Since the frequencies of gametes from Nx and chiasma tetrads given in Table 12 were calculated by using the rates of disjunction and nondisjunction, they are in proportion within each arm. Hence by summing the Nx gametes we obtain the total Nx produced in the heterozygous translocation. The total chiasmata configurations can be obtained the same way. In translocations 8 and 31 crossover data were obtained from a limited number of gametes nondisjunctional for the nontranslocated arm. In other translocations these data are lacking. However, in 2, and probably in 9, nondisjunction of the nontranslocated arm is too infrequent to affect markedly the frequency of chiasmata in this arm. The percentages of no-chiasma and chiasma tetrads from actual and corrected data vary, as seen in Table 12, but each of the three sets of values for any one translocation, and for all translocations, is consistent in that no-chiasma tetrads are more frequent for the translocated arm. Comparing translocations, it is obvious that the frequency of no-chiasma tetrads in the translocated fragment decreases with increasing length of the fragment. In the nontranslocated arm, the frequency of no-chiasma tetrads is low in all cases, but in translocations with the break near the centromere, in which the intact arm probably undergoes considerable nondisjunction, the Nx frequency is slightly higher.

The relation of disjunction to chiasma formation is apparent when the data are calculated for the two arms of the chromosome at the same time (Tables 14 and 15). The variation between the two sets of disjunctional data in 8 and 31 can be attributed to random sampling but the difference between chiasma frequencies in disjunctional and nondisjunctional gametes is real. The positive correlation between absence of chiasmata from one arm and nondisjunction is obvious from these tables.

In homozygous translocations the distribution of crossing over is definitely related to the position of the break with respect to the centromere. Beadle (1932a) in a homozygous 3–4 translocation attributed the reduction in crossing over in certain regions to the greater proximity of the spindle fiber of the fourth chromosome. In homozygous X–4 translocations Stone (1934) found a similar reduction, and found further that on the opposite side of the break crossing over was either not changed or higher than normal. Pipkin (1940) in a homozygous 2–3 translocation with no change in chromosome length or distance from the centromere found most regions studied to be normal in chiasma frequency.

In the homozygous 3–4 translocations described above there is an appreciable reduction in chiasma frequency only in those cases which have marked regions moved closer to the centromere, i.e., 8L and 2Rr. In 2Rl, where no change in centromere position is involved, the frequency of chiasma formation is increased, not only above the value for heterozygous 2Rl but above normal. That the difference in change in chiasma frequency in homozygous 8L and homozygous 2Rl is not a question of the presence of foreign chromatin is indicated by the fact that 2, like 8, is a mutual translocation with the distal end of the fourth chromosome attached to 2Rl. These changes in chiasma frequency are in agreement with those found by Stone (1934) in certain X–4 translocations. Among these X–4 cases, in translocation 4, chiasma formation in Xl in the homozygous cross is reduced below the heterozygous value. In homozygous Xr, as in homozygous 2Rl, crossing over is increased. The reduction in chiasma formation in heterozygous and homozygous 8L and 4l can be attributed to the change in centromere position, but the greater reduction in the homozygous as compared to the heterozygous cross requires further explanation. In the homozygous cross, both homologues have chiasma frequency reduced by change in centromere position; in the heterozygous cross, one homologue only. Hence it appears that each pair of sister chromatids of a tetrad exercises its capacity to form chiasmata independently of the other pair.

These same relations have been expressed by Mather (1936, 1937, 1938) in terms of differential and interference distance with the added limitations of a time sequence and position determination of chiasma formation. In 8L, where chromosome length is decreased from the proximal end, the differential distance is moved by change in centromere position into regions previously affected by interference distance only. To agree with the experimental data, the differential distance, even though shorter than in the normal third chromosome, must decrease crossing over more than does the interference distance. This is simply another way of stating that the centromere influence on the reduction of crossing over in a given region is greater than the interference brought about by the formation of chiasmata in the same or adjoining region in the absence of the centromere influence. The lesser decrease in chiasma formation in heterozygous 8L as compared to homozygous 8L is explained simply by assuming that the differential distance is restricted to its own chromosome.

It has been postulated that the distribution of chiasmata is causally related to the position on the chromosome at which crossing over begins (Kikkawa 1932, Mather 1936, 1937, 1938). The change found in homozygous 2Rl can be explained by assuming that chiasma formation begins at either end of the chromosome. On Mather's hypothesis chiasma formation begins at the centromere, at a given differential distance related to the length of the chromosome. Since chromosome length is decreased in 2Rl, the differential distance is decreased, and chiasma formation now takes place closer to the centromere. The interference distance remaining

the same, more chiasmata can form in the regions in question than could previously. Therefore the frequency of chiasma formation in homozygous 2Rl can exceed not only the heterozygous value but the control value as well.

However, the increase in chiasma formation in homozygous 2Rl can be explained by assuming that chiasma formation begins at the distal end as assumed by Kikkawa. The regions in 2Rl will usually be occupied by the second chiasma, which is restricted by the interference between it and the first. In the absence of the end region, removed by translocation to another chromosome, the first chiasma can form in the regions usually occupied by the second chiasma without the limiting influence of the original first chiasma.

According to Charles (1938) the curves obtained by Mather can be obtained if crossing over begins at either end of the chromosome, or is determined jointly from the centromere and from the free end. From a comparison of tetrad exchange frequencies and distances along the salivary gland maps Charles found that the average position of the exchange, in single chiasma tetrads, is approximately the midpoint of the chromosome; and in double chiasma tetrads, the average positions of the two exchanges are approximately the midpoint of the left and right halves of the chromosome. Hence his data do not support the conclusion of Mather that there is a time sequence in the formation of chiasmata beginning at the centromere. Charles suggests the possibility that the numbers and positions of chiasmata may be determined by conditions throughout the tetrad, with the centromere and the free end of little or no more importance than other portions of the tetrad.

It has been shown in certain forms (Darlington 1937) that in long chromosomes chiasma frequency is proportional to length, but in short chromosomes there is a tendency to form one chiasma. The data for the normal third chromosome show approximately one chiasma in each arm. In heterozygous translocations the total crossover configurations and total chiasmata are reduced in all except 31. The reduction always involves double chiasmata. Single chiasmata are sometimes decreased, frequently normal, coincidence usually being reduced. Hence interference in these cases must be increased by some factor which does not necessarily reduce the frequency of single chiasmata. In homozygous translocations double chiasmata are reduced in all cases in the translocated arm, partly because one region is lost through random segregation. Compared to the modified control double chiasmata are reduced only in 8. On the basis of interference calculations coincidence is decreased in regions 1–2 at a considerable distance from the break, in 8L, but not in regions 5–6, near the break, in 2Rl. Yet chromosome length is decreased in both cases. However, in 8L chromosome length is decreased from the proximal end; in 2Rl, from the distal end. The position with respect to the centromere of the regions of double chiasma formation is changed only in 8. Therefore it is not the change chromosome length which is responsible for the change

in chiasma frequency and in coincidence, but the change in position of the centromere.

In Table 16 are given the percentages of no-chiasma, single, double and triple chiasma configurations calculated for homozygous translocations and their controls. Comparison with the expected configurations, calculated by Haldane's method, reveals the degree of interference which exists. Since the expected configurations, and the expected single:double ratios, on the basis of no interference, vary with the frequency of chiasmata, comparison is best made between crosses having an equal chiasma frequency. It is evident that with one exception homozygous translocations differ little from their respective controls. Only 8L has a single:double ratio which is abnormally high. The chiasma frequency is reduced below normal, yet the total frequency is no less than in 2Rl, a fragment of comparable length. Hence interference is determined not alone by the length of the chromosome, not by the total number of chiasmata, but by the distribution of chiasmata in relation to the centromere.

The independence of crossing over in the two arms of the second and third chromosomes has been concluded from interference calculations by Kikkawa (1932), Graubard (1934), Mather (1936) and Stevens (1936). It has been concluded also from the writer's own data from calculations, not presented here, in which the arms of the third chromosome were considered mutually dependent. In these calculations the coincidence of regions across the centromere is usually one. The relation is clearly seen in the examination of experimental crossover data. Half of the exchanges in one arm involve an additional exchange in the other arm. It has been postulated by Mather (1936) that the absence of interference between the two arms is due to a special property of the centromere. However, this independence of the two arms may merely reflect the fact that, as measured on the salivary gland map as well as the oögonial chromosomes, the median region in which crossing over is usually studied has a physical length equivalent to that of approximately forty-five to fifty crossover units measured from the free end of either arm. Namely, the *st-cu* region is physically equal in length to the remaining section of either arm. The *st-p* region is not much shorter. This actual distance may be sufficient to make double chiasmata across the centromere independent without resorting to a postulated effect of the centromere on coincidence in the two arms.

The data for homozygous 8 and 2 show that interference is conditioned not solely by the frequency of chiasmata in the several regions under consideration. The single:double ratios bear out this observation. The change in chiasma formation in 8L is proof that the centromere reduces crossing over. The data indicate that the centromere also increases interference. Therefore, there is no reason to assume that the centromere in a median position destroys interference between the two arms. The fact that crossing over does not occur freely in the *st-cu* region does not imply that this long distance will not influence interference. In view of evidence that the centromere can increase interference, whereas there is no evidence that it

can reduce interference, it is all the more likely that distance alone can explain the absence of interference across the centromere.

Mather (1936, 1937), in relating the position of chiasma formation to the centromere, emphasizes the necessity of considering each arm separately. He states (1937) "the two arms probably act independently in crossing over so preventing comparison of one and two armed chromosomes." In view of his belief that cytological chiasmata are the result of crossing over (1936, 1937) the independence of the two arms in regard to crossing over should facilitate comparison with one armed chromosomes. After calculating the mean chiasma distance separately for each arm in Drosophila chromosomes Mather (1937) cites data from Levan (1935) on chiasma frequency in several V-shaped chromosomes of *Allium zebdanense* to prove that chiasma frequency is proportional to length, yet fails to separate the chromosomes into their two component arms. If the two arms of a V-shaped chromosome are independent, and if each chromosome, no matter how small, will form at least one chiasma, the frequency of chiasmata should be proportional, not to the length of the whole chromosome, but to the length of each arm measured separately. There should be a minimum of one chiasma per arm, and the formation of a chiasma in one arm should not interfere with the formation of a chiasma in the other arm. In the data cited, the chiasma frequency is less than two for two of the two-armed bivalents. The length—chiasmata frequency of these tetrads, which form less than one chiasma per arm part of the time, should not agree with that of the bivalents which form a minimum of one chiasma per arm; and when plotted, should not necessarily fall on the same straight line.

A comparison of the chiasma frequencies in translocations with breaks near the centromere fails to reveal any significant role of the heterochromatin in chiasma formation. In homozygous 36L, where the inert region is removed, both the amount and distribution of chiasmata, as well as the single:double ratio, are equally as normal as in homozygous 31L where most of the inert region is retained, and in 27R, where no heterochromatin is involved. All three cases are approximately normal when compared to the modified control in regard to single, double, and total chiasmata. If anything, 31 shows more change than 36. In the heterozygous tests, 36L shows no greater reduction in chiasma formation than does 27R and does not differ greatly from 31 disjunctional. Hence there is no reason to suppose that the heterochromatin has any appreciable effect on chiasma frequency or distribution.

Mather (1936) explains the difference in position of curves plotted from genetical distance against salivary gland cytological distance on the absence of inert regions in salivary gland maps. He states that "crossing over is known to occur in the inert regions of the X chromosome in the male"; yet proof that such crossing over occurs with a frequency sufficient to effect values of euchromatic crossing over is not at hand. Philip (1935) found the frequency of crossing over in the *bobbed* region between the X and the Y in the male to be one in 3,000. In a crossover experiment with

f car bb ey in the female (Brown 1940) so few crossovers occurred in either male or female that the rate of chiasma formation in the heterochromatin cannot be considered to be of any consequence in euchromatic crossover rate.

Unless crossing over occurs where it is not detectable, chiasma frequency in homozygous 8 is little more than fifty per cent of the normal value. The chromosome break is approximately at *st,* hence little or no undetected crossing over can be expected to occur between *st* and the centromere, especially in view of the reduction in crossing over found near the centromere. In the normal third chromosome the frequency of chiasma formation between *st* and the centromere is only 1.26 per cent. Nondisjunction in homozygous 8 females is only 0.8 per cent. If it is postulated that at least one chiasma forms in each tetrad in which disjunction is normal (Mather 1937), then the 44.7 per cent no-chiasma tetrads in the data from homozygous 8 must have formed a chiasma beyond *ru* to insure normal disjunction. Even if the slight decrease in egg hatch in homozygous 8 were due to nondisjunction, there would still have to be a large per cent of chiasmata beyond *ru* to equal the nondisjunction rate. Consequently a high frequency of chiasma formation must occur here in the normal chromosome to explain why it can occur in the translocation. If by a decrease in differential distance chiasma formation is increased in distal regions, as beyond *ru,* chiasma formation should have been increased between *ru* and *h* also. In the absence of an increase here, there is scant reason to postulate an increase in the region beyond. In translocation 2 the crossover count for the homozygous Rr fragment is admittedly poor, since the marked genes *Pr* and *ca* are not at the ends of the fragment. Crossing over could have occurred beyond the markers, especially beyond *ca,* but in view of the centromere influence on crossing over, such exchanges could not have been frequent. In 9, crossing over was not studied in the homozygous translocation, but data from similar cases suggest that chiasma formation in the translocated fragment is decreased rather than increased. Yet in 9 and 2 the egg hatch from homozygous females is normal, giving no indication of nondisjunction. Therefore in view of evidence that no-chiasma tetrads do not necessarily mean nondisjunction, and in the absence of more convincing evidence that at least one chiasma always occurs in disjunctional gametes, use of *Drosophila* data to prove the occurrence of localized chiasmata is not warranted.

Crossing over as ordinarily understood is obviously not necessary for disjunction in the male of *Drosophila.* The data from homozygous translocations indicates that neither is crossing over necessary for disjunction in the female. In view of Darlington's (1934) demonstration of a specialized type of chiasma formation, ostensibly for disjunction, in the sex chromosomes of *D. pseudoobscura,* perhaps the possibility of its occurrence in females should not be excluded. However, in the absence of evidence to indicate its existence, the assumption of genetically undetectable chiasma formation in females would serve only to complicate the problem further. From the above study of heterozygous and homozygous

3–4 translocations the following relations between disjunction and chiasma formation appear. Normal disjunction, as distinguished from chance disjunction resulting fifty per cent of the time from random distribution of a chromosome, can occur in *Drosophila* without the formation of chiasmata when both homologues have the same length and same gene number and sequence. This may apply to normal chromosomes as well as to translocations, since no-chiasma tetrads are found even in the control. The ten per cent inviable eggs in the control cannot be attributed to nondisjunction due to no-chiasma tetrads because, if this were the case, the inviability in homozygous translocations could not remain uniformly ten per cent in spite of an increase in no-chiasma frequency. In the presence of a chromosomal abnormality, such as a heterozygous translocation, disjunction is not normal because the homologues are no longer equivalent in structure. In the male, nondisjunction of the nontranslocated arm, which remains completely homologous to the normal third chromosome from one end of the chromosome to the centromere, is infrequent. The translocated arm, now attached to a centromere which is not duplicated on the normal third chromosome, segregates almost at random. In the female, disjunction is correlated with chiasma formation in such a way that in special cases, such as heterozygous translocations, the formation of chiasmata in the translocated arm may result in an association between homologues which might otherwise be lacking.

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SUMMARY

A. Crossing Over and Coincidence

1. In heterozygous 3–4 translocations the frequency of single chiasma tetrads is reduced in the translocated arm when the break is within the euchromatin of the arm. The reduction is found on both sides of the break. When the break is near the centromere the frequency of single chiasmata is normal or increased.

2. The frequency of double chiasma tetrads is reduced in the broken arm in all cases. The increase in interference is inversely proportional to the length of the translocated fragment.

3. In the non-translocated arm the distribution of chiasmata may be altered without change in total frequency. A slight reduction in double and total chiasmata is found in translocations with the break near the centromere.

4. In nondisjunctional gametes, the frequency of no-chiasma tetrads is high for the nondisjoining arm. The frequency of no-chiasma tetrads is proportional to the rate of nondisjunction of the translocated fragment.

5. In nondisjunctional gametes in which chiasma formation has occurred interference relations are the same as in disjunctional gametes of heterozygous translocations.

6. In homozygous translocations a significant reduction in chiasma formation is found only when a marked change in centromere position has occurred. In one case, with the break near the distal end of the chromosome, chiasma formation in the proximal fragment is increased.

7. Breaking the third chromosome into two fragments causes shifts in chiasma distribution due to changes in local interference. Total interference, as measured by single: double ratios, is increased in homozygous translocations only when the centromere position is changed.

B. Disjunction and its Relation to Chiasmata

8. The rates of nondisjunction are not the same in males and females, nor in all 3–4 translocations.

9. In males the translocated arm or fragment segregates almost at random in all translocations regardless of the length of the fragment. The non-translocated arm nondisjoins rarely, the combined rates of nondisjunction of both fragments not exceeding fifty per cent.

10. In females, disjunction in heterozygous translocations is correlated with chiasma formation. When no chiasmata are formed in the translocated fragment, the fragment segregates approximately at random. When a chiasma is formed, disjunction usually occurs, the rate of nondisjunction decreasing as chiasma formation approaches the normal value. As a consequence of these relations, the amount of recombination is lower in nondisjunctional than in disjunctional gametes as Nx tetrads contribute an equal number to each but chiasma tetrads contribute mostly to disjunctional gametes. If we translate these relations into chiasma configurations at metaphase and anaphase, the distribution may be described thus (the segregation of the 4-chromosome is ignored as it could not be followed satisfactorily): If a bivalent and a univalent are formed, the univalent goes at random. If a chain of three chromosomes is formed, these disjoin 5–10 times as often as they undergo nondisjunction. Apparently in Dobzhansky's (1933) case of 2–3 translocation a chain of four chromosomes was usually formed and any one of the four arms of the synaptic cross configuration could fail to form a chiasma to give rise to the chain with equal frequency. This accounts for Dobzhansky's results if the chain usually segregated so that two pairs of adjacent centromeres go to the opposite poles. (See Figure 4, also Pipkin (1940). This is similar to Burnham (1932) and Brink and Cooper's (1932) results with maize.

11. Nondisjunction of the non-translocated arm increases in females as nondisjunction of the translocated arm decreases. In translocations with the break at the centromere the nondisjunction rates of the two fragments may be nearly equal.

12. The combined rates of nondisjunction of the two fragments in females decrease below fifty per cent as the length of the translocated fragment increases.

13. Disjunction in the female is determined by the chiasmata present to a great degree although other forces enter and are present in the male. It cannot be determined by axis of separation as Glass (1936) postulated nor by synapsis as Dobzhansky suggested. The only measure of intimacy of synapsis, that is, crossing over, shows that synapsis was as intimate in nondisjunctional as in disjunctional gamete formation as the single:double ratio was the same. Pipkin (1940) has discussed the relations in a 2–3 translocation at some length.

14. In homozygous translocations disjunction is normal in both sexes. There is little if any correlation between disjunction and chiasma frequency here even in the female.

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APPENDIX

Derivation of Formulae

For the calculation of chiasma frequency in the control, in heterozygous and homozygous translocations, and in the disjunctional arms of chromosomes in exceptional gametes, the following formulae were used:

$$Nx = Nob - Sob + Dob - Tob \quad (1)$$
$$Sx = 2(Sob - 2Dob + 3Tob) \quad (2)$$
$$Dx = 4(Dob - 3Tob) \quad (3)$$
$$Tx = 8(Tob) \quad (4)$$

where Nx, Sx, Dx and Tx = the total non-, single, double and triple chiasma tetrads, respectively; and Nob, Sob, Dob and Tob = the total number of non-, single, double and triple crossover flies observed.

$$Sx_a = 2[S_aob - (D_{ab}ob + D_{ac}ob) + T_{abc}ob] \quad (5)$$
$$Sx_b = 2[S_bob - (D_{ab}ob + D_{bc}ob) + T_{abc}ob] \quad (6)$$
$$Sx_c = 2[S_cob - (D_{ac}ob + D_{bc}ob) + T_{abc}ob] \quad (7)$$
$$Dx_{ab} = 4(D_{ab}ob - T_{abc}ob) \quad (8)$$
$$Dx_{ac} = 4(D_{ac}ob - T_{abc}ob) \quad (9)$$
$$Dx_{bc} = 4(D_{bc}ob - T_{abc}ob) \quad (10)$$
$$Tx_{abc} = 8(T_{abc}ob) \quad (11)$$

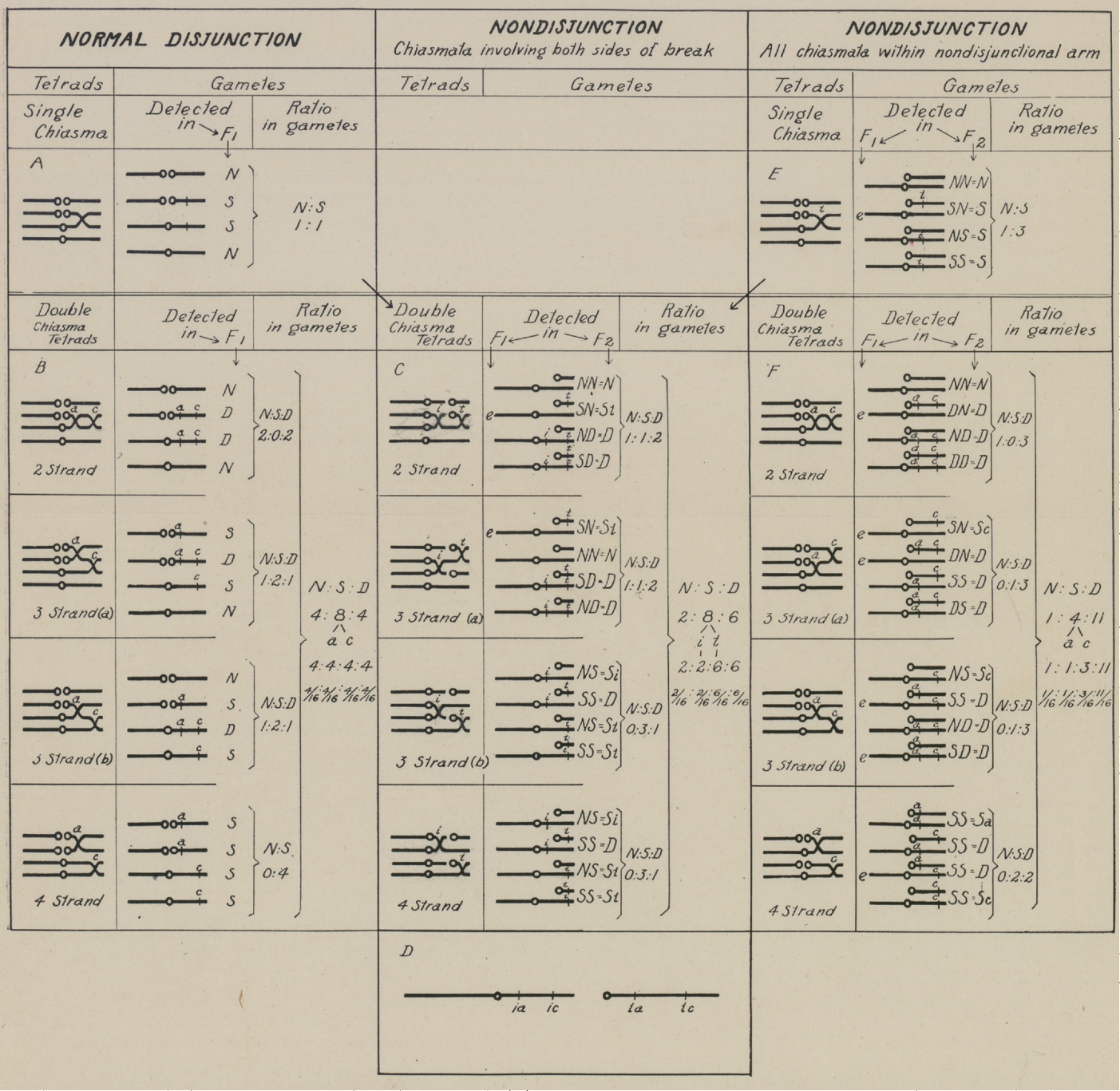
where Sx_a, Sx_b and Sx_c = the number of tetrads with a single chiasma in the specific regions *a*, *b* and *c*, respectively; Dx_{ab}, Dx_{ac} and Dx_{bc} = the number of tetrads with two chiasmata, one in each of the specific regions indicated, respectively; Tx_{abc} = the number of tetrads with chiasmata in the three specific regions indicated; S_aob, S_bob and S_cob = the number of flies observed with a crossover in regions *a*, *b*, and *c*, respectively; $D_{ab}ob$, $D_{ac}ob$ and $D_{bc}ob$ = the number of flies observed with two points of crossing over, one each in regions *a* and *b*, *a* and *c*, and *b* and *c*, respectively; $T_{abc}ob$ = the number of flies observed with crossing over in regions *a*, *b* and *c*.

The above formulae were derived from the generalized tetrad formula of Weinstein (1932, 1936). They differ only in symbols from those similarly derived by Mather (1933). They are given here in a form slightly more specific than necessary in order to facilitate comparison with formulae applying to nondisjunctional gametes.

For the calculation of chiasma frequency in exceptional gametes special formulae were derived to take into consideration the recovery of two strands, or parts of strands, from the four chromatids in a tetrad. The diagrams in Figure 3 show typical single and double chiasma configurations and the strands recovered upon nondisjunction of the translocated fragment. The ratios of non-, single and double crossover gametes are based on the assumption of random two strand, three strand and four strand exchange, without chromatid interference or sister strand crossing over, and with only two strands undergoing chiasma formation at any one level (Anderson 1925, Bridges and Anderson 1925, Emerson and Beadle 1933, Beadle and Emerson 1935). As the two arms of the third chromosome were considered independent in regard to crossing over no multiple crossovers involving chiasmata in both arms at once are represented.

The inclusion of two strands from the female in exceptional gametes increases the chance of detecting crossing over in a given tetrad. From disjunctional meiosis (Figure 3(A)) a single crossover strand is recovered from single chiasma tetrads fifty per cent of the time; whereas following nondisjunctional segregation (Figure 3(E)) three-fourths of the resulting gametes, when adequately marked, show crossing over. The number of non-crossover gametes is decreased proportionately. In double chiasma configurations two, three or all four strands of a tetrad may be involved.

FIG. 3 DIAGRAMS OF GAMETES AND RATIOS



This figure shows diagrams of single and double chiasma tetrads and all possible strands recovered from them in disjunctional gametes and in nondisjunctional gametes where the normal third chromosome and the translocated fragment are recovered from heterozygous translocations. The locus of the centromere is represented by a circle. The point of exchange is represented in the tetrads by a chiasma; on the crossover strands by a short vertical line. In (B), (D), and (F) the letters *a* and *c* designate the points of exchange proximal and distal with respect to the centromere. In (C), (D), and (E) the letters *i* (interstitial) and *t* (terminal) represent points of exchange intermediate between the centromere of the third chromosome and the break, and distal to the break, respectively, in the broken arm. The diagrams so laballed are applicable to translocations of either the right or the left arm, or a fragment thereof. The letter *e* to the left of gametes indicates the detection in the F_1 of recessive gene markers made homozygous by crossing over followed by nondisjunction; i.e., the so-called equationals. The letters *N*, *S*, and *D* indicate strands or gametes with no, one or two points of exchange. Note that two singles in different regions in the two strands of a nondisjunctional gamete, (C) and (F), are evidence that they were derived from a double chiasma tetrad. Of the two columns of letters under the heading detected in F_2 in (C), (E), and (F), the left column, *NN*, *NS*, *SS*, etc., designates the two strands of the non-disjunctional gametes as detected in the F_2. The right column, *N*, *Sa*, *Sc*, and *D*, indicates whether each gamete is *demonstrably* from a non, single or double chiasma tetrad. Under *ratio in gametes* in the left column are given the ratios of nons to singles, and of nons to singles to doubles as recovered in the F_1 following disjunction and in the F_2 following nondisjunction from each type of tetrad. In (A), note that the ratio is 1:1, whereas in (E), in two-strand nondisjunctional gametes, the ratio is 1:3. In the right-hand column of (B), (C), and (F) are given the combined ratios of non, single, and double exchange gametes from all types of double chiasma tetrads. Note that in disjunctional gametes, (B), the nons, the two types of singles, and the doubles are equal in frequency, each 4/16 of the total. In (C), in two-strand nondisjunctional gametes recovered from double chiasma tetrads with one exchange in the disjoining fragment, as in (A), and one chiasma in the nondisjoining fragment, as in (E), the ratio of nons is decreased to 2/16; the total singles remain 8/16 but the frequency of the two types is unequal, 2/16 to 6/16; and the doubles are increased to 6/16. In (F) in two-strand nondisjunctional gametes recovered from double exchange tetrads with both exchanges in the nondisjoining fragment, the nons are reduced to 1/16; the two types of singles differ in frequency and together equal only 4/16; and the doubles are increased to 11/16. From these ratios the conversion formulae found in the appendix are deduced.

With random recovery of any two homologues in a nondisjunctional gamete (Figure 3(F)) the two strands in the gametes resulting from the three types of exchange may be as follows: both non-crossovers, a single and a non-crossover, a double and a non-crossover, a single and a double crossover, two double crossovers and two single crossovers. In the latter case, even though each strand has taken part in the formation of only one chiasma, when the two singles involve different regions the gamete is genotypically a double crossover. A material increase in the proportion of doubles recovered from double chiasma tetrads in exceptional as compared to disjunctional offspring can be attributed to gametes of this kind. Obviously, the proportion of single and non-crossovers recovered is necessarily decreased.

A further difference between gametes recovered from disjunctional and nondisjunctional segregation is evident from Figure 3(C)(F). The two classes of singles recovered from double chiasma tetrads are not equal. The crossover distal to the centromere is recovered as a single more often than is the proximal crossover. Following three strand crossing over the proximal crossover is never recovered without the distal crossover, hence is represented in double crossover gametes only.

The same principles will apply in triple and higher multiple crossovers. However, since these were not recovered within the limits of one arm in exceptional gametes, they will not be considered here.

From figure 3(F) we see that of the sixteen kinds of gametes recovered from tetrads with two chiasmata in the nondisjoining arm, eleven are revealed in the F_2 as double crossovers. Therefore,

$$\mathrm{dDx} = 11/16\ \mathrm{Dx} \tag{12}$$

where dDx = the double crossover gametes recovered from double chiasma tetrads, and Dx = the theoretical number of double chiasma tetrads. In the absence of triple crossovers, all double crossovers observed in the experimental data are derived from double chiasma tetrads. Therefore, since all formulae must eventually be expressed in terms of observed values,

$$\mathrm{dDx} = 11/16\ \mathrm{Dx} = \mathrm{Dob} \tag{13}$$

where Dob = the number of flies recovered in the F_2 as double crossovers. Therefore,

$$\mathrm{Dx} = 16/11\ \mathrm{Dob} \tag{14}$$

The double chiasma tetrads yield also single and non-crossover gametes, which can be expressed in terms of Dx, hence in terms of Dob. From figure 3(F),

$$\mathrm{sDx} = 4/16\ \mathrm{Dx} \tag{15}$$

where sDx = the total single crossover gametes recovered from double chiasma tetrads. By substitution of (14) in (15), we obtain

$$\begin{aligned}\mathrm{sDx} &= 4/16 \times 16/11\ \mathrm{Dob} \\ &= 4/11\ \mathrm{Dob}\end{aligned} \tag{16}$$

However,

$$\mathrm{s_aDx_{ac}} = 1/16\ \mathrm{Dx_{ac}} \tag{17}$$

and

$$\mathrm{s_cDx_{ac}} = 3/16\ \mathrm{Dx_{ac}} \tag{18}$$

where $\mathrm{s_aDx_{ac}}$ = the proximal, and $\mathrm{s_cDx_{ac}}$ = distal single crossover gametes recovered from a double chiasma tetrad with chiasmata in regions a and c. By substitution of (14) in (17) and (18),

$$\begin{aligned}\mathrm{s_aDx_{ac}} &= 1/16 \times 16/11\ \mathrm{D_{ac}ob} \\ &= 1/11\ \mathrm{D_{ac}ob}\end{aligned} \tag{19}$$

and

$$\begin{aligned}\mathrm{s_cDx_{ac}} &= 3/16 \times 16/11\ \mathrm{D_{ac}ob} \\ &= 3/11\ \mathrm{D_{ac}ob}\end{aligned} \tag{20}$$

Likewise [Figure 3(F)],

$$\mathrm{nDx} = 1/16\ \mathrm{Dx} \tag{21}$$

where nDx = the non-crossover gametes recovered from double chiasma tetrads. Hence, by substitution of (14) in (21),

$$\begin{aligned} nDx &= 1/16 \times 16/11 \text{ Dob} \\ &= 1/11 \text{ Dob.} \end{aligned} \tag{22}$$

From single chiasma tetrads [Figure 3(E)] three-fourths of the gametes recovered in the F_2 reveal the crossover. Or,

$$sSx = 3/4 \text{ Sx} \tag{23}$$

where sSx = the single crossover gametes recovered from single chiasma tetrads, and Sx = the number of single chiasma tetrads. Therefore,

$$Sx = 4/3 \text{ sSx} \tag{24}$$

However, not all observed singles are derived from single exchanges; a certain proportion are derived from double chiasma tetrads. Hence,

$$sSx = Sob - sDx \tag{25}$$

where Sob = the total single crossover flies observed. By substitution of (16) in (25), and of (25) as modified in (24), we obtain,

$$Sx = 4/3 \ (Sob - 4/11 \text{ Dob}). \tag{26}$$

To determine the number of chiasmata occurring in each region separately, let a = a specific region, proximal to the centromere, and c = a specific region distal to the centromere. Let b = a specific region intermediate between a and b. Since

$$s_aDx_{ac} = 1/11 \ D_{ac}ob, \tag{19}$$

and

$$s_cDx_{ac} = 3/11 \ D_{ac}ob, \tag{20}$$

by analogy,

$$s_aDx_{ab} = 1/11 \ D_{ab}ob \tag{27}$$
$$s_bDx_{ab} = 3/11 \ D_{ab}ob \tag{28}$$
$$s_bDx_{bc} = 1/11 \ D_{bc}ob \tag{29}$$
$$s_cDx_{bc} = 3/11 \ D_{bc}ob \tag{30}$$

where b is the distal single recovered from Dx_{ab} and the proximal single recovered from Dx_{bc}.

The total number of tetrads with a single chiasma occurring in regions a, b and c, respectively, is therefore

$$Sx_a = 4/3 \ [S_aob - 1/11 \ (D_{ab}ob + D_{ac}ob)] \tag{31}$$
$$Sx_b = 4/3 \ (S_bob - 3/11 \ D_{ab}ob - 1/11 \ D_{bc}ob) \tag{32}$$
$$Sx_c = 4/3 \ [S_cob - 3/11 \ (D_{ac}ob + D_{bc}ob)] \tag{33}$$

The total number of single chiasmata can thus be calculated in toto by formula (26) or can be obtained by regions by the summation of formulae (31), (32) and (33).

Single chiasma tetrads yield also non-crossover gametes, which can be expressed in terms of Sx, hence in terms of Sob. From Figure 3(E),

$$nSx = 1/4 \text{ Sx} \tag{34}$$

By substitution of (26) in (34) we obtain,

$$\begin{aligned} nSx &= 1/4 \times 4/3 \ (Sob - 4/11 \text{ Dob}) \\ &= 1/3 \ (Sob - 4/11 \text{ Dob}). \end{aligned} \tag{35}$$

To obtain the number of tetrads having no chiasmata we subtract from the non-crossovers observed, Nob, the non-crossovers obtained from all crossover tetrads.

$$Nx = Nob - nSx - nDx \ . \ . \ . \tag{36}$$

By substitution of (35) and (22) in (36), we obtain,

$$Nx = Nob - 1/3 \ (Sob - 4/11 \text{ Dob}) - 1/11 \text{ Dob.} \tag{37}$$

The above formulae apply to cases where nondisjunction of a whole arm, either right or left of the centromere, takes place.

In translocations 9 and 2 the right arm of the third chromosome is broken within the limits of the arm. The right fragment is in each case the translocated section, hence the fragment which undergoes nondisjunction more frequently. Since the left fragment disjoins normally in these circumstances [Figure 3(C)], the configurations involving this fragment only can be calculated from the formulae for normal disjunction (1) to (11). Similarly, the configurations involving the right fragment only can be calculated from formulae (14), (26), (31), (32), (33) and (37). [Compare (C) with (A) and (E) in Figure 3.] Hence only the tetrads involving chiasmata in both fragments at once need further consideration.

Let Dx_{it} represent double chiasma tetrads involving both fragments; i.e., one chiasma between the centromere and the break, and one chiasma distal to the break in the translocated fragment. Then from Figure 3(C),

$$dDx_{it} = 3/8\ Dx_{it} \tag{38}$$

where dDx_{it} = the double crossover gametes recovered from double chiasma tetrads involving *i* and *t*.

$$\begin{aligned} Dx_{it} &= 8/3\ dDx_{it} \\ &= 8/3\ D_{it}ob \end{aligned} \tag{39}$$

in the absence of higher configurations giving rise to double crossovers.

The double chiasma tetrads in the broken arm will therefore be the sum of formulae (3), (39) and (14), as follows:

$$Dx = 4\ D_{ii}ob + 8/3\ D_{it}ob + 16/11\ D_{tt}ob \tag{40}$$

where $D_{ii}ob$ and $D_{tt}ob$ = the number of observed flies which are double crossovers in the disjoining, and in the nondisjoining fragments only.

For the number of single chiasmata, from Figure 3(C) we have,

$$sDx_{it} = 1/2\ Dx_{it} \tag{41}$$

where sDx_{it} = the total single crossover gametes recovered from double chiasma tetrads. By substitution of (39) in (41),

$$\begin{aligned} sDx_{it} &= 1/2 \times 8/3\ D_{it}ob \\ &= 4/3\ D_{it}ob \end{aligned} \tag{42}$$

Similarly,

$$s_iDx_{it} = 1/8\ Dx_{it} \tag{43}$$

where s_iDx_{it} = the gametes with a single crossover in the disjoining fragment recovered from Dx_{it}.

By substitution of (39) in (43),

$$s_iDx_{it} = 1/8 \times 8/3\ D_{it}\ ob = 1/3\ D_{it}\ ob \tag{44}$$

Similarly

$$s_tDx_{it} = 3/8\ Dx_{it} \tag{45}$$

where s_tDx_{it} = the gametes with a single crossover in the nondisjoining fragment recovered from Dx_{it}. Therefore,

$$\begin{aligned} s_tDx_{it} &= 3/8 \times 8/3\ D_{it}ob \\ &= 1/1\ D_{it}ob = D_{it}ob. \end{aligned} \tag{46}$$

Since to obtain the number of single crossover gametes derived from single chiasma tetrads, all single crossover gametes derived from double chiasma tetrads must be subtracted as in formula (25), we obtain, by incorporating formula (44) in (2), and (46) in (26),

$$Sx_i = 2\ (S_iob - 2\ D_{ii}ob - 1/3\ D_{it}ob) \tag{47}$$

$$Sx_t = 4/3\ (S_tob - D_{it}ob - 4/11\ D_{tt}ob) \tag{48}$$

for the total single chiasma tetrads in the disjunctional and nondisjunctional fragments, respectively, of the broken arm.

To obtain the number of chiasmata in specific regions, let *ia* and *ic* be specific regions of chiasma formation in the disjoining fragment, proximal and distal,

respectively, to the centromere of chromosome 3; and let *ta* and *tc* be specific regions of chiasma formation in the nondisjoining fragment, proximal and distal, respectively, to the centromere of chromosome 4 (Figure 3(D)). By utilization of certain of the above formulae we obtain the following:

$$Sx_{ia} = 2\ (S_{ia}ob - D_{iaic}ob - 1/3\ D_{iat}ob) \qquad (44)\ in\ (5) \qquad (49)$$

$$Sx_{ic} = 2\ (S_{ic}ob - D_{iaic}ob - 1/3\ D_{ict}ob) \qquad (44)\ in\ (5) \qquad (50)$$

$$Sx_{ta} = 4/3\ (S_{ta}ob - D_{ita}ob - 1/11\ D_{tatc}ob) \qquad (46)\ in\ (31) \qquad (51)$$

$$Sx_{tc} = 4/3\ (S_{tc}ob - D_{itc}ob - 3/11\ D_{tatc}ob) \qquad (46)\ in\ (33) \qquad (52)$$

where $D_{iat}ob$ and $D_{ict}ob$ = observed number of flies with one crossover in region *ia* or *ic*, respectively, and another in either *ta* or tc; and $D_{ita}ob$ and $D_{ict}ob$ = the observed number of flies with one crossover in *ta* or *tc*, respectively, and another in either *ia* or *ic*.

The number of tetrads with no chiasma in either fragment of the broken arm is obtained as above by subtracting the non-crossover gametes derived from single and double chiasma tetrads from the observed value of non-crossovers. Therefore,

$$Nx = Nob - nSx_i - nSx_t - nDx_{ii} - nDx_{it} - nDx_{tt} \ldots \qquad (53)$$

Since the non-translocated fragment undergoes normal disjunction whereas the translocated fragment nondisjoins, we obtain from Figure 3(A), (E) and (C),

$$nSx_i = sSx_i$$
$$= S_iob - 2\ D_{ii}ob - 1/3\ D_{it}ob \qquad (54)$$

and

$$nSx_t = 1/3\ sSx_t$$
$$= 1/3\ (S_tob - D_{it}ob - 4/11\ D_{tt}ob). \qquad (44)\ in\ (33) \qquad (55)$$

From normal disjunction (Figure 3(B)) we obtain, in the absence of triples,

$$nDx_{ii} = dDx_{ii} = D_{ii}ob \qquad (56)$$

and from figure 3(C),

$$nDx_{it} = 1/3\ dDx_{it}$$
$$= 1/3\ D_{it}ob \qquad (57)$$

in the absence of triples. As obtained previously from Figure 3(F),

$$nDx_{tt} = 1/11\ D_{tt}ob. \qquad (22)$$

By substitution of formulae (54) to (57) and formula (22) in (53) we obtain,

$$Nx = Nob - (S_iob - 2\ D_{ii}ob - 1/3\ D_{it}ob) - 1/3\ (S_tob - D_{it}ob - 4/11\ D_{tt}ob) - D_{ii}ob - 1/3\ D_{it}ob - 1/11\ D_{it}ob \qquad (58)$$

for the total number of tetrads with no chiasmata in either fragment of the broken arm.

For calculating the chiasma frequency in both arms simultaneously, as given in Tables 14 and 15, the following formulae were used for the nondisjunctional gametes. These formulae are identical with those derived for the broken arm of 9 and 2 with the additional complication of triple crossovers and triple chiasma tetrads. As in the formulae above, *t* represents the locus of a crossover, or a chiasma, in the nondisjoining fragment, which in 36, 31 and 27 is either the whole left or right arm of the third chromosome. The sub-script *i* represents the locus of a crossover, or a chiasma, in the disjoining arm.

$$Tx_{tti} = 32/11\ T_{tti}ob \qquad (1)$$

$$Tx_{tii} = 32/6\ T_{tii}ob \qquad (2)$$

$$Dx_{tt} = 16/11\ (D_{tt}ob - T_{tti}ob) \qquad (3)$$

$$Dx_{tii} = 8/3\ (D_{ti}ob - 4/11\ T_{tti}ob - 2\ T_{tii}ob) \qquad (4)$$

$$Dx_{ii} = 4\ (D_{ii}ob - 1/3\ T_{ii}ob) \qquad (5)$$

TABLE 1. FREQUENCY OF RECOVERED CROSS OVERS

Cross-over Regions	Total	0	1	2	3	4L	4R	5L	5R	6	7L	7R	12	13	14L	14R	15L	15R	16	17L	17R	23	24L	24R	25L	25R	26	27L	27R
Control	4264	1109	438	345	9	—85—		—217—		148	—630—		26		—32—		—81—		65	—271—		1	—18—		—91—		74	—246—	
8 Heterozygous	6242	1947	748	228	1		84	—490—		362	—1076—		10			30	—133—		134	—355—				17	72		53	—90—	
Disjunctional	859	262	112	28		—12—		—92—		37	—148—		2		—4—		—21—		19	—50—					2		2	—13—	
Homozygous	7091	1202	318	148	2	—1310—		—335—		174	—634—		3	1	—351—		—90—		62	—171—			—132—		—35—		25	—61—	
Non-disjunction of 3L	647	259	15	8			9	—70—		47	—146—					1	—11—			—8—				1	—7—		2		
36 Heterozygous	4348	1289	509	339	7	40	38	—242—		205	—611—		12	1	9	6	—82—		87	—257—			9	5	—80—		49	—159—	
Homozygous	6954	959	389	280	10	—921—		—193—		147	—539—		26	1	—381—		—85—		71	—219—		1	—274—		—83—		56	—173—	
Non-disjunction of 3L	121	43	12	8				—13—		4	—24—						—4—		1	—4—				1	—2—		2	—2—	
31 Heterozygous	5057	1550	460	500	14	100	48	—285—		208	—534—		8	2	18	14	—140—		90	—259—			10	20	—117—		90	—217—	
Disjunctional	2930	830	359	243	8	—55—		—141—		112	—462—		9	3	—13—		—52—		49	—193—		1	—3—		—41—		34	—152—	
Homozygous	6675	996	422	315	8	—992—		—179—		169	—514—		26	2	—372—		—70—		51	—205—		1	—301—		—46—		50	—146—	
Non-disjunction of 3L	563	182	40	41	4	2	6	—36—		36	—106—		4	1		3	—14—		7	—17—				1	—10—		4	—17—	
27 Heterozygous	7880	2562	886	740	15	72	24	—284—		255	—1316—		44	3	23	5	—106—		136	—518—		1	12	3	—85—		93	—379—	
Homozygous	7090	1032	416	324	9	—1089—		—188—		157	—580—		29	3	—380—		—87—		68	—220—			—301—		—62—		62	—172—	
Non-disjunction of 3R	185	70	22	32		2		—3—		3	—22—		2		1		—2—		2	—4—					—4—			—7—	
9 Heterozygous	6057	1981	866	919	54	—205—		5	8	34	—870—		166	10	—97—		2	6	16	—353—		4	—35—		5	10	14	—379—	
Non-disjunction of 3R	2716	1153	465	535	10	—79—			5	13	—142—		65	2	—29—		3	2		—38—			—14—			5	5	—95—	
2 Heterozygous	5893	2854	1119	1013	29	—186—		—138—		41	11	30	95	11	—71—		—65—		16	4	4	1	—44—		—61—		17	4	12
Disjunctional	3121	1436	686	477	25	—117—		—81—		9	—32—		74	8	—28—		—19—		12	—9—		3	—19—		—33—		11	—9—	
Homozygous	3924	1412	628	556	19	—122—		—296—		205			66	5	—63—		—133—		89			1	—26—		—135—		65		
Non-disjunction of 3R	1496	723	294	253	10	—66—		—37—		8	3	3	31		—12—		—12—		10		1	1	—8—		—9—			1	2

Cross-over Regions	34L	34R	35(R)	36	37(R)	4L5	4R5	4L6	4R6	4L7	4R7	47R	56	5L7	5R7	57L	57R	67(R)
Control	—1—		4		5	—10—		—15—		—51—			3	—73—				33
8 Heterozygous		1	1				8		16		47		4	—156—				65
Disjunctional								—1—		—3—				—22—				13
Homozygous			1		1	—291—		—240—		—660—			13	—98—				18
Non-disjunction of 3L										1	5		1	—39—				11
36 Heterozygous				2	4	7	2	7	4	17	21		2	—94—				22
Homozygous			1	1	2	—187—		—172—		—533—			8	—106—				51
Non-disjunction of 3L											1							
31 Heterozygous		1	3	3	9	20	2	14	5	34	29		5	—88—				19
Disjunctional			1		5	—11—		—5—		—26—			2	—41—				12
Homozygous	—7—				4	—208—		—153—		—523—			2	—64—				15
Non-disjunction of 3L	1		2		1			1			4		1	—5—				2
27 Heterozygous				1	7	12	2	8	3	44	13			—15—				26
Homozygous	—8—		2	1	3	—151—		—170—		—604—			2	—64—				29
Non-disjunction of 3R										3				—1—				
9 Heterozygous				1	13			—1—		—56—				1	1			6
Non-disjunction of 3R			1		4					—25—					1			2
2 Heterozygous	—2—		5	1	2	—4—		—2—				7				2	4	1
Disjunctional	—1—					—7—		—2—						—3—				
Homozygous	—3—		7	1		—26—		—23—					5					
Non-disjunction of 3R			1	1		—5—												

Cross-over Regions	123	124L	124R	125(R)	126	127(R)	134(R)	135	136	137(L)	14L5	14R5	145L	14L6	14R6	14L7	14R7	147R	156	15L7	15R7	157L	157R	167
Control				11	5	18		3		1	—6—			—9—		—16—			1	—24—				11
8 Heterozygous				3		1									2	—15—				—44—				12
Disjunctional				2																—9—				4
Homozygous		—1—		1							—88—			—49—		—211—				—30—				5
Non-disjunction of 3L						1														—4—				
36 Heterozygous			1	4		4									2	3	5		2	—38—				11
Homozygous		—15—		9	9	15	3				—84—			—59—		—232—			2	—39—				16
Non-disjunction of 3L																								
31 Heterozygous		3		3	3	5	1	1	1	2	4	2		3	1	9	3			—17—				12
Disjunctional		—1—		4		3				3	—2—			—1—		—6—				—19—				8
Homozygous		—18—		7		7			1	1	—66—			—74—		—167—			1	—28—				6
Non-disjunction of 3L			1	3	1	1					1					1				—2—				
27 Heterozygous				4	3	17		1		3	1			2		14	3		1	—14—				15
Homozygous		—8—		5	4	7	1		1		—94—			—64—		—229—				—16—				6
Non-disjunction of 3R																3				—1—				
9 Heterozygous	1	—6—			3	46			1	5			1	—1—		—31—				1	1			1
Non-disjunction of 3R				1	1	6				2				—2—		—5—								
2 Heterozygous		—6—		4	2	2	1	2		1	—2—			—2—				2				2	1	
Disjunctional		—5—		4		1		1	1		—1—			—3—						—1—				
Homozygous		—4—		11	4			2	1		—6—			—3—					3					
Non-disjunction of 3R				1							—1—												1	

Cross-over Regions	234	235	236	237	24L5	24R5	24L6	24R6	24L7	24R7	247R	256	25L7	25R7	257R	267	345	34L6	347(R)	357	367	456	4L57	4R57	45R7	457L	4L67	4R67	567
Control					—5—		—5—		—8—			1	—21—			19				1	1		—2—				—5—		
8 Heterozygous						2		1	—5—			1	—7—			7								4			—3—		
Disjunctional																													1
Homozygous					—27—		—22—		—65—			2	—11—			3			1			2	—113—				—31—		
Non-disjunction of 3L													—1—																
36 Heterozygous								1	2	2		3	—30—			7				1	1			2			1		2
Homozygous					—48—		—46—		—161—			3	—37—			11		1	1			5	—98—				—53—		1
Non-disjunction of 3L																													
31 Heterozygous					5		1	3	4	4		3	—29—			5				5	1		3	1				1	
Disjunctional		1			—1—		—2—		—1—				—6—			5							—1—				—1—		
Homozygous	1				—49—		—49—		—161—			3	—18—			1	1	1	4	1		2	—55—				—28—		1
Non-disjunction of 3L										1			—1—			2							1						
27 Heterozygous			1		1				1	4		1	—18—			7		1			2		5				2		
Homozygous					—58—		—52—		—145—			2	—13—			6		3	2	1	1	2	—56—				—17—		1
Non-disjunction of 3R													—1—																
9 Heterozygous				1			—2—		—19—				1	1		2													
Non-disjunction of 3R														1		2									1		—1—		
2 Heterozygous					—4—		—2—				1				2				1										
Disjunctional					—1—								—1—																1
Homozygous			1		—1—		—1—					1																	
Non-disjunction of 3R					—1—																					1			

Cross-over Regions	1236	1237	1245L	1246	124(L)7	1256	1257	1267	1345	1347	1357	1456	14L57	14R57	14(L)67	1567	2456	24L57	24R57	24R67	2567	4567	12347	12457	12467	13457	124567
Control							4			1		1			2	1				1							
8 Heterozygous							2	1						1					1	1	1						
Disjunctional																											
Homozygous		1	1	1	2								—29—		8			—5—		5							1
Non-disjunction of 3L																											
36 Heterozygous					1		1	3										1									
Homozygous			8	7	13		7	3				1	—35—		16		2	—30—		7	1	1	1	3	1		1
Non-disjunction of 3L																											
31 Heterozygous							2	1					2								1						
Disjunctional								1							1												
Homozygous	1	1	8		11			3	1	2			—23—		7		2	—19—		3		1		1			
Non-disjunction of 3L																											
27 Heterozygous						1	2	2											1								
Homozygous			3	1	17		1	1	1		1		—16—		10			—23—		3		1		2	2	1	
Non-disjunction of 3R																											
9 Heterozygous					3																						
Non-disjunction of 3R			1																								
2 Heterozygous																											
Disjunctional																											
Homozygous																											
Non-disjunction of 3R																											

$$Sx_t = 4/3\ [S_tob - (D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) - 4/11\ (D_{tt}ob - T_{ttt}ob) - 4/11\ T_{ttt}ob - T_{ttt}ob] \quad (6)$$

$$Sx_t = 2\ [S_tob - 2\ (D_{tt}ob - 1/3\ T_{ttt}ob) - 1/3\ (D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) - 1/11\ T_{ttt}ob - 2/3\ T_{ttt}ob] \quad (7)$$

$$Nx = Nob - 1/11\ T_{ttt}ob - 1/3\ T_{ttt}ob - 1/11\ (D_{tt}ob - T_{ttt}ob) - 1/3\ (D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) - (D_{tt}ob - 1/3\ T_{ttt}ob) - 1/3\ [S_tob - (D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) - 4/11\ (D_{tt}ob - T_{ttt}ob) - 4/11\ T_{ttt}ob - T_{ttt}ob] - [S_tob - 2\ D_{tt}ob - 1/3\ (D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) - 1/11\ T_{ttt}ob - 2/3\ T_{ttt}ob] \quad (8)$$

Use of the above formulae is simplified if values obtained for the observed configurations in parentheses are substituted in each succeeding formula, since only the constants change. For example, let

$T_{ttt}ob = a$
$T_{ttt}ob = b$
$(D_{tt}ob - T_{ttt}ob) = c$
$(D_{tt}ob - 4/11\ T_{ttt}ob - 2\ T_{ttt}ob) = d$
$(D_{tt}ob - 1/3\ T_{ttt}ob) = e$

Then

$$Sx_t = 4/3\ (S_tob - d - 4/11\ c - 4/11\ a - b)$$

and

$$Sx_t = 2\ (S_tob - 2\ e - 1/3\ d - 1/11\ a - 2/3\ b)$$

Then

$$Nx = Nob - 1/11\ a - 1/3\ b - 1/11\ c - 1/3\ d - e - 1/3\ f - g,$$

Where $f = (S_tob - d - 4/11\ c - 4/11\ a - b)$ and $g = (S_tob - 2\ e - 1/3\ d - 1/11\ a - 2/3\ b)$.

Method of Calculating Rates of Nondisjunction

Values for the rate of nondisjunction of the two fragments of the third chromosomes were obtained as follows. Using translocation 36 as an example, in the cross heterozygous males by normal females the hatch was 53 per cent (Table 7). As the normal hatch is only 88 per cent a part of the eggs from heterozygous translocations fail to hatch from causes other than nondisjunction. We shall assume that this proportion is the same. Therefore the actual rate of normal disjunction must be 100/88 of the value determined from the count. In 36 the heterozygous female value is 53 per cent. Corrected the rate of normal disjunction is .602. The sum of the rates of nondisjunction of the left and right fragments, L + R, is therefore 1.00—.602 = .398. In the cross heterozygous females by normal males the hatch is 60.8 per cent, or when corrected by 100/88, 69.1 per cent; hence the corresponding values for the rate of disjunction and for L + R are .691 and .309. The expected frequency of disjunctional zygotes from the cross heterozygous females by heterozygous males is therefore .602 × .691 = .416. We see in Table 8 that among 8630 offspring from the cross of heterozygous males by heterozygous females, the disjunctional zygotes number 8044. Since these are all derived from gametes which were disjunctional in both sexes, the 8044 zygotes must represent .416 of the random combinations of the gametes produced in both sexes. The zygotes resulting from nondisjunction of 36L in both sexes number 578 (Table 8). However, since nondisjunction results in gametes with a deficiency or with a duplication of part of the third chromosome, only zygotes resulting from the union of reciprocal gametes of the same types of nondisjunction survive. Since hypo- and hyperploid gametes are produced in equal numbers,

half the zygotes, those resulting from the union of two hypoploid gametes and of two hyperploid gametes, die.

♀ \ ♂	disjunction .602	N. D. L. L♂ + R♂ = .398	N. D. R.
disjunction .691	survive 8044 .416	all die	all die
N. D. L. L♀ + R♀ = .309	all die	50% die 578 x 2 = 1156 .06008	all die
N. D. R.	all die	all die	50% die 8 x 2 = 16 .0008274

Hence the 578 *D Ly* and *Pr* flies in Table 8 represent only half the zygotes which result from the union of gametes resulting from nondisjunction of the left fragment. Therefore,

$578 \times 2 = 1156$ = the number of zygotes resulting from nondisjunction of L.

Since the 8044 disjunctional zygotes represent .416 of the total gametes, the 1156 zygotes nondisjunctional for L represent 1156 divided by 8044/0.416 or .06008 of the total gametes. Similarly, for the zygotes resulting from nondisjunction of the right fragment,

$8 \times 2 = 16$

and this represents

16 divided by 8044/0.416 or .0008274 of the total gametes.

We can now set up the following equations:

$L♂ + R♂ = .398$
$L♀ + R♀ = .309$
$L♂ \times L♀ = .06008$
$R♂ \times R♀ = .0008274$

Where L♂ and R♂ represent the rate of nondisjunction of L and of R in males, and L♀ and R♀, the rate of nondisjunction of L and R in females.

Substituting in the equation $L♂ + R♂ = .398$

$$\frac{.06008}{L♀} + \frac{.0008274}{R♀} = .398$$

$$.398(R♀)^2 - .063729\ R♀ + .000255666 = 0$$

Therefore, solving for R♀ with the equation $\frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$

$$R♀ = \frac{.0637294 \pm \sqrt{.004061436 - .0004070212}}{.796}$$

$$= .156 \text{ or } .004118$$

The values for L♀, L♂ and R♂ can then be obtained by substituting the values for R♀ in the original equation.

Since it seems probable that the correction for egg hatch may be too great or too small, there is some justification for using as the correct value that corrective factor for which the roots are equal or coincident.

TABLE 2. CALCULATED CHIASMA FREQUENCY IN PERCENT

		Total	1	2	3	4L	4R	5L	5R	6	7L	7R	1-2	1-3	1-4L	2-3	2-4L	3-4L	123	124L	4R5	4R6	4R7(R)	5 6	5L7	5 7L	5 7R	5R7	6 7(R)	4R57	4R67	567	Recovered % Non-disjunction in 3L	No. chiasma Tetrads L	Total Singles L	Total Doubles L	Total Triples L	Total chiasma Tetrads L	Total Chiasmata L	Recovered % Non-disjunction in 3R	No. chiasma Tetrads R	Total Singles R	Total Doubles R	Total Triples R	Total chiasma Tetrads R	Total Chiasmata R	Ratio of Singles to Doubles L	Ratio of Singles to Doubles R
	Control	42.64	38.41	34.09	0.70	1.50	0.61	13.32		10.50	46.01		6.00	0.47	3.27	0.09	1.97				0.65	0.94	3.28	0.47	11.62					6.09		0.56	0.19		13.46	74.71	11.81		86.52	98.33		5.72	70.45	23.07	0.75	94.27	118.84	6.3:1	3:1
	Control-Region 4 omitted	42.64	41.69	36.06	0.70			13.97		11.44	49.29		6.00	0.47		0.09								0.46	11.63				6.66			0.19		14.96	78.47	6.56		85.03	91.59		6.33	74.71	18.76	0.18	93.65	112.77	11.9:1	3.9:1
	Control-Region 7 omitted	42.64	38.41	34.09	0.70	1.50	3.89	24.95		16.60			6.00	0.47	3.27	0.09	1.97				0.65	1.50		0.65										13.46	74.71	11.81		86.52	98.33		51.73	45.45	2.81		48.26	51.07	6.3:1	16.1:1
8	Heterozygous	62.42	46.68	15.02	0.09		1.47	15.44		14.25	37.55		1.09								0.25	0.96	3.65	0.25	12.92				5.12	0.77	0.51	0.13		37.10	61.80	1.09		62.89	63.98		6.66	68.72	23.16	1.41	93.30	119.27	56:1	2.9:1
	Disjunctional	8.59	50.05	9.54			2.79	20.25		9.54	37.48		1.87									0.46	1.40	-0.46	13.97				7.45			0.93		38.53	59.60	1.87		61.47	63.34		6.17	70.08	22.81	0.93	93.82	118.49	31:1	3.0:1
	Homozygous	70.91	39.57	15.06	0.14			15.99		13.73	40.95		0.45	0.0		-0.05			0.11					0.90	16.08				3.89			0.11		44.71	54.77	0.39	0.11	55.27	55.89		8.33	70.68	20.87	0.11	91.66	112.75	140:1	3.3:1
	Non-disjunction of 3 L	6.47	7.94	3.89		0.20	1.85	13.29		11.44	29.67		0.22										3.09	0.62	27.20				6.80				11.97	87.70	12.08	0.22		12.30	12.53	0.76	6.03	56.25	37.71		93.96	131.67	54:1	1.4:1
36	Heterozygous	43.48	44.25	29.39	0.64	2.21	0.73	11.26		13.56	37.99		2.21	0.09	1.01		1.01			0.18	0.0	0.55	2.29	0.46	14.80				3.76	0.37	0.18	0.36		18.99	76.49	4.32	0.18	80.99	85.67		13.61	63.57	21.89	0.92	86.38	110.11	17:1	2.9:1
	Homozygous	69.54	43.42	31.55	0.34			9.37		11.33	39.68		6.62	0.16		0.0			0.11					0.98	20.18				8.85			0.46		17.76	75.32	6.78	0.11	82.21	89.22		9.12	60.39	30.02	0.46	90.87	121.81	11:1	2.0:1
	Non-disjunction of 3 L	12.1	23.15	16.55			0.0	31.40		11.58	47.90												3.31										6.69	60.30	39.70			39.70	39.70	0.09	5.78	90.90	3.31		94.21	97.52		27:1
31	Heterozygous	50.57	37.01	37.54	1.14	4.64	1.46	16.68		14.23	33.85		1.50	0.55	2.61		1.34			0.47	0.23	0.63	2.68	0.55	11.39				2.84	0.16	0.16	0.16		13.11	80.40	6.01	0.47	86.88	93.84		14.92	66.24	18.35	0.47	85.06	104.36	13:1	3.5:1
	Disjunctional	29.30	44.57	31.53	0.41	2.73	1.22	12.27		11.74	49.28		2.46	0.82	1.77	0.27	0.41					0.41	2.32	0.27	9.82				3.14					15.00	79.24	5.73		84.97	90.70		9.48	74.53	15.97		90.50	106.47	13:1	4.6:1
	Homozygous	66.75	42.12	32.42	0.57			12.52		14.32	44.22		4.73	0.29		0.0			0.24					0.47	12.40				3.65			0.24		19.61	75.11	5.03	0.24	80.38	85.89		12.14	71.07	16.53	0.24	87.84	104.85	14:1	4.3:1
	Non-disjunction of 3 L	5.63	18.81	18.00	1.57	0.88	2.13	19.89		15.63	44.40		2.58	0.26	0.51			0.26					3.55	0.71	6.39				2.84				7.75	57.09	39.29	3.61		42.91	46.53	0.17	4.44	82.06	13.49		95.55	109.04	10.8:1	6.0:1
27	Heterozygous	78.80	39.79	31.72	0.38	2.23	0.20	9.56		11.16	53.60		3.50	0.35	2.03	0.11	0.71	.05			0.05	0.15	0.96	0.15	5.73				2.74	0.10				19.11	74.13	6.75		80.88	87.63		15.56	74.50	9.80	0.10	84.40	94.40	10.9:1	7.6:1
	Homozygous	70.90	42.82	32.24	0.62			12.77		14.21	48.29		4.51	0.45										0.22	10.83				4.11			0.22		19.35	75.69	4.96		80.65	85.61		9.30	75.26	15.17	0.22	90.65	106.27	15:1	4.9:1
	Non-disjunction of 3 R	1.85	27.00	45.40		1.08		6.28		3.60	27.51		4.32		8.65										2.35								0.10	13.51	73.51	12.97		86.49	99.47	5.71	60.23	37.41	2.35		39.76	42.11	5.6:1	15:1
9	Heterozygous	62.45	32.60	35.65	1.53	1.37	3.77	0.32	0.67	1.98	53.00		13.58	0.96	3.90	0.25	1.47		0.13	0.64		0.06	3.99		0.19			0.19	0.57					7.90	71.18	20.16	0.77	92.10	113.76		35.81	59.75	4.41		64.16	68.55	3.5:1	13.5:1
	Non-disjunction of 3 R	2.716	31.80	42.00	0.82	2.28	4.12	0.37	0.62	0.91	14.07		10.60	0.55	2.50		0.88			0.29		0.10	1.37				0.16	0.27				0.06	8.17	77.00	14.60	0.29	91.89	107.07	18.12	77.99	20.10	1.90		22.00	23.90	5.2:1	10.5:1
2	Heterozygous	58.93	37.09	33.85	0.81	1.26	5.09	9.31		2.64	0.54	1.46	6.99	1.02	2.44	0.07	1.74			0.41		0.13	0.34			0.27	0.47	0.07					14.30	73.01	12.27	0.41	85.69	98.78		79.67	19.03	1.28		20.31	21.59	5.9:1	148:1
	Disjunctional	31.21	40.69	28.70	0.83	2.69	5.38	9.16		2.24	3.01		10.25	1.28	1.92	0.38	1.02			0.51		0.25		-0.13	0.51			-1.3				0.25		11.69	72.92	14.86	0.51	88.29	104.17		79.42	19.79	0.51	0.25	20.55	21.56	4.9:1	38.8:1
	Homozygous	39.24	38.93	34.25	0.92	1.83	4.18	29.35		18.32			8.26	0.82	3.46	0.20	1.32	0.10		0.41	1.63	1.25		0.92									9.48	75.94	14.16	0.41	90.50	105.48		44.34	51.88	3.77		55.65	59.42	5.3:1	137:1
	Non-disjunction of 3 R	14.96	38.10	33.10	1.47	3.61	5.74	8.77		2.54	0.40	0.44	8.54		1.87	0.26	1.34									0.27	0.18						0.04	13.91	74.00	12.08		86.08	98.16	29.06	81.64	17.90	0.44		18.34	18.78	6.1:1	40.7:1

The algebriac condition for this is when $b^2 - 4ac = 0$, and by setting up equations for this condition the corrective factor can be obtained and the results recalculated on that basis.

An approximation, sufficiently close for the purposes of these data, may be obtained as follows: (translocation 36 is still used as an example.)

Let the value of R♀ from uncorrected data be X, the value from corrected data be X′, and the value when the roots are coincident be X″.

The equations of the uncorrected and of the corrected data are

$$Y = .470\ X^2 - .13857\ X + .000247, \quad \text{and}$$
$$Y' = .398\ X'^2 - .06373\ X' + .000256$$

Setting the first derivative equal to zero to obtain maximum or minimum values,

$$.94\ X = .1386$$
$$X = .147 \text{ and } Y \text{ (by substitution)} = -.00997$$
$$.796\ X' = .0637$$
$$X' = .0801 \text{ and } Y' = -.00229$$

The point where the line connecting these points crosses the X axis would be given by the equation

$$X'' = X - Y\left(\frac{X - X'}{Y - Y'}\right)$$

$= .060$, the value of R♀ when the roots are coincident. The values for L♀, and L♂, and R♂, as well as the corrective factor may be obtained by substitution.

TABLE 3

Total Chiasmata Per Region in Per Cent

		NxL	1	2	3	4l	4r	5l 5r	6	7l 7r	NxR
	Control	13.46	48.15	42.15	1.26	6.74	6.04	26.24	18.74	67.74	5.72
	Control region 4 omitted	14.96	48.15	42.15	1.26			26.24	18.74	67.74	6.33
	Control region 7 omitted	13.46	48.15	42.15	1.26	6.74	6.04	26.24	18.74		51.73
8	heterozygous	37.10	47.77	16.11	0.09		7.61	29.76	21.22	60.65	6.66
	disjunctional	38.53	51.92	11.41			4.65	34.69	17.92	61.23	6.17
	homozygous	44.71	40.13	15.57	0.20			33.08	18.63	61.03	8.33
	nondisjunctional	87.70	7.71	4.11		0.20	4.94	41.11	18.86	66.76	6.03
36	heterozygous	18.99	47.74	32.79	0.71	4.41	4.11	27.24	18.87	59.74	13.61
	homozygous	17.76	50.31	38.28	0.61			30.99	21.62	69.17	9.12
	nondisjunctional	60.30	23.15	16.55			3.31	31.40	11.58	51.21	5.78
31	heterozygous	13.11	42.14	40.85	1.69	9.06	5.32	29.17	18.57	51.24	14.92
	disjunctional	15.00	49.61	34.66	1.50	4.91	3.95	22.36	15.56	64.56	9.48
	homozygous	19.61	47.38	37.39	1.10			25.63	18.68	60.51	12.14
	nondisjunctional	57.09	22.16	20.58	2.09	1.65	5.68	26.99	19.18	57.18	4.44
27	heterozygous	19.11	45.67	36.04	0.89	5.02	1.46	15.59	14.20	63.13	15.56
	homozygous	19.35	47.78	36.75	1.07			24.04	18.76	62.45	9.30
	nondisjunctional	13.51	39.97	49.72		9.73		8.63	3.60	29.86	60.23
9	heterozygous	7.90	51.81	51.72	2.87	7.38	7.22	0.51 0.86	2.61	57.34	35.81
	nondisjunctional	8.17	45.74	53.77	1.37	5.95	5.59	0.36 0.78	1.27	15.86	77.99
2	heterozygous	14.30	47.95	43.06	1.90	5.85	5.56	10.05	2.84	0.81 2.34	79.67
	disjunctional	11.69	54.65	40.86	2.49	6.14	5.63	9.79	2.48	3.90	79.42
	homozygous	9.48	51.87	44.43	2.04	7.11	7.06	31.90	20.49		44.34
	nondisjunctional	13.91	48.51	43.24	1.73	6.82	5.79	9.22	2.54	0.67 0.62	81.64

TABLE 4

Chiasma Formation in Exceptional Gametes with Nondisjunction of the Intact Arm

	L Disjoining Arm							R Nondisjoining Arm					
	Total	Nx		Sx		Dx		Nx		Sx		Dx	
		No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
8	58 D	2	3.4	56	96.5	0	0.0	14.5	24.9	27.6	47.5	17.4	30.0
31	18 D	2	11.1	12	66.6	4	22.2	10	55.5	8	44.4	0	0.0

TABLE 5

Chiasma Relations in the l and r Portions of the Broken Arm

	Left Arm			Right Arm Divided Into Rl and Rr					
	Nx	C	X	Nx l	C	X	Nx r	C	X
Control region 5	13.46	86.52	98.33	85.26	13.95	14.60	11.42	87.79	102.06
9 heterozygous disjunctional	7.90	92.09	113.76	92.25	7.73	7.73	39.90	60.08	60.84
9 nondisjunctional	8.17	91.89	106.07	94.02	5.96	5.96	82.48	17.50	17.90
Control region 7	13.46	86.52	98.33	36.40	62.82	71.86	54.54	44.68	44.68
2 heterozygous disjunctional	14.30	85.69	98.78	81.13	18.87	19.28	97.66	2.34	2.34
2 nondisjunctional	13.91	86.08	98.16	82.26	17.55	17.82	99.35	0.62	0.62

TABLE 6

Coincidence

Regions =	1–2	5–6	5–7	6–7
Control	0.30	0.13	0.66	0.53
8 heterozygous	0.14	0.05	0.77	0.44
disjunctional	0.31	—	0.70	0.76
N. D. 3L	0.65	0.08	0.99	0.53
homozygous	0.08	0.16	0.84	0.35
36 heterozygous	0.14	0.15	0.95	0.38
N. D. 3L	—	—	—	—
homozygous	0.34	0.21	0.96	0.62
31 heterozygous	0.11	0.12	0.78	0.33
disjunctional	0.14	0.07	0.67	0.31
N. D. 3L	0.56	0.13	0.41	0.25
homozygous	0.28	0.14	0.81	0.31
27 heterozygous	0.21	0.07	0.59	0.31
N. D. 3R	0.21	—	0.91	—
homozygous	0.25	0.09	0.72	0.36
			5(1)–7 5–7(r)	6–7
9 heterozygous	0.54	—	0.66 0.39	0.38
N. D. 3R	0.44	—	— 1.45	1.44
			5–7(1) 5–7(r)	6–7(r)
2 heterozygous	0.35	—	3.37 2.04	1.16
disjunctional	0.48	—	not calculated	
N. D. 3R	0.40	—	— —	—
homozygous	0.37	0.14	random segregation	in region 7

TABLE 7

Egg and Hatch Count

	Cross	No. of Eggs	No. of Flies	Per Cent Hatch	Corrected Hatch
	Control +/+ ♀ x +/+ ♂	9871	8686	88.0	100.0
8	homozygous ♀ x +/+ ♂	3812	2936	77.0	87.5
	homozygous ♂ x +/+ ♀	5256	semi-sterile		
	heterozygous ♀ x +/+ ♂	4762	2785	58.4	66.4
	heterozygous ♂ x +/+ ♀	4632	2211	47.7	54.2
36	homozygous ♀	5916	5291	89.4	101.6
	homozygous ♂	4536	4000	88.1	100.1
	heterozygous ♀	4262	2595	60.8	69.1
	heterozygous ♂	3200	1698	53.0	60.2
31	homozygous ♀	3872	3443	88.9	101.0
	homozygous ♂	3339	3030	90.7	103.1
	heterozygous ♀	4324	3006	69.5	79.0
	heterozygous ♂	2928	1508	51.5	58.5
27	homozygous ♀	4908	4292	87.4	99.3
	homozygous ♂	4291	semi-sterile		
	heterozygous ♀	5390	3473	64.4	73.2
	heterozygous ♂	4596	2169	47.2	53.6
9	homozygous ♀	3745	3218	86.2	98.0
	homozygous ♂	2308	semi-sterile		
	heterozygous ♀	4311	2596	60.2	68.3
	heterozygous ♂	4554	2152	47.2	53.6
2	homozygous ♀	5123	4459	87.0	98.8
	homozygous ♂	4511	3745	83.0	94.3
	heterozygous ♀	5260	2748	52.2	59.2
	heterozygous ♂	4444	2143	48.2	54.7

TABLE 8

Progeny from Heterozygous Female by Heterozygous Male

Progeny of $\frac{\_\ \_}{\text{rucuca}}$ females x $\frac{\text{D PR}}{\text{Ly}}$ males

Translocation	Disjunctional Ly D Pr	N. D. 3L D Ly Pr	N. D. 3R D Pr Ly	Total
8	9915 7016 87.25 per cent	958 1365 11.97 per cent	125 24 0.76 per cent	19403
36	4051 3993 93.20 per cent	262 316 6.69 per cent	6 2 0.09 per cent	8630
31	11355 11305 92.06 per cent	866 1044 7.75 per cent	36 7 0.17 per cent	24613
27	5571 5461 94.18 per cent	8 4 0.10 per cent	389 280 5.71 per cent	11713
9	3073 1886 81.80 per cent	2 2 0.06 per cent	584 515 18.12 per cent	6062
2	6968 6248 70.89 per cent	8 0 0.04 per cent	2659 2759 29.06 per cent	18642

TABLE 9

Equational Exceptions

Translocation	8	36	31	27	9	2
Total D	125	6	36	389	584	2659
cu sr e^s ca	1					
sr e^s ca	3	1		4		
e^s ca	4	1	1	9	2	
ca	13		4	28	20	3
cu sr e^s	3					
sr e^s	4			1		
e^s	2					
Total D equationals	30	2	5	42	22	3
Total Pr	1365	316	1044	4	2	0
ru	25	6	43			
ru h	9	9	39			
ru h th			1			
ru h th st		2	3			
h			9			
h th st			2			
st			1			
Total Pr equationals	34	17	98	0	0	0

TABLE 10

Possible Relations in Gamete Formation

N. D. Gamete Formation		Recovery in Zygotes Heterozygous males x heterogous females		
♂	♀	L♂L♀>R♂R♀	L♂L♀=R♂R♀	L♂L♀<R♂R♀
1. L > R	L > R	possible	impossible	impossible
2. L > R	L = R	possible	impossible	impossible
3. L > R	L < R	possible	possible	possible
4. L = R	L > R	possible	impossible	impossible
5. L = R	L = R	impossible	possible	impossible
6. L = R	L < R	impossible	impossible	possible
7. L < R	L > R	possible	possible	possible
8. L < R	L = R	impossible	impossible	possible
9. L < R	L < R	impossible	impossible	possible

TABLE 11

Calculated Nondisjunction Rates

	Experimental Data in Per Cent						Data Corrected for Egg Hatch					
Translocation No. =	8	36	31	27	9	2	8	36	31	27	9	2
Disjunction	27.8	32.2	35.8	30.4	28.4	25.2	36.0	41.6	46.1	39.3	36.6	32.4
N. D. L L	7.6	4.6	6.0	0.1	0.4	0.03	9.9	6.0	7.8	0.1	0.1	0.04
N. D. R R	0.5	0.1	0.1	3.7	12.6	20.6	0.6	0.1	0.2	4.7	16.2	26.3
N. D. L + R ♂	52.3	47.0	48.5	52.8	52.8	51.8	45.8	39.8	41.5	46.4	46.4	45.3
N. D. L + R ♀	41.6	39.2	30.5	35.6	39.8	47.8	33.6	30.9	21.1	26.8	31.7	40.8
N. D. rate L ♂	50.4	46.8	47.7	42.4	19.2	8.3	36.7	39.3	36.1	28.4	−3.7	.....
R ♂	1.8	0.2	0.8	10.4	33.6	43.5	9.1	0.5	5.3	17.9	50.2	.....
L ♀	15.1	9.9	12.6	0.1	2.4	0.4	26.9	15.3	17.8	0.3	−1.6	.....
R ♀	26.5	29.3	17.9	35.4	37.4	47.4	6.7	15.7	3.3	26.5	33.3	.....
L ♂	18.9	11.9	20.1	0.2	3.2	0.4	.....	19.7	.....	0.5	.....	.....
R ♂	33.4	35.1	28.4	52.6	49.6	51.4	.....	20.1	.....	45.9	.....	.....
L ♀	40.1	39.0	30.0	28.6	14.4	7.6	.....	30.5	.....	16.4	.....	.....
R ♀	1.5	0.2	0.5	7.0	25.4	40.1	.....	0.4	.....	10.4	.....	.....

Values Where the Two Roots Are Equal

Translocation	8	36	31	27	9	2
Corrective Factor	88.1	84.4	89.2	83.3	88.2	103.3
L ♂	36.4	35.7	39.0	2.9	−2.3	0.4
R ♂	9.4	1.5	3.2	40.4	48.8	52.9
L ♀	26.8	22.0	17.8	3.2	−1.3	7.2
R ♀	6.8	6.0	5.3	18.4	33.2	42.2

TABLE 12

Gamete Frequencies Calculated from Table 11

		Uncorrected Egg Hatch														Corrected Egg Hatch										
		8		36		31		27		9			2			8		36		31		27		9		
		L	R	L	R	L	R	L	R	L	Rl	Rr	L	Rl	Rr	L	R	L	R	L	R	L	R	L	Rl	Rr
Zygotes	Disjunctional	37.1	6.6	19.0	13.6	13.1	14.9	19.1	15.6	7.9	92.2	39.9	14.3	81.1	97.7	37.1	6.6	19.0	13.6	13.1	14.9	19.1	15.6	7.9	81.1	39.9
from Nx tetrads in per cent	N. D. L.	87.7	6.0	60.3	5.8	57.1	4.4									87.7	6.0	60.3	5.8	57.1	4.4					
	N. D. R.	3.4	24.9			11.1	55.5	13.5	60.2	8.2	94.0	82.5	13.9	82.2	99.3	3.4	24.9			11.1	55.5	13.5	60.2	8.2	82.2	82.5
N. D. rate in female		15.0	26.5	9.9		12.6	17.9		7.0		25.4	25.4		40.1	40.1	26.9	6.7	15.3		17.8	3.3		10.4		33.3	33.3
Egg hatch from heterozygous female		58.4		60.8		69.5		64.4		60.2			52.2			66.4		69.1		79.0		73.2		68.3		
Gametes from Nx tetrads	Disjunctional	2166.6	385.4	1154.6	826.9	911.1	1035.5	1230.0	1002.2	475.6	5550.4	2402.2	746.5	4233.4	5097.8	2463.4	438.2	1312.2	939.8	1035.7	1177.1	1398.1	1139.0	539.6	6297.3	2726.1
	actual N. D. L. corrected	1315.5 (1.6) 2166.6	90.0 (1.6) 144.0	597.0 (1.9) 1154.6	57.4 (1.9) 109.1	719.3 (1.2) 911.1	55.0 (1.2) 66.0									2359.1 (1.0)	161.4 (1.0)	922.6 (1.4) 1312.2	88.7 (1.4) 124.2	1016.2 (1.0)	78.3 (1.0)					
	actual N. D. R. corrected	90.1 (.58) 52.2	659.8 (.58) 385.4			198.7 (.89) 176.8	993.4 (.89) 839.5	94.5 (2.3) 217.3	421.6 (2.3) 1002.2	208.3 (1.1) 229.1	2387.6 (1.1) 2626.4	2095.0 (1.1) 2402.2	557.4 (1.3) 724.6	3296.2 (1.3) 4285.0	3984.0 (1.3) 5097.8	22.8	166.8			36.6	183.1	140.4 (1.8) 252.7	626.4 (1.8) 1139.0	273.0 (1.0)	3130.2 (1.0)	2746.6 (1.0)
Gametes from chiasma tetrads	Disjunctional	3673.4	5454.6	4925.4	5265.1	6038.8	5914.4	5210.0	5438.0	5544.4	469.6	3618.0	4473.5	986.6	122.1	4176.6	6220.4	5597.8	5970.2	6864.3	6722.9	5921.9	6181.0	6281.4	532.7	4104.8
	actual N. D. L corrected	184.5 (1.6) 295.2	1410.0 (1.6) 2256.0	393.0 (1.9) 746.7	932.6 (1.9) 1771.9	540.7 (1.2) 648.8	1195.0 (1.2) 1434.0									330.9	2528.6	607.4 (1.4) 850.4	1441.3 (1.4) 2017.8	763.8	1701.7					
	actual N. D. R. corrected	2559.9 (.58) 1484.7	1990.1 (.58) 1154.2			1591.3 (.89) 1416.2	796.5 (.89) 708.9	605.5 (2.3) 1392.6	278.4 (2.3) 489.6	2331.7 (1.1) 2564.9	152.4 (1.1) 167.6	445.1 (1.1) 489.6	3452.6 (1.3) 4488.4	709.8 (1.3) 922.7	26.0 (1.3) 33.8	647.2	503.2			293.4	146.8	899.6 (1.8) 1619.3	413.6 (1.8) 744.5	3066.9	199.8	583.4
Ratio of disjunctional to N. D. gametes from chiasma tetrads	actual	20.0:1	2.7:1	12.5:1		11.2:1	7.4:1		19.5:1			8.1:1			4.7:1	12.6:1	12.3:1	9.2:1		9.0:1	45.8:1		14.9:1			7.0:1
	corrected	12.4:1	4.7:1	6.6:1		9.3:1	8.3:1		8.3:1			7.3:1			3.6:1			6.6:1					8.3:1			
Per cent Nx tetrads	actual corrected	35.7 44.6	11.3 9.3	24.8 28.9	12.5 11.7	18.3 19.0	20.9 19.4	18.5 18.0	19.9 25.3	8.0 8.0	92.8 92.8	52.3 53.9	14.1 14.1	81.2 70.0	98.4 98.5	48.4	7.6	26.5 28.9	12.2 11.7	20.9	14.4	18.4 17.9	21.1 24.7	8.0	92.9	53.9
Per cent chiasma tetrads	actual corrected	64.2 55.4	88.6 90.6	75.2 71.1	87.5 88.3	81.7 81.0	79.1 80.6	81.4 82.0	8.1 74.7	92.0 92.0	7.2 7.2	47.5 46.1	85.9 85.9	18.7 16.1	1.6 1.5	51.5	92.3	73.5 71.1	87.8 88.2	79.1	85.6	81.6 82.0	78.9 75.2	92.0	7.2	46.1

TABLE 13

Single : Double Ratios in Heterozygous Translocations

		Total Flies	Total Chiasmata in L in Per Cent	Nx L	Sx L	Dx L	Tx L	Sx : Dx L	Actual: Expected	Total Chiasmata in R in Per Cent	Nx R	Sx R	Dx R	Tx R	Sx : Dx R	Actual: Expected
	actual	4264	98.3	13.5	74.7	11.8		6.3 : 1	3.1	118.8	5.7	70.4	23.1	0.7	3.0 : 1	1.8
	Control expected			37.4	36.8	18.1	5.9	2.0 : 1			30.5	36.2	21.5	8.5	1.7 : 1	
8	heterozygous	6242	64.0	37.1	61.8	1.1		56.0 : 1	18.7	119.3	6.7	68.7	23.2	1.4	2.9 : 1	1.6
				52.8	33.7	10.8	2.3	3.0 : 1			30.3	36.2	21.6	8.6	1.7 : 1	
	disjunctional	895	63.3	38.5	59.6	1.9		31.0:1	10.3	118.5	6.2	70.1	22.8	0.9	3.0 : 1	1.8
				53.1	33.5	10.6	2.3	3.0 : 1			30.6	36.2	21.5	8.5	1.7 : 1	
	N. D. L	647	12.5	87.7	12.1	0.2		54.0 : 1	4.0	131.7	6.0	56.2	37.7		1.4 : 1	1.3
				88.3	10.9	0.7	0.04	13.3 : 1			26.8	35.3	23.2	10.2	1.1 : 1	
36	heterozygous	4348	85.7	19.0	76.5	4.3	0.2	17.0 : 1	7.1	110.1	13.6	63.6	21.9	0.9	2.9 : 1	1.6
				42.5	36.3	15.6	4.5	2.4 : 1			33.2	36.6	20.2	7.4	1.8 : 1	
	N. D. L	121	39.7	60.3	39.7					97.5	5.8	90.9	3.3		27.0 : 1	13.5
				67.2	26.7	5.3	0.7	5.0 : 1			37.7	36.7	17.9	5.8	2.0 : 1	
31	heterozygous	5057	93.8	13.1	80.4	6.0	0.5	13.0 : 1	6.2	104.4	14.9	66.2	18.3	0.5	3.5 : 1	1.8
				39.2	36.7	17.2	5.4	2.1 : 1			35.3	36.7	19.1	6.7	1.9 : 1	
	disjunctional	2930	90.7	15.0	79.2	5.7		13.0 : 1	4.0	106.5	9.5	74.5	16.0		4.6 : 1	2.4
				40.1	36.6	16.6	5.0	3.3 : 1			34.5	36.7	19.5	6.9	1.9 : 1	
	N. D. L	563	46.5	46.5	57.1	39.3	3.6	10.8 : 1	2.6	109.0	4.4	82.1	13.5		6.0 : 1	3.3
				62.9	29.1	6.8	1.1	4.2 : 1			33.6	36.6	20.0	7.3	1.8 : 1	
27	heterozygous	7880	87.6	19.1	74.1	6.7		10.9 : 1	4.7	94.4	15.6	74.5	9.8	0.1	7.6 : 1	3.8
				41.7	36.4	16.0	4.7	2.3 : 1			38.8	36.7	17.3	5.5	2.0 : 1	
	N. D. R	185	99.5	13.5	73.5	13.0		5.6 : 1	2.8	42.1	60.2	37.4	2.3		15.0 : 1	3.2
				37.0	36.8	18.3	6.1	2.0 : 1			65.7	27.5	5.8	0.8	4.7 : 1	

		Total	Total Rl Chiasmata	Nx Rl	Sx Rl	Dx Rl	Tx Rl	Sx : Dx Rl	Actual: Expected	Total Rr Chiasmata	Nx Rr	Sx Rr	Dx Rr	Tx Rr	Sx : Dx Rr	Actual: Expected
9	heterozygous	6245	7.7	92.2	7.7					60.8	39.9	59.3	0.8		74 : 1	25.0
				92.6	7.0	0.3	0.007	23 : 1			54.4	33.0	10.1	2.0	3 : 1	
	N. D. Rr	2716	6.0	94.0	6.0					17.9	82.5	17.1	0.4		42 : 1	2.6
				94.3	5.4	0.3	0.006	18 : 1			83.7	14.8	1.4	0.1	16 : 1	
2	heterozygous	5893	19.3	81.1	18.5	0.4		46 : 1	3.0	2.3	97.6	2.3				
				82.5	15.8	1.5	0.1	15 : 1			97.8	2.1	1.0	0.02	2 : 1	
	N. D. Rr	1496	18.2	82.1	17.4	0.3		58 : 1	5.8	0.61	99.3	0.61				
				82.9	15.5	1,5	0.1	10 : 1			99.4	0.5	0.02		25 : 1	

TABLE 14

Chiasmata Frequency in Per Cent Where Both Arms of Chromosome 3 Are Considered Together

		Nx	Sxl	Sxr	Dxll	Dxlr	Dxrr	Txlll	Txllr	Txlrr	Txrrr	Qxllrr	Qxlrrr
8	heterozygous	0.8	5.1	23.6	0.6	51.3	6.6		−0.4	10.6	−0.1	0.8	1.0
	disjunctional	−3.6	9.8	30.5	−0.9	44.7	4.6		1.9	12.1	0.9		
	N. D. 3L	6.7	−0.5	46.9	−1.4	8.4	33.2		0.4	4.8			
	N. D. 3R	−3.5	15.2	6.9		39.2				30.0			
31	heterozygous	9.7	5.4	−1.6	−3.5	72.2	−1.6	0.5	5.1	12.0	0.0	1.6	0.3
	disjunctional	1.4	7.8	9.9	−2.2	68.5	−2.2		4.4	11.2		1.1	
	N. D. 3L	0.8	4.0	49.5	−0.5	28.5	6.9		4.1	6.6			
27	heterozygous	5.8	7.9	10.7	−0.5	65.4	−0.2		4.3	5.6		1.0	0.2
	N. D. 3R	6.2	47.8	8.8	4.2	21.3	−0.7		8.5	3.1			

TABLE 15

Chiasma Relations Between L and R

		Total Flies	One or More Chiasmata in Both Arms at Same Time in Per Cent	One or More Chiasmata in Left Arm Only in Per Cent	One or More Chiasmata in Right Arm Only in Per Cent
8	heterozygous	6242	63.3	5.8	30.1
	disjunctional	859	58.7	8.8	36.0
	N. D. 3L	647	13.6	1.9	80.1
	N. D. 3R	58	69.2	15.2	6.9
31	heterozygous	5057	91.2	2.3	—3.2
	disjunctional	2930	85.2	5.7	7.8
	N. D. 3L	563	39.2	3.5	56.4
27	heterozygous	7880	76.4	7.3	10.2
	N. D. 3R	185	32.9	52.0	8.1

(obtained by summing per cent of single, double and triple chiasma tetrads)

TABLE 16

Single: Double Ratio in Homozygous Translocations

	Total flies	Total chiasmata L in per cent	Nx L	Sx L	Dx L	Tx L	Sx : Dx L	Actual: expected	Total chiasmata R in per cent	Nx R	Sx R	Dx R	Tx R	Sx : Dx R	Actual: expected
actual config.	4264	98.3	13.5	74.7	11.8		6.3 : 1		118.8	5.7	70.4	23.1	0.7	3.0 : 1	
Control expected config.			37.4	36.8	18.1	5.9	2.0 : 1	3.1		30.5	36.2	21.5	8.5	1.6 : 1	1.9
actual config. Control Region 4 omitted	4264	91.6	15.0	78.5	6.8		11.9 : 1		112.8	6.3	74.7	18.8	0.2	3.9 : 1	
expected config.			39.8	36.6	16.8	5.1	2.2 : 1	5.4		32.3	36.5	20.6	7.7	1.7 : 1	2.3
actual config.	7091	55.9	44.7	54.8	0.4	0.1	140.0:1		112.7	8.3	70.7	20.9	0.1	3.3 : 1	
8 homozygous expected config.			57.2	31.9	8.9	1.7	3.6 : 1	40.0		32.3	36.5	20.6	7.7	1.7 : 1	2.0
actual config.	6954	89.2	17.8	75.3	6.8	0.1	11.0 : 1		121.8	9.1	60.4	30.0	0.5	2.0 : 1	
36 homozygous expected config.			41.0	36.5	16.3	4.8	2.2 : 1	5.0		29.6	36.0	21.9	8.9	1.7 : 1	1.2
actual config.	6675	85.9	19.6	75.1	5.0	0.2	14.0 : 1		104.8	12.1	71.1	16.5	0.2	4.3 : 1	
31 homozygous expected config.			42.4	36.3	15.6	4.5	2.3 : 1	6.0		35.1	36.7	19.2	6.7	1.8 : 1	2.4
actual config.	7090	85.6	19.3	75.7	5.0		15.0 : 1		106.3	9.3	75.3	15.2	0.2	4.9 : 1	
27 homozygous expected config.			42.5	36.3	15.5	4.4	2.3 : 1	6.5		34.6	36.7	19.5	6.9	1.9 : 1	2.6
actual config. Control Region 7 omitted	4264	98.3	13.5	74.7	11.8		6.3 : 1		51.1	51.7	45.4	2.8		16.0 : 1	
expected config.			37.4	36.8	18.1	5.9	2.0 : 1	3.1		60.0	30.6	7.8	1.3	3.9 : 1	4.1
actual config.	3924	105.5	9.5	75.9	14.2	0.4	5.3 : 1		59.4	44.3	51.9	3.8		13.7 : 1	
2 homozygous expected config.			34.9	36.7	19.4	6.8	1.9 : 1	2.7		55.2	32.8	9.7	1.9	3.4 : 0	4.0

II. CHIASMA FORMATION IN THE *BOBBED* REGION OF THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

META SUCHE BROWN*

Department of Zoology, The University of Texas, Austin, Texas

In *D. melanogaster* the X-chromosome is well marked for crossover studies from the distal end to the locus of *bobbed*. However, the frequency of crossing over occurring to the right of bobbed has long been in question. To obtain evidence on this point this study of crossing over between *forked* and the centrosome was undertaken.

MATERIAL AND METHODS

Two translocations, T1, 4 A-11 and T1, 4 A-14, which showed little or no reduction of crossing over (Stone 1934), were used in the experiment. Cytologically the chromosomes involved in these translocations are attached at or near the centromeres (Painter and Stone 1935), and have been interpreted as fusions of the fourth and X-chromosomes, the latter retaining the entire heterochromatin in each case. Subsequent work has proved that the fourth chromosome is V-shaped (Griffin and Stone 1938, Panshin and Khrostova 1938). Consequently these supposed fusions are probably in reality translocations.

Individual males of 11 and 14 were crossed to homozygous *f car bb ey*† females, selected for complete expression of *bb*. The F_1 females were backcrossed individually to single *f car bb ey* males. Among the F_2 progeny crossovers were counted in the female only, since a normal allele of *bb* is present in the Y-chromosome. Data from each F_1 female were recorded separately.

All F_2 females in which the distinction between *ey* and non-*ey* was doubtful were backcrossed to *f car bb ey* males; and all non-*bb ey* females, i.e., apparent crossovers between *bb* and the centromere, were tested for the presence of a Y. In the absence of patroclinous males among the offspring of non-*bb* females crossed to *Bar* males (the occurrence of non-*Bar* females was not conclusive evidence since not all F_2 females were virgin) the presence or absence of a Y in the original females was further tested by backcrossing their male offspring. From each apparent crossover female six to fifteen F_1 males were crossed individually to selected *f car bb ey* females. If both *bb* and non-*bb* appeared among the female offspring of several males of a test group, a Y-chromosome was judged to be responsible for the suppression of *bb*. If no *bb* females were recovered from all males of a group, the normal allele was considered present in the X chromosome.

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*Now Division of Agronomy, Texas Agricultural Experiment Station, College Station, Texas.

†*f* forked 1-56.5; *car* carnation 1-62.5; *bb* bobbed I-66.0; *ey* eyeless 4—. Bridges 1938. In the absence of data determining the locus of the centromere with respect to mutant genes, its location four units to the right of *bb* remains highly tentative.

In these tests the random segregation of *ey* and non-*ey*, the latter having been introduced by the *Bar* males, indicated that the non-*bb* phenotype in the case of non-*f*, non-*car* females was neither a mutation to *ey* nor a crossover of *ey* into the fourth chromosome involved in the translocation. The original female was therefore considered the result of crossover between *bb* and the centromere as marked by *ey*. Five cases were further verified by cytological examination, which showed two normal X's and no Y in the oögonial metaphase plates.

Results

Detectable crossing over in the right end of the X proved to be very infrequent. Among a total of 10,000 F_1 females in translocation 14, 9,302 showed no recombination (Table 1). In the *f-car* region 377 exchanges between *f* and *car* were recovered; in the *car-bb* region, 312 exchanges between *car* and *bb*. To the right of *bb* only five possible cases of recombination between *bb* and *ey* remained after tests were completed. Of the *f car bb* females, all 55 proved to be eyeless when backcrossed, and are so classified in Table 1. Of the non-*bb ey* class, six females carried a Y and five were verified as crossovers between *bb* and *ey*. Between *f* and *bb* only one double crossover was recovered. No double crossovers involving the *f car* and the *bb ey* regions were recovered. Of the possible crossovers between *car* and *ey* all twelve carried a Y. Of the *bb* non-*ey* class six proved to *ey* and hence single crossovers between *car* and *bb*. Two were actually *bb* non-*ey*. The complete linkage of X and fourth chromosomes was proof that these were not cases of reversal of *ey* to normal in the free fourth, but double crossovers in regions *car-bb* and *bb-ey*. The *car ey* case was according to all tests a triple crossover.

For translocation 11, 412 crossovers were recovered in the *f-car* region, and 235 in the *car-bb* region. In the *bb-ey* region the three non-*bb ey* females gave no evidence of a Y. Of the reciprocal class, 35 *f car bb* females proved to be *ey* and therefore non-crossovers. No double crossovers involving the *f-car* and *car-bb* regions were recovered. Of the possible crossovers involving regions *f-car* and *bb-ey*, one *f ey* female was verified as a double crossover, but all seven *car-bb* flies proved to be *ey* and therefore single crossovers between *f* and *car*. Of eleven *f car ey* females eight were XXY, but in three *bb* had been crossed out by a double exchange involving regions *car-bb* and *bb-ey*. No triple crossovers were recovered in translocation 11.

The per cent of recombination for each region is given in Table 2. Table 3 shows the detectable crossover data converted into chiasma frequencies by Weinstein's (1936) method. This table reveals that most crossovers in the region beyond *bb* are multiple crossovers involving one or more regions to the right of *forked*. Table 4 gives a comparison of the actual and expected percentages of multiple chiasma formation. It is obvious from this table that not only is coincidence involving the two regions to either side of *bb* unusually high, but it is higher for the two adjacent regions than the *f-car* region, farther to the left, is involved.

Discussion

The occurrence of chiasmata in the *bobbed* region, although exceedingly rare, has adequate experimental confirmation. Their occurrence in this region was first inferred from detachments of X chromosomes obtained by Stern (1927) and Stern and Ogura (1931) from attached-X females. Proof that such detachments resulted from exchanges between the X and the Y was given by Kaufmann (1933) and later by Neuhaus (1936), who showed the attachment of one arm of the Y to a detached X. However, the relative stability of attached-X stocks indicates that X-Y exchange in the female is not very frequent. The separation of the Theta fragment from the X, and its attachment to one arm of the Y (Stern and Doan 1936) demonstrated the same phenomenon occurring in the male. These exchanges, however, give no evidence regarding the exact loci where the chiasmata are formed. Crossovers to the left or to the right of *bobbed* could not readily be distinguished in X-Y exchanges. Grüneberg (1935) inferred, but did not prove, the occurrence of crossing over beyond *bb* in a long inversion in the X in the female. In view of the increase in crossing over obtained in regions moved away from the centromere, his assumption that the difference in crossing over remaining in the *y-car* area after the subtraction of the normal values for *y-rb* and *car-bb* represents crossing over beyond *bb* is not proved. The work of Philip (1935) in crossing over in the *bb* region in the male gave conclusive evidence that chiasmata are formed on both sides of this locus. Philip recovered only double crossovers, approximately one in 3,000.

There remains the question of the frequency of chiasma formation in the heterochromatin of the X chromosomes in the female. Compared to linkage data given with Bridges' (1938) map of the X chromosome, recombination in the three regions studied was slightly reduced. Granting that the presence of the fourth chromosome caused this reduction, nevertheless there was little or no change in length of heterochromatin left of the centromere. Whatever its cause, the reduction in crossing over is scarcely sufficient to invalidate the data as a source of information on the question of chiasma formation in the region tested. Here the critical classes are those involving *bb* and either adjacent locus. Of *ey* and non-*ey* there can be no question since all doubtful cases were tested. In the two cases of *bb* non-*ey* the complete linkage of the X and the fourth chromosomes is proof that *bb* is present in the translocation. Unless arising by mutation, *bb* could have been obtained only by double crossing over. In view of Philip's (1935) examination of 39,000 flies without finding a mutation of *bb* and her recovery of double crossovers involving this locus in the male, the *bb* non-*ey* flies can be regarded as crossovers. In the case of the non-*bb* reciprocal class, a crossover involving this locus could be simulated by the presence of a Y chromosome, by a double crossover in the male, or by a nonreciprocal exchange in the male which linked the X with the arm of the Y containing the normal allele of *bb*. The first can be detected genetically as well as cytologically, and the third can be ruled out by cytological examination of metaphase plates. The *f ey*, *car ey* and

three of the non-*f*, non-*car*, non-*bb ey* cases were examined cytologically and proved to have normal X's and no Y. Granting the adequacy of the tests to establish the genotype of the flies involved, we have a verified maximum of 548 crossovers to the left of *bb*, nine crossovers to the right, and six crossovers involving both regions simultaneously among the 20,000 females examined. Hence detectable crossing over in the heterochromatin, in particular between *bb* and the centromere, is very infrequent in the female.

It has been proposed (Darlington 1934a, b) that in Drosophila species disjunction in the male is brought about by the formation of reciprocal chiasmata in the centromere region. In *D. melanogaster* such exchanges would take place in the heterochromatin forming the Y and the right end of the X. Presumably this same material when in the female should retain its capacity to form chiasmata, hence under suitable conditions might show recombination of marked loci. In view of Darlington's (1934) cytological demonstration of reciprocal chiasma formation in the centromere region of *D. pseudo-obscura,* we might accept, for the purpose of discussion, his hypothesis that normal disjunction of the X and Y is oriented thereby, and assume that any comparable chiasma formation in the heterochromatin of the X chromosome in the female is likewise reciprocal and localized, probably to the right of *bb*. Hence, any detectable crossing over within the heterochromatin, whether between two X's or between X and Y, would be exceptional either by being nonreciprocal or by occurring outside the usual chiasma-forming area. On such an assumption the low chiasma frequency experimentally obtained would not be unexpected. However, Gershenson's (1933) demonstration of only 61.4 per cent normal disjunction in the male with a deficiency of heterochromatin including *bb* (and block A) suggests that in *D. melanogaster* the synaptic area extends a considerable distance to the left of the centromere. Further, although the hypothesis of reciprocal chiasma formation may apply to the sex chromosomes in *D. pseudo-obscura* where both X and Y chromosomes are V-shaped, but not to the autosomes which are rods, it does not necessarily hold in *D. melanogaster* where the X is at best a J with a very short arm.

From the small number of recovered crossovers the distribution of chiasmata is difficult to determine. However, the data indicate that interference relations in the heterochromatin are radically different from those in other regions of the same chromosome. A chiasma to the right of *bb* is always accompanied by a chiasma to the left. Namely, all crossovers involving *bb* are multiples. Philip (1935) interprets similar double crossovers recovered from the male as being consistent with Darlington's hypothesis. In this event, no evidence presented above, the centromere region must be interpreted as including practically all of the heterochromatin. The postulation that the heterochromatin consists of a few genes which elaborate relatively large blocks of extragenic material (Muller and Prokofyeva 1935, Muller and Gershenson 1935, Muller *et al.* 1936) suggests a physical basis for the localization of chiasmata. It is to be noted

that block A, which is responsible for the greater part of the bulk of the heterochromatin, lies to the right of *bb* where detectable crossing over is low. However, its nature and relative size during meiotic prophase are open to question, and no conclusions regarding crossing over can be based on the structure of the heterochromatin at other stages in the cycle.

The possibility remains that these translocations may be mutual, in which case the region normally to the right of the centromere may be changed or absent. In this event the data do not rule out the possibility, in the normal condition, of a reciprocal double exchange involving both sides of the centromere, so conditioned that a chiasma does not form to the left of the centromere in the absence of a chiasma to the right. In any event there may always be a genetically undetectable reciprocal double exchange to the right of *bb*.

Since crossing over in the euchromatin is relatively free in the female, and suppressed in the male, it could be postulated that a comparable mechanism operates in the reverse manner on the heterochromatin in the two sexes. Chiasmata in the male would serve only to orient disjunction. In the female, crossing over supplements segregation as a mechanism for increasing variation within the species. As such its restriction in the female to the region rich in gene loci would have a selective basis. On this interpretation the heterochromatin might be regarded as a region selected to protect the euchromatin from the centromere influence which reduces crossing over. In *D. melanogaster* heterochromatin is present adjacent to the centromere in the second and third chromosomes as well as in the X. However, in *D. pseudo-obscura* chiasmata are not necessary for disjunction of the autosomes in the male (Darlington 1934a). Further, the absence of crossing over in the male in Drosophila is probably a derived condition, since in most species crossing over occurs in both sexes. Finally, there is no evidence from crossing over data from translocations involving the heterochromatin of the third chromosome in *D. melanogaster* that this region has any buffering effect (Brown 1940, this bulletin).

Although there may possibly be frequent reciprocal double exchange near the centromere in the X chromosome in the male of *D. melanogaster* the data indicate that in the material tested this region crosses over very rarely in the female. The apparent values may even be higher than the actual in the event mutation or double crossing over with the Y has occurred in the P_1 male. The calculated frequencies therefore probably represent the maximum possible values, unless genetically undetectable reciprocal exchanges to the right of *bb* on each side of the centromere are frequent. Whatever special properties may be responsible for the low recombination values in the heterochromatin, the simplest explanation remains that this region is adjacent to the centromere and hence is subject to its inhibiting effect on crossing over.

The writer wishes to express her appreciation to Dr. Wilson Stone for many helpful suggestions in the execution and analysis of the problem.

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TABLE 1

		Translocation 14		Translocation 11		Totals
Non-crossovers	+ + + + *f car bb ey* *f car bb ey* with Y	6,663 2,627 12	3* 55*	6,373 2,965 8	35*	18,648
Crossovers						
Singles						
Region 1 *f–car*	*f* non-*ey* *car bb ey* *car bb ey* with Y	207 166 4	13*	232 175 5	7*	789
Region 2 *car–bb*	*f car* non-*ey* *bb ey* *bb ey* with Y	187 118 7	6*	145 90	9*	547
Region 3 *bb–ey*	*f car bb* non-*ey* + + + *ey*	5		3		8
Doubles						
Region 1–2	*f bb ey* *car* non-*ey*	1				1
Region 1–3	*f ey* *car bb* non-ey			1		1
Region 2–3	*f car ey* *bb* non-*ey*	2		3		5
Triples						
Region 1–2–3	*f bb* non-*ey* *car ey*	1				1
Totals		10,000		10,000		20,000

*Tested for eyeless; included in totals.

TABLE 2

Recombination in Per Cent

	1 *f–car*	2 *car–bb*	3 *bb–ey*
Tl, 4 A–14	3.79	3.16	0.08
Tl, 4 A–11	4.13	2.38	0.07
Sum	3.96	2.77	0.075
Standard	5.80	3.50	(4.00?)

TABLE 3

Number and Per Cent of Chiasmata by Regions

	Nx	Sx1	Sx2	Sx3	Dx 1–2	Dx1–3	Dx2–3	Tx1–2–3
T1, 4 A–14	8,610	754	620	8	0	—4	4	8
T1, 4 A–11	8,700	822	464	—2	0	4	12	0
Sum	17,310	1,576	1,084	6	0	0	16	8
Per cent of chiasmata		7.88	5.42	0.03	0.0	0.0	0.08	0.04

Per cent of chiasmata Region 1 *f–car* 7.92; Region 2 *car–bb* 5.54; Region 3 *bb–cy* 0.15

TABLE 4

Multiple Chiasma Formation

Regions	1–2	1–3	2–3
Actual per cent	0.04	0.04	0.12
Expected per cent	0.438	0.011	0.008
Coincidence	0.09	3.6	15.0

III. SEGREGATION AND CROSSING OVER IN A 2,3 TRANSLOCATION IN *DROSOPHILA MELANOGASTER*

SARAH BEDICHEK PIPKIN[1]
Department of Zoology, The University of Texas, Austin, Texas

Segregation in heterozygous translocations involving the 2 and 3 chromosomes of *Drosophila* has been studied genetically by Sturtevant and Dobzhansky (1931), Dobzhansky (1933), and Glass (1933, 1935). Pairing of translocated chromosomes with their normal homologues at the pachytene stage in *Drosophila* was thought to be in the form of a cross configuration similar to that found in maize translocation hybrids figured by McClintock (1930). Segregation of alternate members of the cross configuration to the same pole at the first meiotic division distributes a full haploid chromosome complement to the resulting so-called orthoploid gamete. If adjacent chromosomes of the cross pass to the same pole at the first meiotic division, the gamete formed at the end of the second meiotic division is aneuploid because it does not possess a full haploid chromosome complement. Since it contains two members of the cross configuration, it is called a two-chromosome aneuploid gamete, which is diploid for the nondisjunctional arm,[2] haploid for each disjunctional arm but lacks any contribution from the chromosome arm opposite the nondisjunctional arm in the cross configuration. Very rarely three chromosomes of an equiaxial[3] cross configuration pass to one pole and one to the other at the first meiotic division. Two complementary kinds of gametes result from such a segregation: one-chromosome and three-chromosome aneuploid gametes, respectively. The former is haploid for two adjacent chromosome arms. The latter is haploid for two adjacent chromosome arms and diploid for the two other adjacent chromosome arms. One- and three-chromosome aneuploid gametes occur in Glass's case, *V5/Gr*, which forms an equiaxial T configuration, with the same frequency as that of each of the two kinds of two-chromosome aneuploid gametes, Glass (1935).

Muller (1930) and Glass (1935) have pointed out that in heterozygous *Drosophila* translocations so far studied, the orthoploid gametes tend to

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[1]Now at The North Texas Agricultural College, Arlington, Texas.

[2]Explanation of terminology: Chromosome arm—either one of four paired homologues of a pachytene cross configuration of a heterozygous translocation, or a single or paired homolgue(s) derived from one of the four paired homologues of the pachytene cross configuration now present in a gamete.

[3]Axis of pachytene cross configuration: Two imaginary lines perpendicular to each other which run between paired homologues of two nonadjacent chromosome arms.

Equiaxial translocation: A translocation which in the heterozygous state produces a cross configuration at pachytene with axes of equal length.

Heteroaxial translocation: A translocation which in the heterozygous state produces a cross configuration at pachytene with axes of unequal lengths.

Orthoploid gamete corresponds to a regular gamete in Dobzhansky's paper.

Aneuploid gamete corresponds to exceptional gamete in Dobzhansky's paper.

The terms orthoploid and aneuploid gamete, heteroaxial and equiaxial translocation are taken from Glass (1935).

occur with a frequency very slightly in excess of that of the total aneuploid gametes. Dobzhansky and Sturtevant (1931) and Glass (1933, 1935) have also found that in cases where one axis of the cross configuration formed by a heterozygous translocation at pachytene is much longer than the other axis, aneuploid gametes nondisjunctional for the long arm of the cross are far in the minority and may be absent altogether. The reason for this, according to a suggestion by Dobzhansky and Sturtevant (1931) is that there is a competition for pairing between adjacent arms in a cross configuration. In a heteroaxial cross configuration, the longer arms have the pairing advantage.

Glass concludes tentatively that equality of axes of a cross configuration causes the two types of two-chromosome aneuploid gametes to be formed with equal frequency, Glass (1935). He cites as evidence his equiaxial T configuration, *V5/Gr*, and Dobzhansky's equiaxial cross configuration. According to the theory of Dubinin and co-workers, 1935, mechanical difficulties in chromosome pairing in heterozygous translocations arise because the meiotic centromeres or spindle attachments form a chromocenter as in salivary gland nuclei. If a chromocenter exists at the meiotic phophase, then Glass's *V5/Gr* would not form an equiaxial T configuration at pachytene, since all the centromeres are not located at the bend of the T. In any case the present author has found that the two types of two-chromosome aneuploid gametes are not produced with equal frequency by flies heterozygous for the equiaxial translocation T_A 2, 3–1. Thus Glass's hypothesis is not confirmed by the present study.

Crossing over in *Drosophila* females heterozygous for a translocation has been extensively studied by means of backcross experiments. That is, females heterozygous for the translocated chromosomes, carrying wild type genes and normal homologues, containing recessive markers, were crossed to males homozygous for the recessive markers and free from the translocation. Crossovers were counted in the progeny. With the exception of Dobzhansky, previous authors calculated crossover percentages from counts of individuals the parental female gametes of which were orthoploid. In a few such cases when breakage is very close to the centromere, crossover percentages do not differ from control values (Dobzhansky, 1931, 1932; Beadle, 1933; Glass, 1933; Brown, 1940). Most of the translocations studied show a reduction in crossing over, strongest on both sides nearest to the point of breakage (Dobzhansky, 1931; Sturtevant and Dobzhansky, 1931; Rhoades, 1931; Beadle, 1933; Glass, 1933; Stone, 1934; Brown, 1940, and Tsubina, 1936). In a V-shaped chromosome this reduction does not extend to the opposite side of the centromere from the point of translocation; crossing over in this arm may be slightly increased above normal (Dobzhansky 1929, 1930, 1931a, 1931b, and others).

Dobzhansky in the investigation previously described studied the crossover composition, not only of orthoploid gametes, but also of the disjunctional and nondisjunctional arms of two-chromosome aneuploid gametes.

From this work he postulated a theory of the formation of orthoploid and two-chromosome aneuploid gametes on the basis of competitive pairing. This theory will be considered after the results of the present study have been given.

The purpose of the present investigation is to extend the knowledge of the relation between disjunction of homologous parts of chromosomes and crossing over in a heterozygous equiaxial 2,3 translocation, following the method of Dobzhansky (1933). Crossing over in the 2 and 3 of females free from the translocation and of females homozygous for the translocation, where no nondisjunction of homologous parts of chromosomes occurs, was determined for comparison.

Description of the Translocation

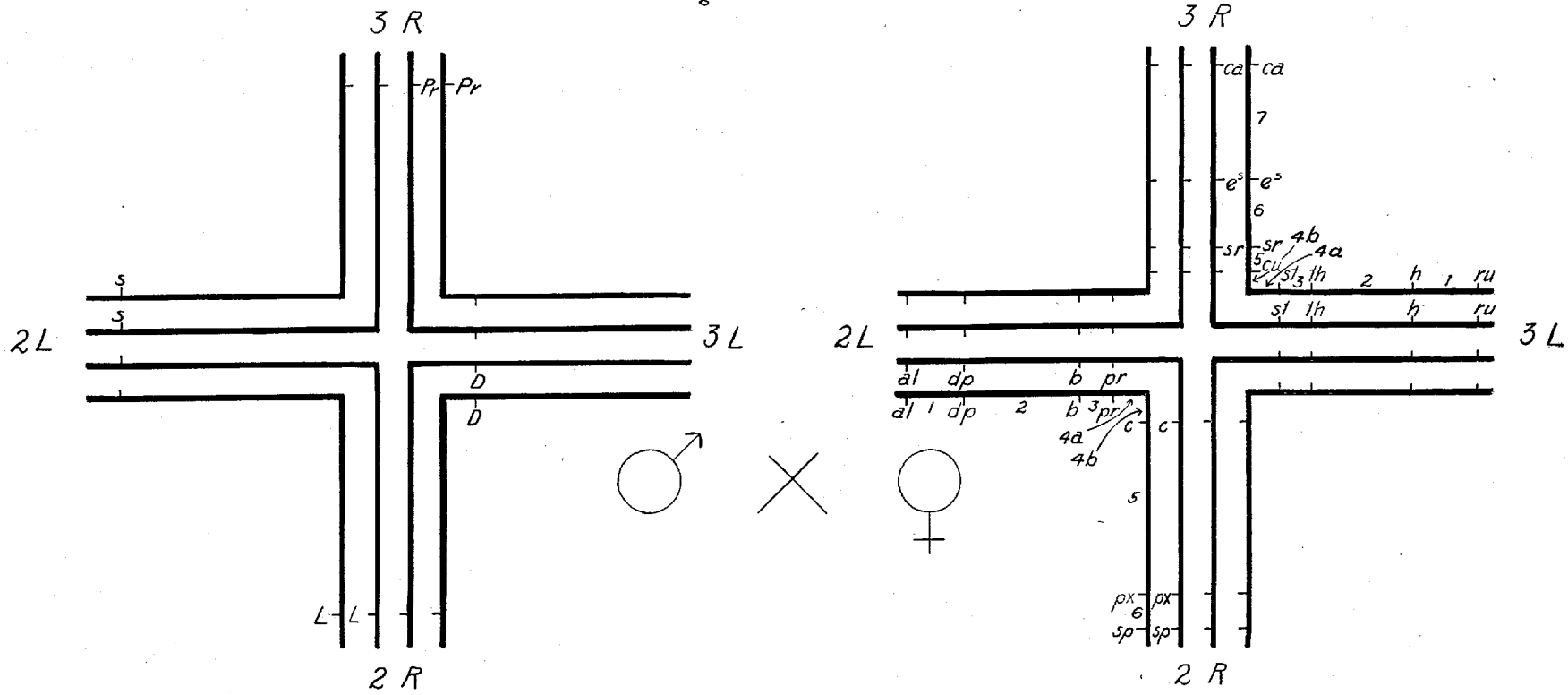
The translocation stock, designated T_A2,3–1, was obtained by X-radiation of normal males (Patterson et al. 1934). The 2 chromosome is broken between the loci of *pr* (54.5) and *c* (75.5), and 3, between *st* (44.0) and *cu* (50.0) on the genetic map. The left arm of the second chromosome (i.e. 2L) is attached to the right arm of the third chromosome (i.e. 3R) and 2R is attached to 3L. A cytological analysis by Dr. T. S. Painter, using the salivary gland technique, showed that both the 2 and the 3 chromosomes had been broken and reattached in their chromocentral regions with the result that the salivary gland configuration of a larval hybrid for the interchange appears the same as that of a normal larva. This translocation has the advantage of being fully viable and fertile in the homozygous condition.

Plan of the Experiments

An experiment in which segregation and crossing over in both 2 and 3 chromosomes could be studied was designed as follows: For each culture, a single male of the constitution *Cy pr/ "all," ru h Dcx ca/ "rucuca"*[4] was crossed with ten females homozygous for the translocation, containing wild type allels in both 2 and 3. Each parent male was numbered and its progeny kept separate in order that later those cultures could be discarded in which crossing over of *c px sp* into the *Cy pr* inverted chromosome had taken place in the *Cy pr/ "all" ru h Dcx ca/ "rucuca"* stock. The F_1 females used from this cross were heterozygous for the translocated 2 and 3 and for a normal 2 and 3 which contained *al dp b pr c px sp* and *ru h th st cu sr e*^{s} *ca*, respectively. These females were crossed at the age of two days to males heterozygous for the translocated chromosomes, carrying the dominant genes *S (Star)* in 2L and *D (Dichaete)* in 3L, and a normal 2 and 3 chromosomes marked by *L (Lobe)* and *Pr (Prickly)*, respectively. The parents were discarded five days after the culture was made. A diagram of this mating is shown below in Fig. 1.

[4]Multiple recessive 2 chromosome stock *"all"* contains the mutants *al dp b pr c px sp;* the 3 chromosome stock *"rucuca"* contains *ru h th st cu sr e*^{s} *ca.*

Fig. 1



Heterozygous ♂ X Heterozygous ♀

Genes distributed as indicated

All zygotes coming from the union of orthoploid gametes were potentially viable. They appeared *S D* or *L Pr* and formed the majority of the offspring. The *S D* flies were either homozygous for the translocation or heterozygous for it. The *L Pr* flies were either heterozygous for the translocation or free from it; i.e., normal. A smaller number of *Pr D*, *S L*, *S Pr*, and *L D* zygotes occurred in the progeny of the cross shown in Fig. 1. They arose from the union of complementary two-chromosome aneuploid gametes. *S Pr* zygotes are produced by the union of eggs containing chromosomes 2L–2R plus 2R–3L with sperm containing 2L–3R plus 3R–3L. These gametes are termed complementary aneuploid gametes, since, when they unite, the resulting zygote possesses the full diploid chromosome complement. Complementary aneuploid gametes result from similar orientations of the translocated and nontranslocated 2 and 3 chromosomes at the first meiotic metaphase. In a like manner, *L D* flies arise from the union of eggs containing 2L–3R and 3R–3L with sperm containing 2L–2R and 2R–3L. *Pr D* flies arise from the union of 2L–2R and 2L–3R eggs with 3R–3L and 3L–2R sperm. Finally *S L* flies arise from the union of 3L–3R and 3L–2R eggs with 2L–2R and 2L–3R sperm. Three-and one-chromosome aneuploid gametes which were produced very rarely by flies heterozygous for this translocation account for the few *S Pr D* and *D* individuals. In these cases, complementary aneuploid gametes must have united in order to produce a potentially viable zygote.

The few recessive genes appearing in the individuals arising from the union of complementary two-chromosome aneuploid gametes are the consequence of crossing over in the four strand stage followed by nondisjunction. These flies are known as equational exceptions and along with similar individuals in the progeny of attached-X females and triploid females heterozygous for recessives constitute proof of the four strand nature of crossing over (Bridges, 1916, and others).

The rate with which the orthoploid and total aneuploid gametes are formed in the male and in the female heterozygous for the translocation was determined by the egg and hatch count method. Two day old females heterozygous for the translocation were crossed with normal males. The eggs, laid on spoons changed twice a day, were counted for a four day period and then discarded. The food from each spoon was transferred to a food vial and the eggs allowed to develop. Finally the offspring hatching from these vials were counted. The experiment was conducted at a constant temperature of 23° C. At the same time that this experiment was being carried out, similar egg and hatch counts were made from the following crosses: T_A 2,3–1/normal male x normal/normal female; T_A 2,3–1/T_A 2,3–1 male x normal/normal female; T_A 2,3–1/T_A 2,3–1 female x normal/normal male; and normal /normal female x normal/normal male. The homozygous translocation and the normal egg and hatch counts were used as controls for the count made from the heterozygous translocation.

The crossover composition of both orthoploid and two-chromosome aneuploid gametes of the heterozygous translocation female was detected

by crossing each of the F_1 male progeny of the cross shown in Fig. 1 to both *al dp b pr c px sp/Cy pr* and *ru h th st cu sr e^s ca/Mé ca* females. In some of the experimental series in which this cross was carried out, the *"all"/Cy pr* and *"rucuca"/Mé ca* females were isolated after fertilization and their progeny examined separately. In other series, the mixed progenies of the *"all"/Cy pr* and *"rucuca"/Mé ca* females were allowed to hatch in the same vial, and doubtful cases were tested again.

Two controls for the study of crossing over in the heterozygous translocation were used: crossing over in normal 2 and 3 chromosomes and crossing over in the homozygous translocation. To study crossing over in normal 2 and 3 chromosomes, a single *Cy pr/ "all" ru h Dcx ca/ "rucuca"* male was crossed to ten females containing wild type genes in their two normal 2 chromosomes and the dominant *Pr* in one of their two normal 3 chromosomes. Ten females from this cross of the composition *+/"all" Pr/ "rucuca"* were crossed either to wild type males or to *S/Cy D/c3x* males. In the first few series, wild type males were used, but later it was found more desirable to mark the chromosome which was not to be tested for crossing over by the dominant *S* or *Cy* for the 2 and *D* for the 3. The F_1 male progeny from this last cross were tested as before for the crossing over which had occurred in their mothers.

In order to study crossing over in the homozygous translocation, the genes *ru h th st* were introduced into the 3L portion of the translocated chromosomes 3L–2R. Likewise *c px sp* was introduced into 2R. The *ru h th st* stock of T_A 2,3–1 could be maintained in the homozygous condition, but the *c px sp* stock of T_A 2,3–1 had to be balanced with the *Cy pr* inversion due to the sterility of these homozygous T_A 2,3–1 males. Hence for each culture, a single male of the composition T_A 2,3–1 *c px sp/Cy pr* was crossed with ten females homozygous for the translocation containing *ru h th st* in both 3L's. The F_1 non-*Cy* females of the composition T_A 2,3–1 *c px sp*/T_A 2,3–1 *ru h th st* were then crossed to single *Cy pr/"all" ru h Dcx ca/ "rucuca"* males. Then a crossover count was made from the *non-Cy non-D* offspring.

Experimental Results

1. Rate of Disjunction

The rates with which orthoploid and aneuploid gamete types may occur may be deduced from the classification according to dominant markers of the offspring of males and females heterozygous for the translocation, shown in Fig. 1. The numbers of the various kinds of offspring of the cross of Fig. 1 are given in Table 1.

Assuming at present that the various kinds of gametes are produced with the same frequency in male and female, we may follow the method of Dobzhansky in calculating these frequencies. Let the frequency of orthoploid gametes be x; of two-chromosome aneuploid gametes nondisjunctional for either 2L or 3L, y; of two-chromosome aneuploid gametes nondisjunctional for either 2R or 3R, z; of three– and one-chromosome

aneuploid gamete types, u and v. The zygotes from union of these gametes survive in the ratio $4x^2$: $2y^2$: $2z^2$: $2u^2$: $2v^2$ = 8234: 212: 738: 3:1. $4x^2$ represents *S D* individuals homozygous for the translocation; *S D* individuals heterozygous for the translocation; *L Pr* individuals heterozygous for the translocation; and *L Pr* individuals free from the translocation. $2y^2$ represents surviving *S L* and *Pr D* individuals; $2z^2$ represents *S Pr* and *L D* individuals. $2u^2$ and $2v^2$ represent the three *S Pr D* individuals and one D individual, respectively. The complementary types *L* and *S Pr L* were not obtained in these experiments.

Solving the above ratio, x:y:z:u:v = 45.3:10.3:19.2:1.2:0.7. Hence 59.06 per cent of all gametes are orthoploid; 13.43 per cent, two-chromosome aneuploid gamete type y (nondisjunctional for either 2L or 3L); 25.03 per cent, two-chromosome aneuploid gamete, type z (nondisjunctional for either 2R or 3R); and 2.47 per cent of the rare three- and one-chromosome aneuploid gametes, types u and v, in which either two chromosome arms are nondisjunctional or completely lacking.

The egg and hatch count experiment previously described provides an independent means of determining the frequency of orthoploid and total aneuploid gametes in males and females heterozygous for the translocation. The results of this experiment are given in Table 2.

The per cent hatch (56.93) (per cent orthoploid gametes) from males heterozygous for the translocation is slightly but significantly higher than the per cent hatch (52.88) from females heterozygous for the translocation, according to Table 2. The difference between the two, 4.05± a standard deviation of 1.10 per cent is only a little more than the difference between homozygous translocation female x normal male and normal female x normal male; i.e., 3.39± 0.66 per cent. These differences may be due to differential viability. The homozygous translocation male x normal female per cent hatch is not significantly different from that of the homozygous translocation female x normal male. (The difference is 0.92± 0.61.)

With the per cent hatch of the control, normal female x normal male, corrected from 88.71 per cent to 100 per cent, the theoretical value, the per cent hatch of heterozygous translocation males and females becomes 64.2 per cent and 59.6 per cent respectively. These values are very close to 59.06 per cent, the per cent of orthoploid gametes previously calculated by Dobzhansky's method upon the assumption that the orthoploid and different types y, z, u, and v of aneuploid gametes are produced with equal frequency in male and female parents heterzygous for the translocation. If the orthoploid gamete frequency calculated by Dobzhansky's method were different from the true orthoploid gamete frequency obtained directly by the egg and hatch count method, it might mean that the different kinds of aneuploid gamete types were being produced with different frequencies in males and in females heterozygous for the translocation.

Brown (1940) estimated the frequency with which the various gamete types occurred in males and females heterozygous for her 3,4 transloca-

tions. Her method of calculating these frequencies may be applied to the data from T_A 2,3–1 as follows:

Let y be the frequency of two-chromosome aneuploid eggs nondisjunctional for 2L or 3L; y′, the frequency of two-chromosome aneuploid sperm nondisjunctional for 2L or 3L. Let z be the frequency of two-chromosome aneuploid eggs nondisjunctional for 2R or 3R; z′, the frequency of two-chromosome aneuploid sperm nondisjunctional for 2R or 3R. Let x be the frequency of orthoploid eggs; x′, the frequency of orthoploid sperm. Then using corrected values from the egg and hatch count experiment,

$$x = 0.596 \quad (1)$$
$$x' = 0.642 \quad (2)$$
$$y + z = 0.404 \quad (3)$$
$$y' + z' = 0.358 \quad (4)$$

From the experiment in which females heterozygous for the translocation were crossed to males heterozygous for the translocation and bearing the dominant markers *S, L, Pr, D,* we may estimate yy′ and zz′. The zygotes survive from this cross in the ratio $4xx' : 2\ yy' : 2zz' = 8234:212:738$. But from the egg and hatch count experiment, $xx' = (0.596)\ (0.642)$ or 0.3826.

Hence $4(0.3826) : 2yy' = 8234:212$

Solving, $yy' = 0.01969 \quad (5)$

Similarly, $4(0.3826) : 2zz' = 8234:738$

Solving, $zz' = 0.06868 \quad (6)$

As shown above, $y + z = 0.404 \quad (3)$

Substituting values for y and z obtained from (5) and (6),

$$0.01969/y' + 0.06868/z' = 0.404 \quad (7)$$

Substituting for y′ the value obtained from (4),

$$0.01969/0.358–z' + 0.06868/z' = 0.404 \quad (8)$$

From equation (8), after clearing fractions, equation (9) is obtained.

$$(z')^2 - 0.479z = -0.0608$$

Solving, z′ is approximately 0.239

Similarly, z is approximately 0.270

y′ is approximately 0.148

y is approximately 0.133

Hence, the two kinds of two chromosomes aneuploid gametes must occur with very nearly the same frequency in males and females heterozygous for the translocation.

In this discussion it has been assumed that at the second meiotic division, sister chromatids always separate to opposite poles. This assumption is justified as the following consideration will show. After a nondisjunctional orientation of the translocated and nontranslocated chromosomes in the heterozygous translocation parent shown in Figure 1, a first oöcyte or first spermatocyte would receive two adjacent members

of the pachytene cross, each consisting of sister chromatids attached to a common centromere. The nondisjunctional arm is represented by four strands or, in other words, two sets of sister chromatids. If sister strands always separate at the second meiotic division, the resulting oötid or spermatid will develop into a two-chromosome aneuploid gamete, as the preceding discussion has assumed. If, on the other hand, sister strands should not necessarily separate at the second meiotic division in such a nondisjunctional second oöcyte or second spermatocyte, then after the second meiotic division, some gametes would be nondisjunctional for two chromosome arms and not have the other two chromosome arms represented. Such gametes would always give rise to inviable zygotes because the complementary gamete type would never be produced. If gametes of this type were produced, owing to the nonseparation in some cases of sister strands at the second meiotic division after a nondisjunctional orientation at the first meiotic division, then we should not expect that the estimate of the total aneuploid gametes calculated by the classification of dominants method would equal the percentage of aneuploid gametes calculated by the egg and hatch count method. These two methods however, each give a percentage of ca 59 per cent as the total aneuploid gamete frequency. Since the two methods agree, the possibility is excluded that sister strands sometimes fail to separate in nondisjunctional second oöcytes or second spermatocytes.

2. *Analysis of Chiasmata Association at the First Meiotic Division in Females Heterozygous for the Translocation*

The crossover composition of progeny from the cross T_A 2,3–1/ *"all" "rucuca"* female $\times$ T_A 2,3–1 *S D/L Pr* males makes possible the determination of chiasmata present in all four arms of the cross configuration at meioses destined to give rise to orthoploid gametes and in three arms only in meioses destined to give rise to two-chromosome aneuploid gametes, types y and z in the female parent. These calculations are based upon the fact that so far as crossing over is concerned, we may consider the right and left arms of 2 and 3 as separate chromosomes; i.e., there is no interference across the spindle attachment (Stevens, 1936).

Weinstein (1932, 1936) and Mather (1933) have shown that where one of the four strands from two homologues passes to a single gamete as when disjunction of all homologous chromosome parts occurs, the number of bivalents in which no, one, and two chiasmata are present may be derived from the following equations:

$$X_0 = (a_0 - a_1 + a_2 - a_3 \text{----------})$$
$$X_1 = 2(a_1 - 2a_2 + 3a_3 \text{----------})$$
$$X_2 = 4(a_2 - 3a_3 \text{----------})$$

where X_0, X_1, X_2 ---------- are the number of bivalents with 0, 1, 2 ---------- chiasmata, and a_0, a_1, a_2 ---------- represent the number of individuals recovered which show 0, 1, 2 ---------- cross overs. In case of zygotes coming

from the union of orthoploid gametes, where only one strand of each of the four arms of the cross is recovered, these equations may be combined by multiplying so as to calculate the chiasmata composition of all four arms of the cross. For example, the number of bivalents with no chiasma in 2L, one in 3R, two in 3L, and none in 2R is

$$X_{0120} = 8\left[\left[(a_{0120}) - (a_{0121} + a_{1120}) + (a_{0122} + a_{2120} + a_{1121}) - (a_{2121} + a_{1122}) + (a_{2122})\right] - 2\left[(a_{0220}) - (a_{0221} + a_{1220}) + (a_{0222} + a_{2220} + a_{1221}) - (a_{2221} + a_{1222}) + (a_{2222})\right]\right]$$

In this formula the subscripts of the X's and the a's correspond to the respective arms of the cross configuration of the heterozygous translocation in the clockwise order 2L, 3R, 3L, 2R. This convention has been followed in describing chiasmata associations in the control also. The above formula is not difficult to apply although it appears unwieldy.

Where two non-sister strands of homologues have passed to the gamete instead of one, as in the case of the nondisjunctional arm of two-chromosome aneuploid gametes, the equation for determining the number of chiasmata present in bivalents at meiosis may be derived by a similar method. From a single chiasma, the different combinations of strands are theoretically recovered in the following ratio: one individual with two non-crossover strands, a_{00},: two individuals with a single and a non-crossover strand, a_{10},: one individual with two single crossover strands, a_{11}. Or, we may say, there are recovered one a_{00} to three individuals recognizable as being derived from a single chiasma at least $(a_{11} + 2a_{10})$. With random exchange, the combinations are recovered from the various kinds of double exchange in the following ratio:

Two strand double exchange or reciprocal comparate double chiasmata	$1a_{22} : 2a_{02} : 1a_{00}$
Three strand double exchange or disparate double chiasmata	$2a_{02} : 2a_{10} : 2a_{(11)d} : 2a_{12}$
Four strand double exchange or complementary comparate double chiasmata	$2a_{11} : 2a_{(11)d}$
Total ratio	$1a_{22} : 4a_{02} : 4a_{(11)d} : 2a_{12} : 2a_{10} : 2a_{11} : 1a_{00}$

In other words, from random double exchange, we expect a ratio of one individual with two non-cross over strands (a_{00}) : four individuals either a_{10} or a_{11}: eleven individuals which could arise as a result of double or higher exchange only; i.e., a_{02}, $a_{(11)d}$, a_{12}, a_{22}. In these subscripts, "1" represents a single crossover strand; "2" a double cross over strand; and $(11)^d$ indicates two single crossover strands which have *different* points of crossing over and therefore could be recovered only from double or higher chiasmata. Thus the individuals receiving two non-crossover chromatids,

a_{00}, arise from all X_0, 1/4 X_1, and 1/16 X_2. Individuals with one single and one non-crossover chromatid or two single crossover chromatids; i.e., a_{10} or a_{11}, are derived from 3/4 X_1 and 4/16 X_2. Finally, individuals recognizable as coming from double exchange at least; i.e., a_{02}, a_{22}, a_{12}, $a_{(11)d}$, result from 11/16 X_2. Therefore the formula for deriving the number of bivalents with no chiasma, X_0, from the crossover composition of the nondisjunctional arm of two-chromosome aneuploids is obtained by solving the following equations simultaneously:

$$a_{00} = X_0 + 1/4\ X_1 + 1/16\ X_2 + \ldots\ldots \text{ etc.}$$
$$(a_{10} + a_{11}) = 3/4\ X_1 + 4/16\ X_2 + \ldots\ldots \text{ etc.}$$
$$(a_{02} + a_{22} + a_{12} + a_{(11)d} = 11/16\ X_2 + \ldots \text{ etc.}$$

Multiplying the second equation by $(-1/3)$ and the last equation by $(+1/33)$, and solving,

$$X_0 = [a_{00} - 1/3\ (a_{10} + a_{11}) + 1/33\ (a_{02} + a_{22} + a_{12} + a_{(11)d} \ldots)]$$

Similarly, $X_1 = 4/3\ [(a_{10} + a_{11}) - 4/11\ (a_{02} + a_{22} + a_{12} + a_{(11)d}) \ \ldots\ ]$

and $X_2 = 16/11\ [(a_{02} + a_{22} + a_{12} + a_{(11)d}) - \ldots\ ]$

In analysing the chiasma composition of future disjunctional arms of two-chromosome aneuploid gametes, the equations of Weinstein and Mather may be used since only one strand is recovered. By combining these sets of equations applying to the two disjunctional arms of two-chromosome aneuploid gametes, we can calculate, for example, the number of diplotene cross configurations with one chiasma in 2L; one in 3R; two in 3L; unknown in 2R, which gave rise to two-chromosome aneuploid gametes nondisjunctional for 3R and disjunctional for 2L and 3L.

$$\text{Thus } X_{112?} = 32/3\ [(a_1\ a_{10+11}\ a_2 - 4/11\ (a_1\ a_{02+22+12+(11)d}\ a_2)$$
$$+ 2\ (a_2\ a_{10+11}\ a_2) - 8/11\ (a_2\ a_{02+22+12+(11)d}\ a_2) \ldots\ldots]$$

Since this process consists of determining what chiasmata associations in cross configurations certain recovered crossover strands represent, the total number of cross configurations must equal the total recovered crossover individuals. This fact constitutes a check on the arithmetical accuracy of the calculations.

The standard deviations of the numbers of different cross configurations with various chiasma associations have been calculated by the following method which the author owes to Professor J. B. S. Haldane:

The mean square deviation is desired of a quantity which may be expressed in general as $X = b_0a_0 + b_1a_1 + b_2a_2 + \ldots$

$= \Sigma\, b_ra_r$, where a_0, a_1, a_2 represent recovered crossover strands of rank 0, 1, 2; X is the number of bivalents containing a given number of chiasmata; and b_r, are constants.

Let $\Sigma a_r = n$, the number in the sample, and let $\propto_r$ be the frequency of r crossovers, so that the expectation of a_r is $n \propto_r$. Then the probability of finding just a_0, a_1, a_2, a_3 . . . etc. individuals in a sample of n is

$$P = n! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \cdot \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_r}}{a_0!\ a_1!\ a_2!\ \ldots\ a_{r-1}!\ a_r!}$$

so that ΣP taken over all possible values $= 1$.

The first moment or mean of X; i.e., $M_x = \Sigma\,[(b_0a_0 + b_1a_1 + \ldots)P]$. $M_x = \Sigma_r b_r a_r \Sigma P$, the second summation being taken over all sets of values of a_r.

$$M_x = \Sigma_r b_r a_r \Sigma n! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_r}}{a_0!\ a_1!\ a_2!\ \ldots\ a_{(r-1)}!\ a_r!}$$

$$M_x = \Sigma_r b_r \Sigma n \propto_r \cdot (n-1)! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_{r-1}}}{a_0!\ a_1!\ a_2\ \ldots\ a_{(r-1)}!\ a_{(r-1)}!}$$

$$M_x = \Sigma_r b_r \propto_r \cdot 1$$

$$M_x = n\Sigma_r b_r \propto_r = \Sigma_r b_r a_r$$

The second moment or variance of X, $M_x^2, = \Sigma\,(b_r a_r)^2\,P$
Adding and subtracting $\Sigma b_r^2 a_r$,

$$M_x^2 = \Sigma\,[\Sigma_r\, b_r^2\, a_r\ (a_r - 1) + 2\Sigma_r\Sigma_s\, b_r b_s a_r a_s + \Sigma_r b_r^2\, a_r] \cdot P$$

$$M_x^2 = \Sigma_r b_r^2\, a_r (a_r - 1)\ n! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_r}}{a_0!\ a_1!\ a_2!\ \ldots\ a_{r-1}!\ a_r!}$$

$$+ 2\Sigma_r\,\Sigma_s\, b_r b_s a_r a_s\, n! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_r}}{a_0!\ a_1!\ a_2!\ \ldots\ a_{r-1}!\ a_r!}$$

$$+ \Sigma b_r^2\, a_r n! \frac{\propto_0^{a_0} \cdot \propto_1^{a_1} \cdot \propto_2^{a_2} \ldots \propto_{r-1}^{a_{r-1}} \cdot \propto_r^{a_r}}{a_0!\ a_1!\ a_2!\ \ldots\ a_{r-1}!\ a_r!}$$

$$M_x^2 = \Sigma_r\, b_r\, n(n-1)\ \propto_r^2 + 2\Sigma_r\,\Sigma_s\, b_r b_s \propto_r \propto_s n(n-1) + \Sigma_r b_r^2 \propto_r . n$$

or $M_x^2 = n(n-1)\ [\Sigma_r\, b_r \propto_r]^2 + n\Sigma_r\, b_r^2 \propto_r$

The mean square deviation of X, $V_x = M_x^2 - (M_x)^2$

$$V_x = n(n-1)\ [\Sigma_r b_r \propto_r]^2 + n\Sigma_r b_r^2 \propto_r - n^2\ (\Sigma_r\, b_r\, \propto_r)^2$$
$$V_x = n\Sigma_r b_r^2 \propto_r - n\,(\Sigma_r b_r \propto_r)^2$$

Our estimate of $\propto_r$ is a_r/n. Substituting this value for $\propto_r$,

$$V_x = \Sigma_r b_r^2 a_r - 1/n (\Sigma_r b_r a_r)^2$$
$$\text{or } \Sigma_r b_r^2 a_r - 1/n (M_x)^2$$

For example, $X_1 = 2a_1 - 4a_2 + 6a_3 \ldots\ldots$

In this case, $b_r = (-1)^{r-1} 2r$

$$V_{x1} = \Sigma_r 4r^2 a_r - 1/n (M_x)^2$$
$$V_{x1} = (4a_1 + 16a_2 + 36a_3 \ldots) - 1/n (2a_1 - 4a_2 + 6a_3 \ldots)^2$$

The standard deviation of X is $= \sqrt{V_x}$

This method is equally applicable in determining the standard deviations of numbers of cross configurations with different chiasmata associations in three and four arms of future aneuploid and orthoploid gametes, respectively, of the heterozygous translocation females.

In Table 3 are given percentages from heterozygous translocation females of cross configurations with various chiasmata associations destined to pass to orthoploid gametes and also percentages of bivalents with different numbers of chiasmata occurring simultaneously in the right and left arms of 2 and 3 in normal control females. Table 4 contains similar percentages of chiasmata associations in cross configurations of future two-chromosome aneuploid gametes. Standard deviations are also given in percentages; i.e., $1/n \sqrt{V_x}$. The negative values in these tables are meaningless; none in the two sets of values of Table 3, which are based upon large samples, is more than twice its standard deviation. Such negative percentages are presumably due to the chance recovery, from the rarer cross configurations with multiple chiasmata in some or all of the arms, of multiple crossover strands rather than lower ranked or non-crossover strands that could also have been recovered from the multiple chiasmata association. There are three negative percentages in Table 4, which is based on smaller samples than Table 3, which are barely more than twice their standard deviations. It is very doubtful if any biological significance should be attached to these three negative values. Absence of statistically significant negative chiasmata frequencies in Table 3 indicates that there is no chromatid interference at least in meioses of the heterozygous translocation female giving rise to orthoploid gametes or in meioses of females free from the translocation, as Mather predicted (Mather, 1933). Mather attributed his small but significant negative frequencies to differential viability rather than to chromatid interference. In the present study the effect of differential viability has been eliminated by testing each crossover individually.

The most satisfactory analyses of chiasmata associations are based upon the *S D* and *L Pr* individuals, numbering 2991, resulting from the union of orthoploid gametes. In the control figures, individuals showing crossing over in regions 4a and 4b in chromosomes 2 or 3; i.e., the regions on either side of the unmarked centromere or spindle attachment, have been excluded, bringing N down from 1558 to 1204. *S Pr* individuals, coming

from the union of two-chromosome aneuploid gametes nondisjunctional for 2R in the female, number 362; *L D* individuals, nondisjunctional for 3R in the female, 398. Tests were obtained for only 131 and 123 *Pr D* and *S L* flies, respectively. The low number of individuals analysed in these last determinations prejudices the accuracy involved.

Although differential viability in crossover individuals due to recessive genes is negligible because each F_1 male was tested for the crossing over which had occurred in the female parent there is another unfortunate source of error in the analysis of *S Pr* and *S L* individuals. The dominant marker *S* (*Star*) located in 2L suppresses the recessive mutant *px* in 2R. Hence in testing *S Pr* and *S L* individuals, all heterozygous for the translocation, it was necessary, in case *c* or *sp* or both had obviously crossed into the translocated 2R, to mate to flies of the composition T_A2,3–1 *c px sp/Cy pr* and then backcross to the same stock so as to get S, in 2L to segregate from the locus of *px* in 2R in the homozygous translocation. As a further complication, males homozygous for the translocation and *c px sp* were of very low fertility, so a number of these tests failed. A record was not kept of the sterile cultures at first. Later it was found that out of 231 *S Pr* flies, 195 tests were complete; 25 failed because of incomplete analysis of the translocated 2R containing *c* or *sp* or both; nine, due to incomplete analysis of 2L or 3L, the translocated 2R being free from *c* or *sp*; and finally, two, on account of incomplete analysis of 2R at least when it was not known whether *c* or *sp* or both had crossed into the translocated 2R. From 117 S L flies tested after a record of sterile cultures was started, 89 were successful. Twenty-two failed because of the incomplete analysis of the translocated 2R containing *c* or *sp* or both; one, from incomplete analysis of 2L and 3R but 2R did not have either *c* or *sp;* and five, because of incomplete analysis of 2R at least, where it was undetermined as to whether or not *c* or *sp* or both were present. Three tests were unsuccessful of 93 *Pr D* and 17 of 304 *L D* flies.

The largest chiasmata percentage in the heterozygous translocation cross configuration of future orthoploid gametes is 32.63 ± 8.2 per cent. This is the proportion having a single chiasma in each of the four arms; i.e., X_{1111}. In the control, 33.22 ± 8.66 per cent bivalents contained a single chiasma in each of 2L, 3R, 3L, and 2R. The two series agree further in having a negligible proportion of diplotene cross configurations with no chiasma in any of the four arms. Frequencies of cross configurations with a single chiasma in either one or two arms only of future orthoploid gametes of the heterozygous translocation are negligible or else very low. Similar frequencies of the control are also negligible or very low. Chiasmata associations involving a double chiasmata in one or more arms of 2 or 3 are subject to high error, as stated previously. Nevertheless, the fact is important that numerous combinations occur and with comparable frequencies in both the heterozygous translocation and the control.

One negative conclusion with respect to formation of aneuploid gametes may be derived from Table 3. A priori, if at least a single chiasma occurs in two nonadjacent arms of the cross configuration of the heterozygous translocation and no chiasma in the other two nonadjacent arms, then the cross will open out into two bivalents at the first metaphase. Disjunction of the two bivalents would be independent and result in orthoploid and two-chromosome aneuploid gametes equally frequently. If this is the source of an appreciable number of the two-chromosome aneuploid gametes, we should expect that a fair-sized percentage of cross configurations destined to give rise to orthoploid gametes contain a single chiasma in two nonadjacent arms with no chiasma in the other two arms. According to Table 3, these percentages are not significant. Thus $X_{1010} = 0.27 \pm 3.44\%$ and $X_{0101} = 3.88 \pm 3.99\%$. Therefore, independent disjunction of two bivalents in a heterozygous translocation female is a trivial source of two-chromosome aneuploid gametes which occur with a total frequency of 38.4 per cent of all the gametes.

Similarly, the three-and-one-chromosome aneuploid gametes should occur with equal frequency as orthoploid gametes from a cross configuration showing at least a single chiasma in two adjacent arms and no chiasma in the other two adjacent arms, if the univalent passed at random to either pole. These types are also of very low frequency: X_{1100} has a frequency of $4.68 \pm 3.94\%$; X_{0110}, $-6.39 \pm 4.02\%$ (absurd value); X_{0011}, $1.60 \pm 3.63\%$; and X_{1001}, $6.02 \pm 3.34\%$. The female gamete which could have contributed to the recovered *D* individual may have been derived from the same kind of chiasma associations as the $4.68 \pm 3.94\%$ of diplotene cross configurations of the composition X_{1100}, with a single chiasma in 2L and 3R and no chiasma in 3L and 2R, which resulted in orthoploid gametes. Likewise the female gamete of the *S Pr D* flies could have come from a chiasma association of X_{0110}, with a single chiasma in 3R and 3L but no chiasma in 2L or 2R. No orthoploid gametes were derived from the latter type of association. Finally, no three- and one-chromosome aneuploid gametes were detected which correspond to the $6.02 \pm 3.34\%$ X_{1001} or the $1.60 \pm 3.63\%$ X_{0011} giving rise to orthoploid gametes.

The number of chiasma (ta) occurring at meiosis can be determined for three arms only when two-chromosome aneuploid gametes resulted. Some deductions may be made as to the nature of the unidentifiable fourth arm, however. In case no chiasma occurred in the arm destined to be nondisjunctional but at least one chiasma was present in each of the disjunctional arms of a future two-chromosome aneuploid gamete, then the missing fourth arm must have contained a chiasma also. Otherwise the cross configuration would have opened out into two bivalents at the first meiotic metaphase and with independent disjunction of these two bivalents, orthoploid and two-chromosome aneuploid gametes would have been formed with equal frequency. As previously shown, X_{1010} and X_{0101} calculated from orthoploid gametes occurs with very low frequency.

Therefore, $X_{1?10}$ calculated from *S Pr* individuals must be X_{1110}; $X_{101?}$ from *L D* individuals, must be X_{1011}. Similarly, $X_{01?1}$ from *Pr D* must be X_{0111}, and $X_{?101}$ from *S L* must be X_{1101}. Now the unidentifiable fourth arm of cross configurations of future two-chromosome aneuploid gametes which contained a single chiasma in each of the three detectable arms either could have had a chiasma in it or not. Therefore, the frequency of $X_{111?}$ from *L D;* i.e. 65.42 ± 10.24%, should be equal to or greater than the frequency of $X_{1?10}$ (X_{1110}) from *S Pr;* i.e., 28.06 ± 9.32%. The frequency of $X_{1?11}$ from *S. Pr;* i.e., 54.91 ± 9.36% should be equal to or greater than the frequency of $X_{101?}$ (X_{1011}) from *L D;* i.e., 8.22 ± 6.19%. The frequency of $X_{?111}$ from *S L;* i.e., 26.02 ± 17.09 should be equal to or greater than the frequency of $X_{01?1}$ (X_{0111}) from *Pr D;* i.e., 41.92 ± 11.95%. Finally, the frequency of $X_{11?1}$ from *Pr D;* i.e., — 11.10 ± 23.63% should be equal to or greater than the frequency of $X_{?101}$ (X_{1101}) from *S L;* i.e., —3.25 ± 14.66%. Thus some two-chromosome aneuploid gametes nondisjunctional for 2R and 3R in the female did arise from cross configuration in which there was a single chiasma in each arm. The value 26.02% ± 17.09 (not significant) for $X_{?111}$ from *S L* is unexpectedly much below 41.92± 11.95% $X_{01?1}$ from *Pr D,* but these percentages are subject to more distortion than the calculations based upon *S Pr* and *L D* flies since they are derived from the rarer two-chromosome aneuploid gamete group.

In general, the analysis of chiasma associations of meioses giving rise to two-chromosome aneuploid gametes is very similar to the analysis of chiasma associations of meioses giving rise to orthoploid gametes. There are probably very few, if any, cross configurations at meiosis which result in two-chromosome aneuploid gametes that have no chiasma in any of the four arms.

Table 5 is a summary of the chiasma associations in the control and the orthoploid gametes from heterozygous T_A 2,3–1. Table 6 is a similar summary for the two-chromosome aneuploid gametes. In Tables 5 and 6 two sets of percentages are given for orthoploid and aneuploid gametes chiasma associations. The first per cent given is the per cent a particular chiasma association forms of its gamete type; the second value given in parentheses is the per cent that chiasma association forms of the total gamete frequency. Thus the per cents of orthoploid chiasma associations are multiplied by 0.5906, the proportion of total gamete frequency represented by orthoploid gametes, to give the percentages in parentheses. The chiasma association percentages of two-chromosome aneuploid gametes nondisjunctional for 2R are multiplied by 0.1251 to give the percentages that such chiasma associations form of the total gamete frequency those of two-chromosome aneuploid gametes nondisjunctional for 3R are likewise multiplied by 0.1251 to give the second set of percentages. Similarly, the percentages of chiasma associations of two-chromosome aneuploid gametes nondisjunctional for 2L are multiplied by 0.0671; the chiasma association percentages of two-chromosome aneuploid gametes nondisjunctional for 3L are also multiplied by 0.0671 to give the percentages of

total gametes formed by the various chiasma associations. Two chromosome aneuploid gametes nondisjunctional for 2R and 3R form 0.2502 of the total gametes; two-chromosome aneuploid gametes nondisjunctional for 2L and 3L form 0.1342 of the total gametes.

In the chiasma associations given in these tables, the subscript $_x$ means that at least one chiasma has occurred in a particular arm. For example, in Table 5, X_{xxxo}, derived from the control, means that at least one chiasma occurred in 2L, 3R, 3L, but none in 2R. X_{xxxo} in this case is equal to $X_{1110} + X_{1120} + X_{1210} + X_{2110} + X_{2120} + X_{2210} + X_{2220}$. In Table 6, $X_{xxo?}$ is equal to the sum of $X_{110?} + X_{120?} + X_{210?} + X_{220?}$. The standard deviation follows the value of the first set of percentages in Tables 5 and 6. It should be noted that for example in Table 6 the chiasma association percentages for $X_{?xxx}$; $X_{x?xx}$; $X_{xx?x}$; and $X_{xxx?}$ include both X_{oxxx}; X_{xoxx}; X_{xxox}; and X_{xxxo}, respectively; i.e., chains of four chromosomes; but also X_{xxxx}, rings of four chromosomes.

In heterozygous T_A 2,3–1, nearly a half of the orthoploid gametes arise from rings of four chromosomes, where a chiasma occurred in each of the four arms of the original cross configuration. Rings of four gave rise to $48.68 \pm 7.61\%$ of orthoploid gametes according to Table 5. This represents 28.75 per cent of the total gametes. Rings of four chromosomes gave rise to between 43 and 52 per cent of two-chromosome aneuploid gametes nondisjunctional for 2R and 3R as may be seen from the following consideration: $X_{x?xo}$ calculated from *S Pr* individuals must be X_{xxxo}. Otherwise the X_{xoxo} group derived from orthoploid gametes would be large since two-chromosome and orthoploid gametes would be formed with equal frequency by random disjunction of two bivalents at the first meiotic division. X_{xoxo}, from orthoploid gametes is zero, according to Table 5. Therefore the per cent of X_{xxxx}, giving rise to the parental female gametes of *S Pr* and *L D* individuals can be estimated from the following: X_{xxxx} equals 81.50 (the per cent of $X_{xxx?}$, calculated from *L D* individuals) minus 38.37 (X_{xxxo}, calculated from *S Pr*) or 43.13. Another estimate of this X_{xxxx} may be derived as follows: $X_{xox?}$ from *L D* must be X_{xoxx} for the reason given above in a similar case. Therefore X_{xxxx} giving rise to parental female gametes of *S Pr* and *L D* individuals is 61.07 ($X_{x?xx}$ from *S Pr*) minus 8.95 (X_{xoxx} from *L D*) or 52.12 per cent. Hence between 43 and 52 per cent of two-chromosome aneuploid gametes come from rings of four chromosomes. These estimates form from 10 to 13 per cent of the total gamete frequency since two chromosome aneuploid gametes nondisjunctional for 2R or 3R have a frequency of 0.2502. Similar reasoning gives widely inconsistent values for an unaccountable reason for the per cent of gametes nondisjunctional for 2L and 3L coming from rings of four chromosomes. The two estimates of the per cent of two-chromosome aneuploid gametes nondisjunctional for 2L and 3L arising from rings of four chromosomes are 4.98 and 32.34 per cent respectively. ($X_{?xxx}$ from *S L* or 34.69 minus $X_{ox?x}$ from *Pr D* or 29.71 equals 4.98 per cent X_{xxxx}. On

the other hand, $X_{xx?x}$ or 46.63 per cent minus $X_{?xox}$ from *S L* or 14.09 equals 32.34 per cent X_{xxxx}.)

Thus rings of four chromosomes give rise to 28.75 per cent of the gametes as orthoploid gametes, and from 10 to 13 per cent of the gametes as two-chromosome aneuploid gametes nondisjunctional for 2R or 3R. It is possible that rings do not give rise to two-chromosome aneuploid gametes nondisjunctional for 2L or 3L and that this gamete type results mainly from chains of four chromosomes. The conflicting estimates of the per cents of X_{xxxx} calculated from these two-chromosome aneuploid gametes nondisjunctional for 2L or 3L does not allow us to be certain about this. In any case, rings are orientated disjunctionally approximately twice as frequently as nondisjunctionally in heterozygous T_A 2,3–1 females.

From studies of crossing over and disjunction in 3,4 translocations, Brown (1940) developed a theory concerning disjunction in chains of chromosomes and applied it to Dobzhansky's 2,3 translocation. According to her theory, one type of nondisjunctional orientation occurs at the first meiotic division in the case of a chain of four chromosomes (where at least one chiasma was present in three arms only of the cross configuration, and no chiasma was present in the fourth arm). In this nondisjunctional orientation, the two end members of the chain are directed toward one pole while the two middle members of the chain are directed toward the other pole. Disjunctional orientations, where alternate members of the chain are directed towards the same pole, occur with the same frequency as this one type of nondisjunctional orientation described. Brown points out that evidence for the truth of this theory lies in the results of Dobzhansky's 2,3 translocation because (1) two-chromosome aneuploid gametes and orthoploid gametes occur with equal frequency and (2) there is a mean chiasma frequency of approximately one which is the normal frequency in the disjunctional arms of two-chromosome aneuploid gametes and a chiasma frequency of approximately one-half in nondisjunctional arms of two-chromosome aneuploid gametes. The disjunction and nondisjunction which occur on the basis of Brown's theory come from an orientation which separates two pairs of adjacent centromeres in the chain. The type of nondisjunction which fails to occur would separate only one pair of adjacent centromeres in the chain.

Apparently there were few rings of four chromosomes in Dobzhansky's 2,3 translocation so that disjunction was determined from chains mainly.

Table 7 was prepared to test the application of Brown's theory of disjunction in chains of four chromosomes to T_A 2,3–1. The first column of Table 7 shows four kinds of chains of four chromosomes: a chain in which a chiasma failed to form in 2L; a chain in which a chiasma failed to form in 3L; a chain in which a chiasma failed to form in 3R; and finally a chain in which a chiasma failed to form in 2R. These chains are shown in disjunctional orientation merely in the interest of saving space. If disjunction is at random in a chain such as these, six possible types of gametes may result. According to Brown's theory, only four of these gametes are actually formed, and these are formed with equal frequency from any one

type of chain: the two complementary orthoploid gametes and the two complementary two-chromosome aneuploid gametes coming from that type of nondisjunctional orientation in which the two end members of the chain pass to one pole and the two middle members pass to the other pole. In Table 7 the centromere of chromosome 2L–2R is numbered 1; of 2R–3L, 2; of 3L–3R, 3 and of 3R–2L, 4. In the second column of Table 6, the centromere numbers indicate the six possible gamete types, assuming random disjunction. In the third column the chiasma associations are given which may be calculated from zygotes having each parental female gamete. Following in the fourth column are chiasma association percentage values taken from Tables 5 and 6. The last column gives the corresponding percentages of the fourth column multiplied by the proportion such gamete class forms of the total number of gametes.

There are present in Table 7 certain classes marked as "don't expect" which should be absent if Brown's theory of disjunction is correct. There is an alternate possibility for their production: they may have come from a type of chiasma association other than a chain of four chromosomes. The unexpected gamete types may possibly have been derived from chains of three chromosomes and a univalent. However, segregation from a chain of three chromosomes and a univalent should give rise most often to three-and one-chromosome aneuploid gametes rather than to two-chromosome aneuploid gametes, unless the three chromosomes in the chain segregated at random which seems unlikely from Brown's results with chains of three chromosomes. It seems more probable that the total of 7.48 per cent "unexpected" gamete types were derived from chains of four chromosomes. Even so the most frequent type of nondisjunction was that postulated by Brown. In the first type of chain in Table 7 with no chiasma in 2L, none of the unexpected classes were present. In the second type of chain with no chiasma in 3L, 2.37 per cent were unexpected and 6.32 per cent were expected gamete types. In the third type of chain with no chiasma in 3R, there were 2.18 per cent not expected to 5.23 per cent expected; and in the fourth type of chain with no chiasma in 2R, 2.93 per cent unexpected and 17.82 per cent expected gamete types were obtained. On the basis of equal frequency of the six gamete types there should have been a 1:2 ratio of unexpected to expected gamete types. Therefore disjunction in a chain of four chromosomes is definitely not proceeding at random but in the direction of what is expected on the basis of Brown's theory.

3. Question of Random Double Exchange in the Nondisjunctional Arm of Future Two-chromosome Aneuploid Gametes

The equations for the derivation of the number of chiasmata present in the nondisjunctional arm of future two-chromosome aneuploid gametes have been based upon randomness of double exchange. In Table 8 appear the numbers of various combinations of recovered cross over strands of two-chromosome aneuploid eggs which united with complementary sperm to give *L D, S Pr, S L, Pr D* flies.

If a single chiasma has occurred in the nondisjunctional arm of a future two-chromosome aneuploid gamete, the combination of single and non-crossover strand in a_{10} individuals is expected to be recovered twice as frequently as the combination of two single crossover strands in a_{11} individuals. If exchange is at random, three-strand double exchange (disparate crossing over) contributes to the a_{10} group as much as four strand double exchange (complementary comparate crossing over) contributes to the a_{11} group. Therefore, theoretically, the a_{11} class should be half as large as the a_{10} class. Actually the number of a_{11} individuals is not significantly different from half of the number of a_{10} individuals.

If double exchange is at random, the various combinations of strands which are recognizably derived from double exchange should be recovered in the ratio 1 a_{22} : 4 a_{02} : 4 $a_{(11)d}$: 2 a_{12}. Table 5 shows that the recovery of a_{12} individuals from three strand double exchange is far below expectation. The a_{02} and $a_{(11)d}$ groups occurred with approximately equal frequency, according to Table 8. Therefore it seems that two and four strand double exchange are occurring with the same rate, but three strand double exchange is much decreased, from the random double exchange expectation. Lowering of three strand double exchange reduces the contribution of three strand double exchange to the number of a_{10} individuals. However, this departure from random double exchange was evidently not large enough to disturb the 1:2 ratio of a_{11} individuals to a_{10} individuals observed. The number of a_{22} individuals was unaccountably high. In spite of the non-random double exchange found in nondisjunctional arms of T_A–2,3–1, double exchange is at random in future nondisjunctional arms of two-chromosome aneuploid gametes of the translocation studied by Dobzhansky, 1933. Throughout this discussion, it has been assumed that recovered double crossover strands arise almost exclusively from double exchange instead of a higher degree of chiasma formation since no triple crossover strands were recovered in the nondisjunctional arms of two-chromosome aneuploid gametes.

4. Crossing Over

Crossover percentages for each chromosome arm may be obtained in the usual way if one strand only is recovered in the eggs as in the case of the control; homozygous translocation, and of the heterozygous translocation, both orthoploid gametes and disjunctional arm of two-chromosome aneuploid gametes. Standard deviations are calculated from the formula, S. D. $= \sqrt{\frac{p\,q}{n}}$. Alternatively, map distances in chiasmata percentages derived by Weinstein's equations (1932, 1936) may be halved to give the ordinary map distances to which we are accustomed. These equations for each crossover region are as follows:

$$X_0 = (a_0 - a_1 + a_2 - a_3 \text{ ----------})$$
$$X_1 = 2(a_1 - a_2 + a_3 \text{ ----------})$$
$$X_2 = 4(a_2 - a_3 + a_4 \text{ ----------})$$
$$X_3 = 8(a_3 - a_4 \text{ ----------})$$

Percentages are then obtained by dividing X_0, X_1, X_2, etc. by the number in the sample. In these equations, X_0 represents the number of bivalents with no chiasma; X_1, the number of bivalents with one chiasma; X_2, the number of bivalents with two chiasmata, and so on. a_0, a_1, a_2, a_3, etc. represent the number of non-crossover individuals, single crossover individuals, double crossover individuals; etc., recovered, respectively.

For regional crossover percentages in the nondisjunctional arm of two chromosome aneuploid gametes, the number of chiasma associations (rings, chains, bivalents) in which a single chiasma occurred in each region of that nondisjunctional arm and also the number of chiasma associations in which a double chiasma occurred involving each region of that nondisjunctional arm must first be calculated. The number of chiasma associations in which a single chiasma occurred in region r and a double involving region r and any other region may be calculated from the following equations:

$$X_{1r} = 4/3(a_{1r} - 1/11a_{2rs} - 3/11a_{2rb} \text{ ----------})$$
$$X_{2r} = 16/11(a_{2rc} \text{ ----------}),$$

In the first equation, a_{1r} = the number of single crossover individuals for region r; a_{2rs}, the number of double crossover individuals involving region r and any region, s, distal to r (s = all regions distal to r); a_{2rb} double crossover individuals involving region r and any region b proximal to or nearer the centromere than r (b = all regions proximal to r). The author is indebted to Dr. Meta Brown for calling to her attention the fact that only 1/16 of the pairs of strands recovered from all types of double exchange appear as single crossover individuals (a_{10} or a_{11}) if the second point of crossing over is proximal to the point of crossing over in the region being considered; 3/16, as single crossover individuals if the second point of crossing over is distal to the point of crossing over in the region being considered. In the second equation above, a_{2rc} means the number of all double crossover individuals involving region r and other regions, c, proximal or distal to r (c all regions proximal and distal to r).

Numbers of chiasma associations are converted into percentages by dividing each by N, the total number of individuals upon which the calculations are based. Then map distances in chiasmata percent are obtained by adding single chiasma and double chiasmata for each region (and any higher ranked chiasmata, if they occur). Finally the map distances in chiasma percentages are halved to give crossover per cents comparable to those of our ordinary maps.

If the crossover percentages for each region of the nondisjunctional and disjunctional arm(s) of two-chromosome aneuploid gametes had been calculated from samples of equal size, the standard deviations of the non-

disjunctional arm crossover percentages would be smaller than the standard deviations of the crossover percentages of the disjunctional arm of aneuploid gametes. This follows from the following facts: three-fourths of the individuals bearing crossover chromosomes resulting from a single chiasma and eleven sixteenths of those resulting from double chiasmata are recognizable as such if two strands are recovered as in the nondisjunctional arm of two-chromosome aneuploid gametes. On the other hand, only one-half the individuals from single and one-fourth the individuals from double chiasmata are recognizable as such if only one strand is recovered as in the disjunctional arm of two-chromosome aneuploid gametes.

The standard deviations of regional crossover percentages of the non-disjunctional arm of two-chromosome aneuploid gametes were obtained by Haldane's method described in the appendix.

Table 9 gives the crossover percentages with their standard deviations for each region of chromosomes 2 and 3 derived from the heterozygous translocation and control, and for the regions of 2R and 3L of the homozygous translocation. From the heterozygous translocation, there are two sets of crossover figures for the disjunctional arms of two-chromosome aneuploid gametes. In case of 2L, 3L, and 3R, these two sets were combined in order to calculate the per cents given in Table 9. Thus the sample for 2L and 3L comes from 362 *S Pr* plus 398 *L D* individuals, making N, the number in the sample equal 760. Similarly, the sample for 3R comes from 131 *Pr D* plus 123 *S L* flies, giving N equal to 254. Owing to the known source of error in crossovers of 2R derived from *S L* flies, the percentages in Table 9 for this arm are calculated from *Pr D* flies alone, with N equal to 131. In Table 10 appear the regional differences plus or minus their standard deviations between pairs of series in Table 9. Unless the difference between two crossover percentages is twice the standard deviation of the difference, the crossover percentages are not regarded as being significantly different from one another. The crossover values of the control, orthoploid gametes of the heterozygous translocation, and the crossover values of the homozygous translocation are based upon samples of 1558, 2991, and 2538 individuals, respectively.

The control and homozygous translocation differ in two regions out of the seven compared; the control value is higher in one region, the homozygous value in the other. The control and orthoploid series differ in seven of fifteen regions. In four of these regions, the percentage of the control is higher; in three, that of orthoploid gametes exceeds the corresponding control value. Orthoploid gamete crossover percents differ from those of the homozygous translocation in five out of seven regions studied. The homozygous translocation values are higher than the orthoploid values in two regions and lower than the orthoploid values in three regions. A comparison of regional crossover percentages of the disjunctional arm of two-chromosome aneuploid gametes with those of homozygous translocation shows one region of seven significantly different; with orthoploid

gametes, five of fifteen; and with control, two of fifteen regions. The differing regions in all these comparisons are higher in the disjunctional arm of two-chromosome aneuploid gametes. Control and nondisjunctional arm of two-chromosome aneuploid gametes differ significantly in four out of fifteen regions. Crossover percentages calculated from orthoploid gametes are significantly higher than nondisjunctional arm values in five out of fifteen regions. Four of fourteen regions have differences which are at least twice the standard deviation of the difference in the comparison between crossover percentages of the disjunctional arm and nondisjunctional arm of two-chromosome aneuploid gametes. Homozygous translocation and nondisjunctional arm values differ in two of six regions. In no comparison is a regional crossover percentage of the nondisjunctional arm of two-chromosome aneuploid gametes of heterozygous translocation females significantly higher than the corresponding percentage of the control, homozygous translocation, or orthoploid gametes or disjunctional arm of two-chromosome aneuploid gametes of the heterozygous translocation. If the standard deviations of crossover percentages of nondisjunctional and disjunctional arms of two-chromosome aneuploid gametes were smaller, it is probable that more small but significant regional differences would be found between either of these two series and the control, homozygous translocation, and orthoploid gametes of heterozygous translocation females. Nevertheless, Table 10 places an upper limit to the magnitude of the differences which do occur.

The numbers of the different chromosome arms 2L, 2R, 3L, and 3R, in which a chiasma failed to form at meiosis are derived by the Weinstein and Mather equations for cases where one strand is recovered and by the modified method previously given in case of recovery of two strands. No-chiasma percentages in Table 11 show in general the same facts as the crossover values in Table 9. Values from the nondisjunctional arm of two-chromosome aneuploid gametes are consistently higher than those from the orthoploid gametes or the control. No-chiasma percentages from the disjunctional arm of two-chromosome aneuploid gametes are lower in 3L, than percentages calculated from the nondisjunctional arm. The negative values in 2L and 2R, disjunctional arm, are supposedly meaningless.

Similar information may be had from a consideration of the total crossover map distance of 2L, 2R, 3L, and 3R in Table 12. In general, the totals of the disjunctional arm of two-chromosome aneuploid gametes are higher than any other totals for the different arms concerned. Totals of the nondisjunctional arms of two-chromosome aneuploid gametes are consistently below those of orthoploid gametes, the homozygous translocation, and the control. The latter three series differ slightly among themselves.

Discussion

As a general rule, one or more chiasma(ta) serve to help orientate the bivalent on the metaphase plate and thus insure normal disjunction of the homologous pairs of chromosomes at the first meiotic division. (Darlington, 1937). A disturbance of chiasma formation might be expected to cause irregularities in disjunction of homologues at the first metaphase. Of course the notable exception is the normal disjunction of the autosomes of the *Drosophila* male, where no chisma are formed (Darlington, 1934). Beadle (1933) and Richardson (1935) have described cases in *Zea* and *Crepsis,* respectively, in which pairing of homologues occurred, but was not followed by chiasma formation, and disjunction at the first anaphase was irregular. Furthermore, if a certain chromosome pair fails to form chiasma(ta) as in the micro-chromosomes of certain *Hemiptera* (cited by Darlington, 1937) or the A pair in certain *Crepsis* plants (Richardson, 1935), the resulting univalents are distributed irregularly at the first anaphase to the two poles, whereas in the same cell division, bivalents held in metaphase association by chiasmata disjoin in regular fashion.

In *Drosophila* females homozygous for the third chromosome recessive mutant *c3G,* crossing over is practically eliminated and disjunction of homologues is irregular, but segregation is nevertheless distinctly non-random. The offspring with diploid autosomes (2A) far exceed those with triploid autosomes (3A), even more than can be accounted for by differential viability. Moreover, the presence of a Y chromosome in the homozygous *c3G* female increases the triploid progeny fourfold (Gowen, 1933).

These examples show an alteration of the normal disjunction phenomena accompanied by extreme reduction or absence of crossing over. On the other hand it has long been known that no crossing over occurs at meiosis in the autosomes of *Drosophila* males, and yet a mechanism has been established ensuring normal disjunction Even though we set aside meiosis in the male *Drosophila* as an exception to the rule that chiasma formation is a necessary condition for normal disjunction, the fact that disjunction is by no means random in homozygous *c3G* females shows other factors besides chiasma formation to be governing disjunction.

Heterozygous translocations involving two non-homologous chromosomes have been widely studied by cytologists in plants (*Zea, Pisum, Campanula, Tradescantia,* and *Oenothera*). First metaphase figures show both disjunctional and nondisjunctional orientations from rings and chains of four, giving rise to orthoploid and aneuploid gametes, respectively. There has been some difference of opinion as to whether or not aneuploid gametes are produced with greater frequency from rings of four which arise owing to one or more chiasma(ta) in each arm rather than from chains of four which arise owing to one or more chiasma(ta) in three arms only. According to Table 5, 48.68 per cent of orthoploid gametes come from rings

of four chromosomes. Two different estimates of the per cent of two-chromosome aneuploid gametes nondisjunctional for 2R and 3R coming from rings of four chromosomes are 43.13 and 52.12 per cent, respectively. In a heterozygous translocation in *Zea* analyzed by Burnham (1932), the configuration was of necessity a T, opening into a chain always, never a ring. Yet adjacent separations were noted in one hundred and ninety-nine nuclei, compared with one hundred and eighty alternate separations. In *Tradescantia edwardsiana,* Sax and Anderson (1933) report a higher percentage of nondisjunction from rings than from chains. Glass (1935) cites several other cases in some of which rings disjoin more regularly than chains, whereas in others the reverse is true. Hence no general conclusion may be drawn regarding the influence on disjunction of chiasmata in three as opposed to four arms of heterozygous translocations.

Aside from mere presence or absence of a chiasma in the arms of a cross configuration of a heterozygous translocation, Gairdner and Darlington (1930) have suggested a way in which chiasmata may affect segregation. In *Pisum* the chiasmata often fail to terminalize and thus give the ring a rigidity which favors nondisjunctional orientations rather than disjunctional. In the case of *Tradescantia edwardsiana,* Sax and Anderson agree with the former authors. Sax and Anderson state "it is clear that the greater rigidity of chains or rings caused by subterminal chiasmata increases the proportion of nondisjunction" (Sax and Anderson, 1933). However, they regard the presence of interstitial chiasmata as only one of several factors influencing the first metaphase orientation of chromosomes in a heterozygous translocation. They cite, among other cases, *T. reflexa* with only forty per cent of the interchange rings and chains possessing interstitial chiasmata although nondisjunction occurs in over eighty per cent of the cells.

It is difficult to estimate the effect of possible interstitial chiasmata in *Drosophila* on disjunction in heterozygous translocations in the absence of cytological evidence. In the case of T_A2,3–1, egg and hatch counts show the percentage of orthoploid gametes to be practically the same in the female, where crossing over occurs, as in the male, where no crossing over occurs. However, disjunction in a chain of four chromosomes is not proceeding at random but in the direction of what is expected on the basis of Brown's theory.

Brown's extensive studies of 3,4 translocations (Brown, 1940) prove a definite relation between the length of the translocated fragment, crossing over, and disjunction. The per cent of orthoploid gametes in heterozygous translocation male and female are definitely different in certain cases. Brown's theory concerning disjunction in chains of three chromosomes fits her cases well.

A special case in which disjunction rates in a heterozygous translocation differ in the two sexes was reported by Dobzhansky (1933). When inversions were introduced into the two arms of the same axis of his 2,3 equiaxial translocation, the new rate of formation of different kinds of

aneuploid gametes was strikingly changed in the heterozygous translocation female. There was a similar but vastly weaker change in the heterozygous translocation male. These differences in disjunction between the sexes may be due to an influence of chiasma formation, perhaps degree of terminalization, which may act as an accessory agent in determining the disjunction rate.

Anderson (1934, 1938) has reported two translocations in maize which give different crossover values when heterozygous with normal in the male and in the female parents, although there is no striking difference in crossing over in the sexes without the translocation. These translocations were referred to as semi-steriles and the exact disjunction rate not given. The difference in crossing over in the two sexes occurs in one of the translocated homologues only and is opposite in direction in the two cases.

Dobzhansky also concluded that the relation between crossing over and disjunction in heterozygous translocations was an indirect one, but he thought that it was possible that both these processes were dependent upon the intimacy of pairing (Dobzhansky, 1933). In this author's equiaxial translocation there were reductions in crossing over in the nondisjunctional arms of two-chromosome aneuploid gametes. A lesser reduction in crossing over was found in orthoploid gametes. Crossing over in the disjunctional arms of two-chromosome aneuploid gametes was "about equal to that in flies free from the translocation." Dobzhansky used the amount of crossing over as an index of the closeness of the pairing. He suggested that, first, two nonadjacent chromosome arms pair normally but there is a reduction of pairing in the other two nonadjacent arms because of a competition for pairing on the part of adjacent members of the cross configuration. At anaphase, disjunction between homologues of the two normally paired arms is independent, giving equal numbers of orthoploid and of two-chromosome aneuploid gametes. Therefore crossing over in the disjunctional arm of two-chromosome aneuploid gametes should be normal; crossing over in the nondisjunctional arm of two-chromosome aneuploid gametes should be reduced (Dobzhansky, 1933). Dependence of disjunction upon the intimacy of pairing has the advantage that it may be applied equally well to males and females heterozygous for the translocation.

This theory does not fit the data from T_A2,3–1 as well as it fits the crossover results of Dobzhansky's translocation. In T_A2,3–1 crossing over in orthoploid gametes is not very different from the control. From a total of fifteen regions studied, significant deviations in four regions are higher in the control than in orthoploid gametes; in three other significantly different regions, the reverse is true. Regional crossover values of the nondisjunctional arm of two-chromosome aneuploid gametes are either very slightly lower or not significantly different from those of orthoploid gametes or the control. The percentages from the disjunctional arm are either the same or very slightly higher, with one exception, than orthoploid gamete values.

In T_A2,3–1, where the one kind of two-chromosome aneuploid gamete (nondisjunctional for 2R or 3R) is produced with practically twice the frequency of the other two-chromosome aneuploid gamete type (nondisjunctional for 2L or 3L), we should expect on Dobzhansky's theory of competitive pairing that crossing over in orthoploid gametes would be more reduced in 2R and 3R than in 2L and 3L, if crossing over is an index of pairing. No such result was found. The no-chiasma percentage in the nondisjunctional arm 3R was increased above the control less than that of any other nondisjunctional arm. Furthermore, on the basis of Dobzhansky's theory, if we set aside 40.96 per cent out of the total 59.06 per cent orthoploid gametes of the heterozygous T_A2,3–1 as coming from a meiosis in which orthoploid and aneuploid gametes were apriori equally likely to be formed, then there would still be 18.10 per cent out of the 59.06 per cent orthoploid gametes which would result from meioses in which *a priori* all the gametes are to be orthoploid.

The explanation for the difference between Dobzhansky's case and this one is in the presence in this case of the numerous cross configurations where chiasma in each arm gave rise to rings of four chromosomes. In this case 59.06 per cent of the gametes were disjunctional and 28.8 per cent of these came from rings of four leaving some 30.3 per cent that came from chains or other configurations. (The 2.5 per cent 3 or 1 chromosome combinations may have come from several different configurations.) 25.03 per cent of the gametes were nondisjunctional for 2R and 3R. From the several sources for comparison, 13.0 per cent (maximum) came from rings leaving 12.0 per cent from chains. Or 10.8 per cent (minimum) came from rings leaving 14.2 per cent from chains.

The 13.41 per cent gametes nondisjunctional for 2L and 3L (the least satisfactory data) had 4.4 per cent (maximum) from rings leaving 9.0 per cent from chains or 0.7 per cent (minimum) leaving 12.7 per cent from chains. Taking both extremes, there were 30.3 per cent disjunctional gametes from chains of four and configurations other than rings to 12.0 per cent (N. D. 2R + 3R) + 9.0% (N. D. 2L + 3L) + 2.5% (3 or 1 chromosome gametes) giving a 30.3:23.5 ratio. The other values are 30.3% to 14.2% + 12.7% + 2.5% or 30.3:29.4 ratio. Therefore nondisjunction and disjunction from configurations other than rings of four chromosomes gave results very similar to Dobzhansky's where very few rings of four occurred. The presence of these rings which gave 28.8 per cent disjunction to 13.0 per cent nondisjunction of 2R and 3R plus 4.4 per cent nondisjunction of 2L and 3L or 28.8:17.4 per cent disjunction to nondisjunction. The other values are 28.8% to 10.8% (N. D. 2R and 3R) + 0.7% (N. D. 2L + 3L) giving 28.8:11.5 ratio of disjunction to nondisjunction from these rings. Therefore these rings give rise to roughly twice as many disjunctional as nondisjunctional gametes and from 3 to 15 times as many nondisjunctional gametes of one kind as the other.

Even though the data are not completely satisfactory it seems most probable that rings of four chromosomes in *Drosophila* disjoin much more frequently than they nondisjoin as in *Oenothera*. Whereas chains of four

chromosomes give equal rates of disjunction and nondisjunction, with nondisjunction of one type predominating as in maize.

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SUMMARY

1. In the mutual translocation T_A2,3–1, chromosomes 2 and 3 had been broken by X-radiation in their chromocentral regions; and the left arm of 2, 2L, attached to the right arm of 3, 3R; and 2R was attached to 3L. T_A2,3–1 is therefore a bimedial equiaxial translocation, according to the terminology of Glass, 1935.

2. The percentage of orthoploid gametes, resulting from segregation of alternate members of the pachytene cross configuration to the same pole, is 59.06, by one experimental method and ca 59 by another method.

3. The percentage of orthoploid gametes produced by males and females heterozygous for the translocation is very near the same.

4. The percentages of the two kinds of two-chromosome aneuploid gametes, resulting from segregation of adjacent members of the pachytene cross configuration to the same pole, are 13.41 and 25.03, assuming that these gamete types are produced with equal frequency in males and females heterozygous for the translocation. The percentage of three-and one-chromosome aneuploid gametes is 2.50.

5. Chiasma associations were determined in four arms of cross configurations giving rise to orthoploid gametes and in three arms of configurations giving rise to aneuploid gametes. Forty-eight per cent orthoploid gametes arise from rings of 4 chromosome; i.e., where at least one chiasma was present in each of the four arms. Around 50 per cent of two-chromosome aneuploid gametes nondisjunctional for 2R and 3R come from rings.

6. The analysis of chiasma associations in the four arms of cross configurations showed that no chromatid interference occurred in meioses destined to give rise to orthoploid gametes. For an unaccountable reason, double exchange in the nondisjunctional arm of two-chromosome aneuploid gametes was not at random.

7. Crossing over was studied in all four arms of chromosomes 2 and 3 in orthoploid gametes and two-chromosome aneuploid gametes of the heterozygous translocation; in 2R and 3L of the homozygous translocation, and in the 2 and 3 chromosomes of flies free from the translocation. Regional crossing over shown by orthoploid gametes and the disjunctional arm of two-chromosome aneuploid gametes differs little or not at all

from the control. Crossing over in the nondisjunctional arm of two-chromosome aneuploid gametes is either reduced slightly or does not differ from the control. The small crossover reduction in the nondisjunctional arms of the two types of two-chromosome aneuploid gametes is about the same, although one type of two-chromosome aneuploid gametes occurs with twice the frequency of the other type (assuming that the aneuploid gamete types occur with the same frequency in the male and female heterozygous for the translocation).

8. It is concluded that the crossover data of T_A2,3–1 do not support Dobzhansky's theory of the formation of orthoploid and aneuploid gametes by heterozygous translocations.

9. It is concluded that the results of the present study contradict the hypothesis of Glass, 1935, that equality of length of axes of a cross configuration of a heterozygous translocation causes the two types of two-chromosome aneuploid gametes to be produced with equal frequency.

10. Disjunction in a chain of four chromosomes in T_A2,3–1 is not at random but in the direction of what is expected on the basis of Brown's theory.

11. Segregation from a ring of four chromosomes in *Drosophila* is more frequently disjunctional as in *Oenothera*.

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APPENDIX

In the appendix is presented various sample calculations. In addition there are several summaries of the data including the recombination data. This is given by chromosome arm, not by region. The mass of raw data from the counts has been filed with the Zoology department of The University of Texas and may be obtained there for study.

Summary of crossover associations in recovered crossover individuals arising from the union of orthoploid gametes.

Zero denotes no crossover; 1, single crossover; 2, double crossover; and 3, triple crossover. The crossover ranks of the various arms are given in order of 2L, 3R, 3L, and 2R respectively.

a_{0000}	245	a_{0022}	1
a_{1000}	205	a_{2200}	1
a_{0100}	256	a_{2000}	7
a_{0010}	205	a_{0200}	23
a_{0001}	215	a_{0020}	9
a_{1100}	208	a_{0002}	8
a_{0110}	158	a_{0300}	2
a_{0011}	165	a_{0130}	2
a_{1001}	146	a_{1002}	6
a_{1010}	146	a_{1020}	6
a_{0101}	195	a_{1200}	17
a_{1110}	143	a_{0210}	15
a_{0111}	147	a_{0201}	15
a_{1011}	97	a_{0120}	10
a_{1101}	115	a_{2100}	6
a_{1111}	91	a_{2010}	6
a_{2110}	4	a_{2001}	3
a_{2101}	6	a_{0021}	16
a_{2011}	2	a_{2111}	2
a_{1012}	7	a_{1211}	13
a_{1021}	4	a_{1121}	4
a_{1201}	7	a_{1202}	1
a_{1210}	9	a_{0221}	1
a_{1102}	8	a_{0212}	1
a_{1120}	6	a_{0122}	1
a_{0112}	3	a_{2211}	1
a_{0121}	6	a_{2121}	1
a_{0211}	14	Grand total	2991

Summary of crossover associations in recovered *S Pr* crossover individuals arising from the union of two-chromosome aneuploid gametes nondisjunctional for 2R and 3R.

Zero denotes no crossover; 1, single crossover; 2, double crossover. Two strands are recovered in the nondisjunctional arm. The crossover ranks of the various arms are given in the order of 2L, 3R, 3L, and 2R.

$a_{0-?-0-00}$	43	$a_{1-?-0-10}$	31
$a_{1-?-0-00}$	47	$a_{1-?-0-11}$	14
$a_{0-?-1-00}$	48	$a_{1-?-1-00}$	48
$a_{0-?-0-10}$	30	$a_{1-?-1-10}$	28
$a_{0-?-0-11}$	10	$a_{1-?-1-11}$	12
$a_{0-?-1-10}$	21	$a_{1-?-2-00}$	5
$a_{0-?-1-11}$	11	$a_{0-?-2-10}$	1

$a_{2-?-0-10}$	1
$a_{1-?-2-10}$	1
$a_{1-?-1-02}$	2
$a_{0-?-0-(11)d}$	2
$a_{0-?-1-02}$	1
$a_{0-?-1-(11)d}$	2
$a_{0-?-1-22}$	1
$a_{1-?-0-02}$	1
$a_{1-?-0-22}$	1
$a_{1-?-0-12}$	1
Total	362

Summary of crossover associations in recovered *L D* crossover individuals arising from the union of two-chromosome aneuploid gametes nondisjunctional for 2R and 3R.

Zero denotes no crossover; 1, single crossover; 2, double crossover. Two crossover strands are recovered in the nondisjunctional arm. The crossover ranks of the various arms are given in the order of 2L, 3R, 3L, and 2R.

$a_{0-00-0-?}$	36
$a_{0-00-1-?}$	31
$a_{1-00-0-?}$	31
$a_{0-10-0-?}$	41
$a_{0-11-0-?}$	16
$a_{0-10-1-?}$	37
$a_{0-11-1-?}$	17
$a_{1-10-0-?}$	44
$a_{1-11-0-?}$	28
$a_{1-00-1-?}$	27
$a_{2-00-0-?}$	2
$a_{1-10-1-?}$	41
$a_{1-11-1-?}$	14
$a_{1-00-2-?}$	1
$a_{2-11-0-?}$	2
$a_{2-10-1-?}$	1
$a_{1-10-2-?}$	1
$a_{0-02-0-?}$	2
$a_{0-(11)d-0-?}$	1
$a_{0-02-1-?}$	1
$a_{0-22-1-?}$	1
$a_{0-(11)d-1-?}$	2
$a_{1-02-0-?}$	5
$a_{1-22-0-?}$	4
$a_{1-(11)d-0-?}$	3
$a_{1-02-1-?}$	1
$a_{1-22-1-?}$	1
$a_{1-(11)d-1-?}$	4
$a_{1-12-1-?}$	2
$a_{2-(11)d-1-?}$	1
Grand total	398

Summary of crossover associations in recovered *S L* crossover individuals arising from the union of two-chromosome aneuploid gametes nondisjunctional for 2L and 3L.

Zero denotes no crossover; 1, single crossover; 2, double crossover; and 3, triple crossover. Two crossover strands were recovered in the nondisjunctional arm. The crossover ranks of the various arms are given in the order of 2L, 3R, 3L, and 2R, respectively.

$a_{?-0-00-0}$	22
$a_{?-0-00-1}$	9
$a_{?-1-00-0}$	15
$a_{?-0-10-0}$	12
$a_{?-0-11-0}$	8
$a_{?-1-10-0}$	16
$a_{?-1-11-0}$	8
$a_{?-0-10-1}$	5
$a_{?-0-11-1}$	1
$a_{?-1-00-1}$	7
$a_{?-2-00-0}$	1
$a_{?-1-10-1}$	5
$a_{?-1-11-1}$	3
$a_{?-2-10-0}$	1
$a_{?-2-00-1}$	3
$a_{?-2-10-1}$	1
$a_{?-3-10-0}$	1
$a_{?-0-02-0}$	1
$a_{?-0-02-1}$	1
$a_{?-1-02-0}$	2
$a_{?-1-(11)d-0}$	1
Grand total	123

Summary of crossover associations in recovered *Pr D* crossover individuals arising from the union of two chromosome aneuploid gametes nondisjunctional for 2L and 3L.

Zero denotes no crossover; 1, single crossover; 2, double crossover. Two crossover strands are recovered in the nondisjunctional arm. The crossover ranks of the various arms are given in the order of 2L, 3R, 3L, and 2R.

$a_{00-0-?-0}$	16	$a_{10-2-?-0}$	2
$a_{00-0-?-1}$	14	$a_{11-0-?-2}$	2
$a_{00-1-?-0}$	10	$a_{10-0-?-2}$	1
$a_{10-0-?-0}$	10	$a_{10-2-?-1}$	3
$a_{11-0-?-0}$	6	$a_{11-2-?-1}$	1
$a_{00-1-?-1}$	13	$a_{10-1-?-2}$	1
$a_{10-1-?-0}$	6	$a_{11-1-?-2}$	1
$a_{11-1-?-0}$	3	$a_{(11)d-0-?-1}$	1
$a_{10-0-?-1}$	13	$a_{(11)d-1-?-0}$	1
$a_{11-0-?-1}$	10	$a_{22-1-?-0}$	1
$a_{00-2-?-0}$	4	$a_{(11)d-1-?-1}$	1
$a_{10-1-?-1}$	6	$a_{22-1-?-1}$	1
$a_{11-1-?-1}$	4	Grand total	131

Summary of crossover associations in recovered crossover individuals of the control.

Zero denotes no crossover; 1, single crossover; 2, double crossover. The crossover ranks of the various arms are given in order of 2L, 3R, 3L, and 2R, respectively. Individuals with a crossover in region 4 of chromosome 2 or 3 are not included in this classification. Such individuals numbered 354.

a_{0000}	132	a_{0201}	4
a_{1000}	90	a_{2001}	3
a_{0100}	115	a_{2010}	3
a_{0010}	108	a_{1200}	11
a_{0001}	72	a_{2100}	1
a_{1100}	112	a_{0021}	2
a_{1010}	77	a_{1111}	31
a_{1001}	40	a_{1120}	3
a_{0110}	72	a_{0121}	1
a_{0101}	52	a_{1210}	4
a_{0011}	53	a_{2110}	3
a_{2000}	4	a_{0112}	1
a_{0200}	12	a_{0211}	2
a_{0020}	2	a_{2011}	1
a_{1110}	61	a_{1021}	1
a_{1101}	34	a_{1201}	3
a_{0111}	40	a_{2200}	1
a_{1011}	41	a_{2111}	2
a_{1020}	2	a_{1121}	1
a_{0120}	5	a_{2210}	1
a_{0210}	1	Grand total	1204

Sample calculation (1). Calculation of the number of cross configurations with the chiasma association X_{0120}, giving rise to orthoploid gametes. In X_{0120}, no chiasma is present in 2L; 1, in 3R; 2, in 3L, and no chiasma in 2R, respectively.

$X_{0120} = 8(a_{0120} - (a_{0121} + a_{1120}) + (a_{0122} + a_{1121}) - a_{2121} + 2\,a_{0221} - 3\,a_{0130})$; where a_{0120}, a_{0121}, etc., represent the numbers of crossover individuals recovered. The subscripts 0, 1, 2, 3 of a represent no crossover, single crossover, double crossover, and triple crossover, occurring in arms 2L, 3R, 3L, and 2R, respectively, in the order given.

Hence $X_{0120} = 8\,(10 - (6 + 6) + (1 + 4) - 1 + 2 - 6)$.

or, $X_{0120} = -16$.

The per cent of $X_{0120} = -16/2991 = -0.53$.

Sample calculation (2). Calculation of the standard deviation of X_{0120}, obtained in sample calculation (1).

In general, the variance of $X_{0120} = \sum_r b^2 a_r - 1/N\ (M_x)^2$; where a_r is a_{0120}, a_{0121}, a_{1120}; etc.; b is the coefficient of each a_r; N, the total number of crossover individuals counted; M_x, the value of X_{0120}.

Variance of $X_{0120} = \{ [8^2 (a_{0120} + a_{0121} + a_{1120} + a_{0122} + a_{1121} + a_{2121}) + 16^2 a_{0221} + 24^2 a_{01\overline{20}}] - [1/2991\ (-16)^2] \}$

Variance of $X_{0120} = 64(28) + 256\ (1) + 576\ (2) - 0.01 = 3199.99$

Standard deviation of $X_{0120} = \sqrt{V_X} = 56.56$

Standard deviation expressed in per cent $= 56.56/2991 = 1.39$ per cent.

Sample calculation (3). Calculation of the number of cross configurations with the chiasma association $X_{0?10}$, giving rise to two-chromosome aneuploid gametes nondisjunctional for 2R, and S Pr zygotes.

$X_{0?10} = 2[a_{0-?-1-00} - 1/3\ (a_{0-?-1-10} + a_{0-?-1-11}) - a_{1-?-1-00} + 1/3\ (a_{1-?-1-10} + a_{1-?-1-11}) + 2/3\,(a_{2-?-0-10}) + 1/33\ (a_{0-?-1-02} + a_{0-?-1-(11)}d + a_{0-?-1-22}) + 2\ a_{1-?-2-00} - 1/33\ a_{1-?-1-02} - 2/3\ a_{1-?-2-10}]$

$X_{0?10} = 2(48 - 10.6667 - 48 + 13.3333 + 0.6667 + 0.1212 + 10 - 0.0606 - 0.6667)$

$X_{0?10} = 25.4545.$

The per cent of $X_{0?10}$ is 25.4545/362 or 7.03 per cent.

Sample calculation (4). Calculation of the standard deviation of $X_{0?10}$.

The variance of $X_{0?10}$ is in general $\sum_r b_r^2 a_r - 1/N\ (M_x)^2$; where a_r represents $a_{0-?-1-00}$; $a_{0-?-1-10}$, $a_{0-?-1-11}$; $a_{1-?-1-00}$; etc.; b_r is the coefficient of each a_r; M_x is the value of $X_{0?10}$; N, the total number of a_r's.

The variance of $X_{0?10} = [4(48) + 4/9(32) + 4(48) + 4/9(40) + 16/9(1) + 4/1089\ (4) + 16(5) + 4/1089(2) + 16/9(1)] - 1/362(25.4545)^2$.

The variance of $X_{0?10} = 305.7843$.

The standard deviation of $X_{0?10} = 17.48$.

The standard deviation of $X_{0?10}$ in per cent $= 17.48/362 = 4.83$.

Sample calculation (5). Method of obtaining regional crossover per cent in the nondisjunctional arm of two-chromosome aneuploid gametes. Example: derivation of the crossover over per cent of region 5, 2R, from S Pr individuals.

First the number of single chiasma in region 5 must be obtained.

$X_5 = 4/3\,(a_5 - 1/11\ a_{5,6} - 3/11\ a_{4b,5})$

$X_5 = 4/3\,(90 - 1/11\,(2) - 3/11\,(7))$

$X_5 = 117.2094.$

The per cent of $X_5 = 117.2094/362$ or 32.3782.

Also, $X_{4b,5} = 16/11(7) = 10.1818$ or 2.81 per cent.

$X_{5,6} = 16/11(2) = 2.9090$ or 0.80 per cent.

The crossover per cent of region 5 is then $1/2\ X_5 + 1/2\ X_{4b,5} + 1/2\ X_{5,6}$ or $16.19 + 1.40 + 0.40$ or 17.19 per cent.

Sample calculation (6). Method of obtaining the standard deviation of regional crossover per cent of nondisjunctional arm of two-chromosome aneuploid gametes. Example: derivation of the standard deviation of the crossover per cent of region 5, 2R, obtained in sample calculation (5).

In general, the variance of $X_5 = \sum_r b_r^2 a_r - 1/N\ (M_x)^2$; where a_r represents a_5; $a_{4b,5}$; and $a_{5,6}$; b_r, the coefficient of each a_r, and M_x, the value of X_5.

Variance of $X_5 = [\ (16/9(90) + 1/121(2) + 9/121(7)\ ) - 1/362(117.21)^2]$

$= 160.08 - 37.95 = 11.04.$

Standard deviation of $X_5 = \sqrt{\text{Variance of } X_5} = 11.04$
In per cent, the standard deviation of $X_5 = 3.05$
Variance of $X_{4b,5} = 256/121(7) = 14.81$
Standard deviation of $X_{4b,5} = 3.84$ or in per cent, 1.06
Variance of $X_{5,6} = 256/121(2) = 4.23$
Standard deviation of $X_{5,6} = 2.06$ or in per cent 0.57.

Now the standard deviation of the single crossover per cent of region 5 is 1/2 the standard deviation of X_5; standard deviation of the double crossover per cent of regions 4b,5 is $1/2\, X_{4b,5}$; and the standard deviation of the double crossover per cent of regions 5,6 is $1/2\, X_{5,6}$. Hence, the standard deviation of the regional crossover per cent of region 5 is 1/2 [16.19/17.99 (3.05) + 1.40/17.99(1.06) + 0.40/17.99(0.57)] or 2.83/2 or 1.41.

TABLE 1.

Phenotypes of progeny of T_A2,3–1 *S D/L Pr* males x T_A2,3–1/ *"all rucuca"* females. A diagram of the cross is shown in Figure 1.
(Note: In group I, a count of the female progeny was not made.)

	From Orthoploid gametes gamete type x				From 2-chromosome aneuploid gametes nondisj. for 2L, 3L; gamete type y				nondisj. for 2R, 3R; gamete type z				From 3-chromosome aneuploids gamete types w and v	
	S D		L Pr		S L		Pr D		S Pr		L D		S Pr D	D
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♀	♀
Group I	551		591		13		11		77		56		0	0
Group II	1709	1733	1843	1807	42	53	53	40	165	168	141	131	3	1
Subtotal A	2260	1733	2434	1807	55	53	64	40	242	168	197	131	3	1
Subtotal B	3993		4241		108		104		410		328		3	1
Subtotal C	8234				212				738				3	1
Grand total	9188													

TABLE 2

Results of Egg and Hatch Count Experiment

Cross	Het. tr. ♀ x + ♂	Het. tr. ♂ x + ♀	Hom. tr. ♀ x + ♂	Hom. tr. ♂ x + ♀	+ ♀ x + ♂
No. eggs	4033	4017	4139	4114	4144
No. flies hatched	2133	2287	3774	3789	3675
Per cent hatch	52.88 ± 0.79	56.93 ± 0.78	91.18 ± 0.45	92.10 ± 0.42	88.21 ± 0.49

TABLE 3

Percentages of Cross Configuration Chiasma Associations Giving Rise to Orthoploid Gametes and of Bivalents Giving Rise to Control Gametes

Chiasma Association	X0000	X1000	X0100	X0010	X0001	X1100	X0110	X0011	X1001	X1010	X0101	X1110	X0111	X1011	X1101	X1111	X2000	X0200
Heterozygous translocation	—0.84 ±1.83	—2.34 ±2.50	+1.20 ±2.88	+3.01 ±2.60	—3.08 ±2.51	+4.68 ±3.94	—6.39 ±4.02	+1.60 ±3.63	+6.02 ±3.34	+0.27 ±3.44	+3.88 ±3.99	*+11.77 ±5.46*	*+14.44 ±5.71*	—0.27 ±4.61	+3.74 ±5.28	*+32.63 ±8.20*	+0.67 ±0.83	—1.87 ±1.49
Normal Control	+0.83 ±2.77	+4.98 ±4.02	+4.32 ±4.36	+3.16 ±3.96	+2.33 ±3.27	*+13.95 ±6.38*	+2.66 ±5.58	+1.32 ±4.63	—2.99 ±4.40	+1.99 ±5.70	+3.65 ±3.91	+13.29 ±8.39	+3.32 ±6.41	+8.64 ±6.40	+1.33 ±6.16	*+33.22 ±8.66*	—0.33 ±1.45	+1.66 ±2.07
Chiasma Association	X0020	X0002	X0030	X0300	X1002	X1020	X1200	X0210	X0201	X0120	X2100	X2010	X2001	X0021	X0012	X0102	X0130	X2110
Heterozygous translocation	—0.67 ±1.13	*+2.41* ±0.80	—0.53 ±0.38	+0.53 ±0.38	—2.14 ±1.25	—0.53 ±1.31	+2.94 ±1.98	+1.87 ±2.02	+2.94 ±2.05	—0.53 ±1.89	—1.87 ±1.39	—0.27 ±1.17	—1.34 ±1.04	+2.67 ±1.69	—2.41 ±1.16	—0.16 ±1.20	+1.07 ±0.76	+3.21 +2.00
Normal Control	—1.00 ±1.37	+0.33 ±0.33	0.00	0.00	0.00	—0.66 ±1.76	+2.66 ±3.39	—1.99 ±2.20	—0.66 ±1.99	+1.33 ±2.10	0.00 ±2.49	+1.99 ±2.42	+2.66 ±1.63	—0.66 ±1.49	—0.66 ±6.64	—0.66 ±6.64	0.00	—1.33 ±3.99
Chiasma Association	X2101	X2011	X1012	X1021	X1201	X1210	X1102	X1120	X0112	X0121	X0211	X0022	X0220	X2200	X2020	X2002	X0202	X2111
Heterozygous translocation	+3.74 ±1.93	+1.60 ±1.60	*+3.74 ±1.55*	+1.07 ±1.85	—3.21 ±2.83	—1.07 ±2.73	+3.21 ±1.85	+2.14 ±2.00	—0.53 ±1.77	—0.53 ±2.33	—1.07 ±3.21	0.00 ±0.76	—0.53 ±0.53	+1.07 ±0.76	+0.53 ±0.53	0.00	—1.07 ±0.76	—2.14 ±3.38
Normal Control	—5.32 ±3.75	—1.33 +2.30	0.00	0.00 ±1.88	+3.98 ±2.30	+2.66 ±3.77	0.00	+2.66 ±2.66	+1.33 ±1.33	0.00 ±1.88	+2.66 ±1.88	0.00	0.00	+1.33 ±1.33	0.00	0.00	0.00	+5.32 ±3.75
Chiasma Association	X1211	X1121	X1112	X1202	X0221	X0212	X0122	X2102	X1220	X2120	X2012	X2021	X1022	X2201	X2210	X2211	X2121	
Heterozygous Translocation	*+11.77* ±4.41	+2.14 ±2.52	0.00	+1.07 ±1.07	+1.07 ±1.07	+1.07 ±1.07	+1.07 ±1.07	0.00 ±1.07	0.00	—1.07 ±1.07	0.00	—1.07 ±1.07	0.00	—1.07 ±1.07	—1.07 ±1.07	+2.14 ±2.14	+2.14 ±2.14	
Normal Control	0.00	+2.66 ±2.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	+2.66 ±2.66	0.00	0.00	

TABLE 4

Percentages of Cross Configuration Chiasma Associations Giving Rise to Two-Chromosome Aneuploid Gametes

Chiasma Associations	0?00	0?10	1?00	0?01	1?01	0?11	1?10	1?11	0?20	2?00	0?02	1?20	0?21	2?01	1?21	0?12	1?02	1?12				
S Pr zygotes n.d. for 2R in ♀; N=362	−2.88 ± 3.99	+7.03 ± 4.83	+1.49 ± 4.34	+1.88 ± 4.85	+2.68 ± 6.46	−6.43 ± 6.61	*+28.06* ± *9.32*	*+54.91* ± *9.36*	−5.52 ± 2.51	−0.37 ± 0.37	−1.21 ± 0.40	*+10.31* ± *4.97*	0.0 ± 2.08	+1.47 ± 1.47	+2.95 ± 2.91	+1.61 ± 1.97	+0.80 ± 1.80	+3.21 ± 2.27				
Chiasma Associations	000?	001?	100?	010?	110?	011?	101?	111?	200?	002?	102?	201?	020?	210?	012?	120?	021?	112?	211?	121?	221?	220?
L D zygotes n.d. for 3R in ♀; N=398	+1.63 ± 3.10	+2.63 ± 4.22	−2.09 ± 3.74	−3.96 ± 5.25	+9.26 ± 8.00	+2.07 ± 7.21	+8.22 ± 6.19	*+65.42* ± *10.24*	+1.64 ± 1.54	−0.67 ± 1.06	+1.34 ± 2.12	−0.61 ± 0.67	−2.19 ± 1.93	+1.83 ± 2.37	−1.34 ± 1.34	+4.39 ± 3.57	−2.19 ± 2.62	+2.68 ± 2.65	+1.71 ± 2.89	+8.77 ± 4.60	+2.92 ± 2.92	−1.46 ± 1.46
Chiasma Associations	00?0	01?0	00?1	10?0	10?1	11?0	01?1	11?1	10?2	12?0	02?1	01?2	12?1	11?2	20?0	20?1	21?0	21?1	00?2	02?0		
Pr D zygotes n.d. for 2L in ♀; N=131	+8.63 ± 5.89	−19.34 ± 9.44	−6.15 ± 8.73	−6.75 ± 8.47	*+31.27* ± *15.17*	+10.18 ± 11.74	*+41.92* ± *11.95*	−11.10 ± 23.63	+4.07 ± 9.10	−8.14 ± 9.97	−8.14 ± 4.06	−4.07 ± 2.87	*+32.57* ± *15.85*	+16.28 ± 11.30	−1.11 ± 2.48	−2.22 ± 3.84	0.00 ± 4.44	+8.88 ± 6.23	−1.02 ± 2.27	*+14.25* ± 6.47		
Chiasma Associations	?000	?001	?100	?010	?011	?110	?101	?111	?200	?020	?210	?201	?310	?300	?021	?120	?211					
S L zygotes n.d. for 3L in ♀; N=123	+3.18 ± 6.33	+8.72 ± 5.96	+9.36 ± 10.68	−2.07 ± 10.38	−2.96 ± 8.43	*+38.83* ± *14.83*	−3.25 ± 14.66	+26.02 ± 17.09	−3.25 ± 6.93	−3.55 ± 2.62	−13.01 ± 9.62	+17.34 ± 11.37	+8.67 ± 8.54	−2.17 ± 2.15	+2.36 ± 2.35	+7.10 ± 4.04	+8.67 ± 8.54					

TABLE 5

Summary of Chiasma Associations in Meioses Giving Rise to Orthoploid Gametes and in the Control. The First Orthoploid Gamete Percentages are of the Total Orthoploid Gametes; the Second Percentage, Given in Parentheses, are of the Total Gamete Frequency.

Arms Containing Chiasmata	Chiasma Associations $X_{\substack{0\ 0\ 0\ 0 \\ 2L\ 3R\ 3L\ 2R}}$	Control Percentages	Orthoploid Gamete Percentages
None	X_{oooo}	0.83 ± 2.77	—0.84 ± 1.83 (–0.50)
2L	X_{xooo}	—5.21 ± 3.93	—1.67 ± 3.27 (–0.99)
3R	X_{oxoo}	—2.66 ± 5.79	—0.14 ± 6.22 (–0.08)
3L	X_{ooxo}	3.06 ± 4.05	1.81 ± 3.79 (1.07)
2R	X_{oox}	2.66 ± 3.27	—0.67 ± 8.67 (–0.40)
2L, 3R	X_{xxoo}	17.94 ± 5.99	6.82 ± 3.28 (4.03)
2L, 3L	X_{xoxo}	3.32 ± 4.52	0.00
2L, 2R	X_{xoox}	—0.33 ± 26.73	2.54 ± 6.32 (1.50)
3R, 3L	X_{oxxo}	2.00 ± 6.63	—4.52 ± 5.10 (–2.67)
3R, 2R	X_{oxox}	2.33 ± 5.37	5.59 ± 3.67 (3.30)
3L, 2R	X_{ooxx}	1.33 ± 5.04	1.86 ± 4.05 (1.10)
2L, 3R, 3L	X_{xxxo}	19.94 ± 6.52	13.91 ± 5.02 (8.22)
2L, 3R, 2R	X_{xxox}	0.00	7.48 ± 3.19 (4.42)
2L, 3L, 2R	X_{xoxx}	7.31 ± 7.14	5.07 ± 1.55 (2.99)
3R, 3L, 2R	X_{oxxx}	7.31 ± 3.82	15.51 ± 5.17 (9.16)
2L, 3R, 3L, 2R	X_{xxxx}	41.20 ± 7.63	48.68 ± 7.41 (28.75)

TABLE 6

Summary of Chiasma Associations in Meioses Giving Rise to Two-Chromosome Aneuploid Gametes. The First Percentage is of the Gamete Type in Question. The Second Percentage, Given in Parentheses, is of the Total Gametes

Type y Gametes, Nondisjunctional for 2L and 3L				Type z Gametes Nondisjunctional for 2R and 3R			
Calculated From SL Flies		Calculated From Pr D Flies		Calculated From S Pr Flies		Calculated From L D Flies	
Association	Per Cent	Association	Per Cent	Association	Per Cent	Association	Per Cent
$X_{?ooo}$	3.18 ± 6.33 (0.21)	$X_{oo?o}$	8.63 ± 5.89 (0.58)	$X_{o?oo}$	—2.88 ± 3.99 (0.29)	$X_{ooo?}$	1.63 ± 3.10 (0.20)
$X_{?xoo}$	3.94 ± 18.47 (0.26)	$X_{xo?o}$	—7.86 ± 7.62 (—0.53)	$X_{x?oo}$	1.12 ± 5.65 (0.14)	$X_{xoo?}$	—0.45 ± 11.76 (—0.06)
$X_{?oxo}$	—5.62 ± 4.76 (—0.38)	$X_{ox?o}$	—5.09 ± 17.75 (—0.34)	$X_{o?xo}$	1.51 ± 23.31 (0.19)	$X_{oxo?}$	—6.15 ± 4.07 (—0.77)
$X_{?oox}$	8.72 ± 5.96 (0.59)	$X_{oo?x}$	—7.17 ± 7.81 (—0.48)	$X_{o?ox}$	0.67 ± 12.89 (0.08)	$X_{oox?}$	1.96 ± 5.30 (0.25)
$X_{?xxo}$	41.59 ± 13.31 (2.79)	$X_{xx?o}$	2.04 ± 18.80 (0.14)	$X_{x?xo}$	38.37 ± 8.15 (4.80)	$X_{xxo?}$	14.02 ± 6.56 (1.75)
$X_{?xox}$	14.09 ± 10.61 (0.95)	$X_{xo?x}$	33.12 ± 15.18 (2.22)	$X_{x?ox}$	4.95 ± 4.08 (0.62)	$X_{xox?}$	8.95 ± 5.96 (1.12)
$X_{?oxx}$	—0.60 ± 32.34 (—0.04)	$X_{ox?x}$	29.71 ± 15.22 (1.99)	$X_{o?xx}$	—4.82 ± 8.16 (—0.60)	$X_{oxx?}$	—1.46 ± 4.06 (—0.18)
$X_{?xxx}$	34.69 ± 14.95 (2.33)	$X_{xx?x}$	46.63 ± 10.86 (3.13)	$X_{x?xx}$	61.07 ± 8.68 (7.64)	$X_{xxx?}$	81.50 ± 8.97 (10.20)

TABLE 7.

Gamete Types Recovered From Chains of Four Chromosomes

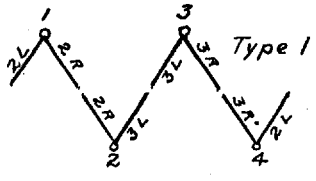
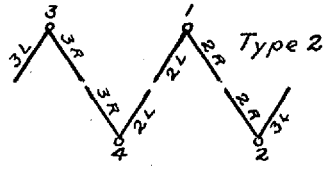
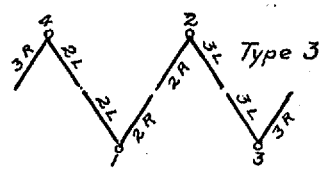
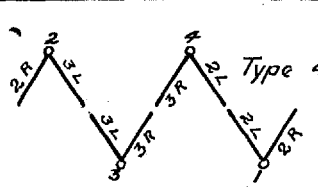
Type of Chain	Centromere number	Chiasma association	Phenotype of zygote	Per cent of each gamete type	Per cent of total gamete frequency	
	1,3	X_{oxxx}	S D	15.51	9.16	
	2,4	X_{oxxx}	L Pr			
	1,2	$X_{o?xx}$	S Pr	—4.82	—0.60	don't expect
	3.4	$X_{oxx?}$	L D	—1.46	—0.18	don't expect
	1,4	$X_{ox?x}$	Pr D	29.71	1.99	
	2,3	$X_{?xxx}$	S L	34.69	2.33	
	1,3	X_{xxox}	L Pr	7.48	4.42	
	2,4	X_{xxox}	S D			
	1,2	$X_{x?ox}$	S Pr	4.95	0.62	don't expect
	3,4	$X_{xxo?}$	L D	14.02	1.75	don't expect
	2,3	$X_{?xox}$	S L	14.09	0.95	
	1,4	$X_{xx?x}$	Pr D	46.63	3.13	
	2,4	X_{xoxx}	S D	5.07	2.99	
	1,3	X_{xoxx}	L Pr			
	1,2	$X_{x?xx}$	S Pr	61.07	7.64	
	3,4	$X_{xox?}$	L D	8.95	1.12	
	1,4	$X_{xo?x}$	Pr D	33.12	2.22	don't expect
	2,3	$X_{?oxx}$	S L	—0.60	—0.04	don't expect
	1,3	X_{xxxo}	L Pr	13.91	8.22	
	2,4	X_{xxxo}	S D			
	1,4	$X_{xx?o}$	Pr D	2.04	0.14	don't expect
	2,3	$X_{?xxo}$	S L	41.59	2.79	don't expect
	1,2	$X_{x?xo}$	S Pr	38.37	4.80	
	3,4	$X_{xxx?}$	L D	81.50	10.20	

TABLE 8

Numbers of Various Combinations of Recovered Crossover Strands of Two-Chromosome Aneuploid Eggs

Composition of Recovered Strands	No. From *L D* Flies	No. From *S Pr* Flies	No. From *Pr D* Flies	No. From *S L* Flies	Total
a_{00}	128	191	57	57	433
a_{10}	165	113	42	41	361
a_{11}	77	47	27	20	171
a_{02}	9	4	0	4	17
$a_{(11)}d$	11	4	3	1	19
a_{12}	2	1	0	0	3
a_{22}	6	2	2	0	10
Total	398	362	131	123	

a_{00} represents individuals bearing two non-crossover strands derived from the nondisjunctional arm of a two-chromosome aneuploid egg. These eggs united with complementary two-chromosome aneuploid sperm to produce zygotes appearing *L D*, *S Pr*, *Pr D*, and *S L* flies. Similarly, a_{10} represents individuals bearing a single crossover strand and a non-crossover strand; a_{11}, with two single crossover strands; a_{02}, with a non-crossover strand and a double crossover strand; $a_{(11)}d$, with two *different* single crossover strands arising from double exchange; and a_{22} with two double crossover strands, and a_{12}, with a single and a double crossover strand.

TABLE 9

Crossover Percentages. (Note that the control crossover percentages are based upon a sample of 1558 for 2L and 2R; upon a sample of 1595, for 3L and 3R, since there were 37 additional crossover individuals analyzed for 3L and 3R but incompletely analyzed for 2L and 2R.)

	2L				2R			3L				3R			
	al–dp 1	dp–b 2	b–pr 3	pr–spa 4a	spa–c 4b	c–px 5	px–sp 6	ru–h 1	h–th 2	th–st 3	st–spa 4a	spa–cu 4b	cu–sr 5	sr–e^s 6	e^s–ca 7
Control	11.50 ± 0.81	26.57 ± 1.12	5.46 ± 0.58	0.45 ± 0.17	18.68 ± 0.99	20.74 ± 1.03	6.80 ± 0.63	24.45 ± 1.09	19.75 ± 1.01	0.13 ± 0.09	1.50 ± 0.31	3.19 ± 0.45	9.59 ± 0.75	8.84 ± 0.72	30.28 ± 1.16
Orthoploid gamete	12.96 ± 0.61	27.19 ± 0.81	3.72 ± 0.35	0.28 ± 0.10	16.22 ± 0.67	23.64 ± 0.79	6.50 ± 0.45	24.40 ± 0.79	17.95 ± 0.70	0.17 ± 0.08	2.51 ± 0.29	1.90 ± 0.25	10.26 ± 0.55	6.40 ± 0.45	35.53 ± 0.87
Nondisj. arm Aneuploid gamete	12.21 ± 2.09	23.31 ± 2.72	4.19 ± 1.13	0.00	(9.75) ± 0.71	17.99 ± 1.41	5.39 ± 0.87	22.77 ± 2.91	13.16 ± 2.28	0.00	2.07 ± 0.93	1.01 ± 0.41	9.49 ± 0.77	6.58 ± 0.94	32.22 ± 1.78
Disj. arm Aneuploid gamete	12.37 ± 1.19	35.39 ± 1.73	5.39 ± 0.82	0.26 ± 0.18	25.19 ± 3.79	27.27 ± 3.89	3.82 ± 1.67	23.15 ± 1.53	21.84 ± 1.50	0.00	4.08 ± 0.72	3.14 ± 1.09	12.59 ± 2.08	7.47 ± 1.65	31.88 ± 2.92
Homozygous translocation					23.69 ± 0.84	20.51 ± 0.80	5.19 ± 0.44	23.01 ± 0.84	21.82 ± 0.82	0.00	2.04 ± 0.28				

TABLE 10

Difference ± Standard Deviation of Difference of Crossover Percentages in Table 9

	2L				2R			3L				3R			
	al-dp 1	dp-b 2	b-pr 3	pr-spa 4a	spa-c 4b	c-px 5	px-sp 6	ru-h 1	h-th 2	th-st 3	st-spa 4a	spa-cu 4b	cu-sr 5	sr-e^s 6	e^s-ca 7
Control/ homozygous translocation					*—5.01 ± 1.30*	+0.23 ± 1.30	*+1.61 ± 0.77*	+1.44 ± 1.37	—2.07 ± 1.30	+0.13 ± 0.09	—0.54 ± 0.42				
Control/ orthoploid	—0.46 ± 0.45	—0.62 ± 0.45	*+1.74 ± 0.67*	+0.17 ± 0.20	*+2.46 ± 1.19*	*—3.90 ± 1.29*	+0.30 ± 0.25	+0.05 ± 1.34	+1.80 ± 1.19	—0.04 ± 0.12	*—1.01 ± 0.42*	*+1.29 ± 0.51*	—0.67 ± 0.93	*+2.44 ± 0.85*	*—5.05 ± 1.45*
Control/ nondisj. arm	—0.71 ± 2.24	+3.26 ± 2.94	+1.27 ± 1.35	*+0.45 ± 0.17*	*(+8.93) ± (1.22)*	+2.75 ± 2.17	+1.41 ± 1.08	+1.68 ± 3.11	+6.59 ± 3.92	+0.13 ± 0.09	—0.57 ± 0.98	*+2.18 ± 0.61*	+0.10 ± 1.08	+2.26 ± 1.18	—1.94 ± 2.13
Control/ disj. arm	—0.87 ± 1.44	*—8.82 ± 2.06*	+0.07 ± 1.00	+0.19 ± 0.25	—6.51 ± 3.92	—6.53 ± 4.02	+2.98 ± 1.79	+1.30 ± 1.88	—2.09 ± 1.81	+0.13 ± 0.09	*—2.58 ± 0.78*	+0.05 ± 1.18	—3.00 ± 2.21	+1.37 ± 1.80	—1.60 ± 3.15
Orthoploid/ nondisj. arm	+0.75 ± 2.18	+3.88 ± 2.84	—0.47 ± 1.27	*+0.28 ± 0.10*	*(+6.47) ± (0.98)*	*+6.65 ± 1.70*	+1.11 ± 0.98	+1.63 ± 3.01	*+4.79 ± 2.39*	*+0.17 ± 0.08*	+0.44 ± 0.98	+0.89 ± 0.48	+0.77 ± 0.95	+0.18 ± 1.04	+3.31 ± 1.98
Orthoploid/ disj. arm	+0.59 ± 1.34	*—8.20 ± 1.92*	—1.67 ± 0.89	+0.02 ± 0.21	*—8.97 ± 3.85*	—2.63 ± 3.97	+2.68 ± 1.73	+1.25 ± 1.72	*—3.89 ± 1.65*	*+0.17 ± 0.08*	*—1.57 ± 0.77*	—1.24 ± 1.12	—2.33 ± 2.15	—1.07 ± 1.71	+3.45 ± 3.05
Disj. arm/ nondisj. arm	+0.16 ± 2.41	*+12.08 ± 3.23*	+1.20 ± 1.47	+0.26 ± 0.18	*+ (15.44) ± 3.86*	*+9.28 ± 4.17*	—1.57 ± 1.89	+0.38 ± 3.29	*+8.68 ± 2.73*	0.00 ±	+2.01 ± 1.18	+2.13 ± 1.17	+3.10 ± 2.22	+0.89 ± 1.90	—0.34 ± 3.42
Orthoploid/ homozygous					*—7.47 ± 1.08*	*+4.13 ± 1.12*	*+1.31 ± 0.63*	+1.39 ± *1.15*	*—3.87 ± 1.08*	*+0.17 ± 0.08*	+0.47 ± 0.40				
Homozygous/ nondisj. arm					*+ (13.94) ± (1.11)*	+2.52 ± 1.70	—0.20 ± 0.97	+0.24 ± 3.03	*+8.66 ± 2.43*	0.00	—0.03 ± 0.97				
Homozygous/ disj. arm					—1.50 ± 3.87	—6.76 ± 3.97	+1.37 ± 1.73	—0.14 ± 1.74	—0.04 ± 1.71	0.00	*—2.04 ± 0.77*				

TABLE 11

No-Chiasma Percentage

	2L	2R	3L	3R
Control	19.26 ± 2.40	14.25 ± 2.42	15.86 ± 2.47	9.97 ± 2.41
Homozygous translocation		19.46 ± 1.94	14.42 ± 1.96	
Orthoploid gametes of heterozygous translocation	17.15 ± 1.80	15.55 ± 1.81	17.29 ± 1.79	8.12 ± 1.82
Nondisjunctional arm of two-chromosome aneuploid gametes	26.07 ± 8.42	38.12 ± 4.86	29.93 ± 8.60	12.11 ± 4.97
Disjunctional arm of two-chromosome aneuploid gametes	—4.74 ± 3.62	—2.29 ± 8.70	6.58 ± 3.62	16.93 ± 6.18

TABLE 12

Total Crossover Map Distances

	2L	2R	3L	3R
Control	43.98	46.22	45.83	51.90
Homozygous translocation		49.39	46.87	
Orthoploid gametes of heterozygous translocation	44.15	47.36	45.03	53.89
Nondisjunctional arm of two-chromosome aneuploid gametes of heterozygous translocation	39.73	33.15	38.00	49.07
Disjunctional arm of two-chromosome aneuploid gametes of heterozygous translocation	53.41	56.28	49.07	55.08

TABLE 13

Summary of data used in the derivation of regional crossover percentages calculated from individuals arising from orthoploid gametes of the heterozygous translocation, 2L. The regions numbered are as follows: *al* 1 *dp* 2 *b* 3 *pr* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1713	513	17.15	.....	.....
1	360	664	22.19	25.92	12.96
2	783	1506	50.35	54.38	27.19
3	94	154	5.14	7.43	3.72
4a	2	2	0.06	0.45	0.28
1,2	19	76	2.54	.....	.....
1,3	8	32	1.06	.....	.....
1,4a	1	4	0.13	.....	.....
2,3	9	36	1.23	.....	.....
2,4a	2	8	0.26	.....	.....
Total	2991				

TABLE 14

Summary of data used in the derivation of regional crossover percentages calculated from individuals arising from orthoploid gametes of the heterozygous translocation, 2R. The regions are numbered as follows: spindle attachment 4b *c* 5 *px* 6 *sp*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1683	465	15.55	------	------
4b	441	794	26.55	32.43	16.22
5	653	1238	41.39	47.27	23.64
6	169	314	10.50	12.10	6.50
4b,5	33	132	4.41	------	------
4b,6	11	44	1.47	------	------
5,6	1	4	0.13	------	------
Total	2991				

TABLE 15

Summary of data used in the derivation of regional crossover percentages calculated from individuals arising from orthoploid gametes of the heterozygous translocation, 3L. The regions are numbered as follows: *ru* 1 *h* 2 *th* 3 *st* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1701	517	17.29	------	------
1	683	1280	42.79	48.80	20.40
2	501	930	31.09	35.89	17.95
3	1	2	0.06	0.33	0.17
4a	50	58	1.94	5.01	2.51
1,2	28	112	3.74	------	------
1,3	2	0	0.00	------	------
1,4a	15	52	1.74	------	------
2,4a	8	32	1.06	------	------
3,4a	0	—8	—0.26	------	------
1,3,4a	2	16	0.53	------	------
Total	2991				

TABLE 16

Summary of data used in the derivation of regional crossover percentages calculated from individuals arising from orthoploid gametes of the heterozygous translocation, 3R. The regions are numbered as follows: spindle attachment 4b *cu* 5 *sr* 6 e^s 7 *ca*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1499	243	8.12	-----	-----
4b	36	38	1.27	3.80	1.90
5	223	278	9.29	20.51	10.26
6	170	302	10.00	12.80	6.40
7	943	1666	55.70	70.66	35.33
4b,5	2	8	0.26	-----	-----
4b,6	1	—4	—0.13	-----	-----
4b,7	16	56	1.87	-----	-----
5,6	3	12	0.40	-----	-----
5,7	79	316	10.56	-----	-----
6,7	17	60	2.00	-----	-----
4b,6,7	2	16	0.53	-----	-----
Total	2991				

TABLE 17

Summary of data used in the derivation of regional crossover percentages for 2L, calculated from *Pr D* individuals arising from two-chromosome aneuploid gametes nondisjunctional for 2L and 3L. The regions are numbered as follows: *al* 1 *dp* 2 *b* 3 *pr* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	57	34.15	26.07	-----	-----
1	21	27.64	21.10	24.42	12.21
2	43	56.73	43.30	46.62	23.31
3	5	5.21	3.98	8.40	4.20
4a	0	0.00	0.00	0.00	0.00
1,2	1	1.45	1.11	-----	-----
1,3	2	2.90	2.21	-----	-----
2,3	2	2.90	2.21	-----	-----
Total	131				

TABLE 18

Summary of data used in the derivation of regional crossover percentages for 2R, calculated from *S Pr* individuals arising from two-chromosome aneuploid gametes nondisjunctional for 2R and 3R. The regions are numbered as follows: spindle attachment 4b *c* 5 *px* 6 *sp*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	191	138.00	38.12	.....	.....
4b	44	57.57	15.90	19.51	9.75
5	90	117.21	32.39	35.99	17.99
6	26	33.21	9.17	12.78	6.39
4b,5	7	10.18	2.81	.....	.....
4b,6	2	2.90	0.80	.....	.....
5,6	2	2.90	0.80	.....	.....
Total	362				

TABLE 19

Summary of data used in the derivation of regional crossover percentages for 3L, calculated from *S L* individuals arising from two-chromosome aneuploid gametes nondisjunctional for 3L and 2L. The regions are numbered as follows: *ru* 1 *h* 2 *th* 3 *st* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	57	36.82	29.93	.....	.....
1	37	48.73	39.61	49.52	24.76
2	21	26.54	21.58	26.31	13.15
3	0	0.00	0.00	0.00	0.00
4a	3	3.64	2.96	4.14	2.07
1,2	4	5.82	4.73	.....	.....
1,4a	1	1.45	1.18	.....	.....
Total	123				

TABLE 20

Summary of data used in the derivation of regional crossover percentages for 3R, calculated from *L D* individuals arising from two-chromosome aneuploid gametes nondisjunctional for 3R and 2R. The regions are numbered as follows: spindle attachment 4b *cu* 5 *sr* 6 e^s 7 *ca*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	128	48.18	12.11	.....	.....
4b	6	7.99	2.01	2.01	1.00
5	37	47.03	11.82	18.76	9.38
6	28	35.51	8.92	12.94	6.47
7	171	218.54	54.91	54.61	27.30
5,6	2	2.91	0.73	.....	.....
5,7	17	24.73	6.21	.....	.....
6,7	9	13.09	3.29	.....	.....
Total	398				

TABLE 21

Summary of data used in the derivation of regional crossover percentages for 2L, calculated from the disjunctional arm of two-chromosome aneuploid gametes giving rise to *S Pr* and *L D* individuals. The regions are numbered as follows: *al* 1 *dp* 2 *b* 3 *pr* 4a spindle attachment.

Region	Number of Crossovers from S Pr	Number of Crossovers from L D	Total Number of Crossovers	Per Cent Crossing Over	Map Distance in Crossover Per Cent
1	34	59	93	12.24	12.37
2	141	124	265	34.87	35.39
3	16	23	39	5.13	5.39
4a	0	1	1	0.13	0.26
1,2	0	4	4	0.53	------
2,3	0	2	2	0.26	------
2,4a	1	0	1	0.13	------
0	170	185	355	------	------
Total	362	398	760		

Number of no-chiasma: —36; per cent of no-chiasma: —4.74.

TABLE 22

Summary of data used in the derivation of regional crossover percentages for 2R, calculated from the disjunctional arm of two-chromosome aneuploid gametes giving rise to *Pr D* individuals. The *S L* individuals were not used in these calculations because the crossover data were obscured by the suppression of *px* by *S*. The regions are numbered as follows: spindle attachment 4b *c* 5 *px* 6 *sp*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	59	—3	—2.29	------	------
4b	29	50	38.17	50.38	25.19
5	35	62	47.33	54.54	27.27
6	3	2	1.53	7.63	3.82
4b,5	3	12	9.16	------	------
4b,6	1	4	3.05	------	------
5,6	1	4	3.05	------	------
Total	131				

TABLE 23

Summary of data used in the derivation of regional crossover percentages for 3L, calculated from the disjunctional arm of two-chromosome aneuploid gametes giving rise to *S Pr* and *L D* individuals. The regions are numbered as follows: *ru* 1 *h* 2 *th* 3 *st* 4a spindle attachment.

Region	Number of Crossovers from S Pr	Number of Crossovers from L D	Total Number of Crossovers	Per Cent of Crossing Over	Map Distance in Crossover Per Cent
1	86	81	167	21.97	23.15
2	73	88	161	21.18	21.84
3	0	0	0	0.00	0.00
4a	15	12	27	3.55	4.08
1,2	4	1	5	0.66	------
1,4a	3	1	4	0.53	------
0	181	215	396	------	------
Total	362	398	760		

Number of no-chiasma: 50; per cent of no-chiasma: 6.58.

TABLE 24

Summary of data used in the derivation of regional crossover percentages for 3R, calculated from the disjunctional arm of two-chromosome aneuploid gametes giving rise to *S L* and *Pr D* individuals. The regions are numbered as follows: spindle attachment 4b *cu* 5 *sr* 6 e^s 7 *ca*.

Region	Number of Crossovers from S L	Number of Crossovers from Pr D	Total Number of Crossovers	Per Cent Crossing Over	Map Distance in Crossover Per Cent
4b	3	0	3	1.18	3.14
5	13	9	22	8.66	12.59
6	4	10	14	5.51	7.47
7	37	29	66	25.98	31.88
4b,6	1	0	1	0.39	------
4b,7	1	2	3	1.18	------
5,6	1	0	1	0.39	------
5,7	2	6	8	3.15	------
6,7	1	2	3	1.18	------
4b,5,7	1	0	1	0.39	------
0	59	73	132	------	------
Total	123	131	254		

Number of no-chiasma: 43; per cent of no-chiasma: 16.93.

TABLE 25

Summary of data used in derivation of regional crossover percentages of the control, 2L. The regions are numbered as follows: *al* 1 *dp* 2 *b* 3 *pr* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	901	300	19.26	.....	.....
1	164	298	19.13	22.99	11.50
2	390	732	46.98	53.14	26.57
3	69	106	6.80	10.91	5.46
4a	6	10	0.65	0.90	0.45
1,2	11	44	2.83	.....	.....
1,3	4	16	1.03	.....	.....
2,3	12	48	3.08	.....	.....
2,4a	1	4	0.25	.....	.....
Total	1558				

Note that 1/42 of the crossovers in region 4 were arbitrarily assigned to region 4a of 2L; 41/42, to region 4b of 2R. The distance between pr and the spindle attachment is 1/42 the distance between *pr* and *c*.

TABLE 26

Summary of data used in the derivation of regional crossover percentages of the control, 2R. The regions are numbered as follows: spindle attachment 4b *c* 5 *px* 6 *sp*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	865	222	14.25	.....	.....
4b	266	486	31.19	37.35	18.68
5	306	582	37.36	41.47	20.74
6	95	172	11.04	13.60	6.80
4b,5	15	56	3.60	.....	.....
4b,6	9	32	2.05	.....	.....
5,6	1	0	0.00	.....	.....
4b,5,6	1	8	0.51	.....	.....
Total	1558				

Note that 1/42 of the crossovers in region 4 were arbitrarily assigned to region 4a of 2L; 41/42, to region 4b of 2R. The distance between pr and the spindle attachment is 1/42 the distance between pr and c.

TABLE 27

Summary of data used in the derivation of regional crossover percentages of the control, 3L. The regions are numbered as follows: *ru* 1 *h* 2 *th* 3 *st* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	894	253	15.86	.....	.....
1	362	668	41.88	48.90	24.45
2	290	530	33.23	39.50	19.75
3	2	4	0.25	0.25	0.13
4a	17	20	1.25	3.00	1.50
1,2	23	92	5.77	.....	.....
1,4a	5	20	1.25	.....	.....
2,4a	2	8	0.50	.....	.....
Total	1595				

Note that 21 of the non-crossovers, 9 of the single crossovers in region 1, 6 of the single crossovers in region 2, and 1 of the single crossovers in region 3 were obtained in individuals in which the crossover composition of chromosome 2 was not determined. Also, 2/6 of the total number of crossovers in region 4 were arbitrarily put in region 4a; these numbered 24. 4/6 of the total number of crossovers in region 4, i.e., 49, were arbitrarily put in region 4b.

TABLE 28

Summary of data used in the derivation of regional crossover percentages of the control, 3R. The regions are numbered as follows: spindle attachment 4b *cu* 5 *sr* 6 e^a 7a *Pr* 7b *ca*.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	803	159	9.97	.....	.....
4b	29	22	1.38	6.38	3.19
5	113	162	10.16	19.18	9.59
6	117	194	12.16	17.67	8.84
7a	299	548	34.36	42.12	21.06 (30.28)
7b	154	234	14.67	24.94	12.47
4b,5	6	24	1.50	.....	.....
4b,6	3	12	0.75	.....	.....
4b,7a	6	16	1.00	.....	.....
4b,7b	5	12	0.75	.....	.....
5,6	2	4	0.25	.....	.....
5,7a	13	40	2.51	.....	.....
5,7b	14	52	3.26	.....	.....
6,7a	3	8	0.50	.....	.....
6,7b	13	56	3.51	.....	.....
7a,7b	8	20	1.25	.....	.....
4b,7a,7b	2	16	1.00	.....	.....
5,6,7a	2	8	0.50	.....	.....
5,7a,7b	2	8	0.50	.....	.....
5,6,7a,7b	1	16	1.00	.....	.....
5,6,7b	0	—8	—0.50	.....	.....
6,7a,7b	0	—8	—0.50	.....	.....
Grand Total	1595				

Note that 17 of the non-crossovers, 1 single crossover in region 4b, 3 singles in region 5, 3 singles in region 6, 11 singles in region 7a, 1 single in region 7b, and 1 double in regions 5,7a were obtained in individuals in which the crossover composition of chromosome 2 was not determined. Also, 2/6 of the total number of crossovers in region 4, i.e., 24, were arbitrarily put in region 4a; 4/6 of the total number of crossovers in region 4, i.e., 49, were arbitrarily put in region 4b.

TABLE 29

Summary of data used in the derivation of regional crossover percentages calculated from the homozygous translocation, 2R. The regions are numbered as follows: spindle attachment 4b *c* 5 *px* 6 *sp*.

Region	Number of Crossovers	Chiasmata Number of	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1401	494	19.46	.....	.....
4b	488	750	29.59	47.39	23.69
5	431	686	27.02	41.03	20.51
6	103	152	5.98	10.38	5.19
4b,5	87	344	13.55	.....	.....
4b,6	26	100	3.94	.....	.....
5,6	1	4	0.15	.....	.....
4b,5,6	1	8	0.31	.....	.....
Total	2538				

Note that 57 of the crossovers in region 4 were arbitrarily put into region 4a; 601, into region 4b.

TABLE 30

Summary of data used in the derivation of regional crossover percentages calculated from the homozygous translocation, 3L. The regions are numbered as follows: *ru* 1 *h* 2 *th* 3 *st* 4a spindle attachment.

Region	Number of Crossovers	Number of Chiasmata	Per Cent of Chiasmata	Map Distance in Chiasmata	Map Distance in Crossover Per Cent
0	1396	366	14.42	.....	.....
1	540	986	38.85	46.01	23.01
2	507	924	36.40	43.64	21.82
3	0	0	0.00	0.00	0.00
4a	38	42	1.65	4.08	2.04
1,2	38	148	5.83	.....	.....
1,4a	10	36	1.02	.....	.....
2,4a	8	28	1.10	.....	.....
1,2,4a	1	8	0.31	.....	.....
Total	2538				

Note that 57 of the crossovers in region 4 were arbitrarily put into region 4a; 601, into region 4b.

IV. MULTIPLE SEX GENES IN THE X-CHROMOSOME OF *DROSOPHILA MELANOGASTER*

SARAH BEDICHEK PIPKIN[1]
North Texas Agricultural College, Arlington, Texas

The theory that sex in *Drosophila* is determined by numerous female tendency genes, mostly in the X-chromosome, interacting with male tendency autosomal genes, originated with Bridges from his discovery of triploid intersexes (Bridges, 1921, 1922). Muller suggested that the specific sex differentiating mechanism was originally a single gene difference which may have gained helper-sex genes in the course of evolution. These helper-sex genes could possibly have come in time to possess a potency equal to that of the original sex differentiator (Muller, 1932). Muller suggested that breakage of the X-chromosome by means of X-rays would furnish a method of discovering what region or regions of the X-chromosome were active in the sex differentiating process (Muller, 1928). Dobzhansky and Schultz (1934), using the triploid method, elaborated the theory that numerous female sex genes occur scattered along the X-chromosome of *Drosophila*. An alternate possibility to the multiple sex gene theory was that a single primary sex gene in the X-chromosome, possibly reënforced by lesser modifying genes, was responsible for the differences between the sexes. The evidence concerning each of these two theories will be reviewed.

Patterson was the first to test on a large scale the influence on sex differentiation of different regions of the X-chromosome present in single or double dose with diploid autosomes (Patterson, 1931). His gynandromorph experiments of 1931 strongly suggested a primary female sex factor restricted to the *garnet-forked* region of the X-chromosome. In later experiments, Patterson, Stone, and Bedichek (1937) disproved the existence of a single primary female sex gene in all regions of the X-chromosome save the *wavy-garnet* region extending from band number 11A6 to 13A6 on Bridge's 1935 salivary gland map. Patterson, 1938, has narrowed down the region of a possible principal sex factor to the *garnet-pleated* section between bands 13A2 to 13A6. This very short section has consistently failed to give viable hyperploid males.

Other contributions to the knowledge of diploid aneuploidy have been made by Dobzhansky and Schultz (1934) and Muller (1930).

In 1934, Dobzhansky and Schultz showed that certain parts of the X-chromosome when present in triplicate in 3A flies while the rest of the X-chromosome was present in duplicate shifted the sex type of the intersex in the female direction. This method of attack upon the sex gene problem had first been suggested by Muller and Stone (1930). In this

[1]A part of this work was done at University College, London, while the author was recipient of a Rockefeller Foundation fellowship.

study, Dobzhansky and Schultz used three translocations involving chromosomes X and 4 and a number of small duplicating fragments of the extreme left and extreme right hand regions of the X-chromosome. The authors concluded that there were numerous female determining sex genes scattered along the X-chromosome (Dobzhansky and Schultz, 1934). They were unable to study the effect of the addition to the intersex chromosome complement (2X3A) of the middle region (*v–m*) alone which in addition to 1X2A produced a sterile hyperploid male.

Punnett criticized Dobzhansky and Schultz for claiming in a preliminary paper that their experiments with short duplications constituted strong evidence in favor of a large number of sex genes. He contended that most of the short fragments contained the extreme left end of the X-chromosome and hence some of the effects produced might be due to a very few genes in this region. Punnett also noted that the mean intersex type of the controls differed by almost as much as the range of the short duplication intersex mean types. The longer duplication 100 (L.V.M.) was conceded to be "in another class" from the short duplicating fragments (Punnett, 1933).

Goldschmidt was unconvinced that the small shifts toward femaleness in the duplication intersexes of Dobzhansky and Schultz were due to multiple sex determiners rather than to environmental agents or the kind of modifers Dobzhansky previously described in ordinary 2X3A intersexes (Goldschmidt, 1935). To satisfy Goldschmidt's arguments, relatively short sections covering each region of the X-chromosome would have to be studied in the duplication intersex, because he predicts that "a triploid intersex plus the section containing the sex gene would be genetically a triploid female but one with considerable deficiency and either not viable or in some respects abnormal, perhaps sterile."

The purpose of the present paper is to extend the knowledge of sex genes in the X-chromosome, using the technique of Dobzhansky and Schultz. X translocations not available at the time of the former authors' work now make possible the production of individuals possessing relatively short or long sections of each region of the X-chromosome and three of each large autosome. For a few regions, 3A flies can be synthesized lacking a section of one of their two X's, and several cases have been obtained in which it could be proven that there was an excess of a portion of the X-chromosome above the 3X3A set.

Materials and General Procedure

The translocation stocks used to produce 3A aneuploidy of the X-chromosome have been described in previous papers. (Patterson et al., 1934, 1935, 1937, and Patterson, 1938). They include X-4 translocations m5, 9, 17, w13, 8, 4, 1, 13, and the X-2 translocation 124. Analyses show that m5 is broken genetically at *w* (1.5) ; 9, between *rg* (11.0) and *cv* (13.7) ; 17 between *t* (27.5) and *lz* (27.7) ; w13, on the left between *lz* (27.7) and *ras* (32.8) and on the right between *bb* (66.0) and the spindle attachment,

the middle portion, XR, being inserted into the broken IV (Muller and Stone, 1930); 8, between *fw* (38.7) and *wy* (40.7); 4, between *sd* (50.5) and *r* (54.5); 1, between *f* (56.7) and *Bar* (57.0); and 13, between *fu* (59.5) and *car* (62.5). It must be noted that translocation w13 is the same stock designated by Dobzhansky and Schultz as X-IV 1 in their experiments. In translocation 124, the *v-g* section is deleted from the X and inserted into the 2 near the *brown* locus, the left-hand break in the X being between *ras* (32.8) and *v* (33.0) and the right-hand break between *g* (44.4) and *pl* (47.9). The frontispiece shows the location of these breaks on the genetic and salivary gland chromosome maps.

Three general methods were used to produce aneuploids of the X-chromosome possessing three of each of the large autosomes:

(1) Males of a translocation base stock were crossed to triploid females homozygous for the recessive markers y^2v *f* or y^2car. Aneuploid individuals receiving either one of the two portions of the broken X-chromosome from their male parent plus 2X3A survive in all cases. If they are even weakly fertile as females, they are called hypotriploid females. Sterile flies of this general type are called duplication intersexes. In a few cases, flies survive with one complete X-chromosome, a fragment of an X, and three of each large autosome. These may be called hypointersexes.

(2) Relatively fertile hypotriploid females; i.e., m5R + 2X3A and 9R + 2X3A obtained by the method just described in (1) were mated with translocation males marked with the dominant *Bar*, the point of breakage of these translocations being not far to the right of the XR fragment in the hypotriploid female parent used. In the progeny, new duplication intersexes could be detected. The duplication intersex individuals were chosen by their gray body color, non-*Bar* eyes, and intersexual appearance. The method is satisfactory only for producing short duplication intersexes of interior regions due to difficulties of identification which will be described later.

(3) Males carrying a short section of the X-chromosome in duplicate were crossed to triploids with recessive markers y^2v *f*. One of these hyperploid stocks ($m5L/y^2$) contains the left-hand fragment (L) of the translocation base stock plus an unbroken X-chromosome carrying y^2. The other hyperploid male stocks used have the left fragment of one translocation and the right portion of another translocation, thus carrying in double dose the genes lying between the two points of breakage of the respective translocation base stocks. A description of the way in which these hyperploid males were obtained may be found in a previous paper (Patterson et al., 1937). The method was first suggested by Muller (1930) and used in one instance by Dobzhansky and Schultz (1934). Hyperploid male stocks w13L + 17R, 8L + w13R, and 1L + 4R were viable and fertile enough for the purpose.

The progeny of triploids crossed with hyperploid males include duplication intersexes and hypertriploids (females with a portion of the X-chromosome in excess of the 3X3A complement).

A special series of crosses which will be described later were used for obtaining duplication intersexes carrying in excess of 2X3A the region between translocations 8 and 4.

3A X-aneuploids were tested for fertility in the following manner: female-like individuals were put singly in vials with three males, and male-like individuals were put with three virgin females. Three days later the vials were checked for death of the aneuploid. Sterile flies not living as long as three days were not considered sufficiently tested for fertility. If the vials developed pupae, even though these did not hatch, the parents were regarded as fertile. Most of the experiments were conducted at 23° C. However, preliminary tests of the effect of low temperature on the phenotypes of certain hypotriploid females (m5R, 124L + R, 9R, w13R and 17R + 2X3A respectively) were carried out at 18° C.

Experimental Results

According to method (1), hypotriploids, duplication intersexes, and in a few cases, hypointersexes, appear in the progeny of translocation base stock males and recessively marked triploid females.

Offspring from such crosses are listed in Table 1.[2] With the exception of stock 13, translocation males were mated with *y²v f* triploids. Stock 13 required *y² car* as markers in the triploid strain. The leftmost column of Table 1 contains the genetic composition of the parent male translocation stock. The experimental series number is indicated in the second column. These crosses were begun in the summer of 1936 at The University of Texas at Austin, using the banana-yeast medium; continued at the Texas State College for Women, Denton, Texas, also using the banana-yeast medium; continued at University College, London, in the fall and spring of 1937–38 on corn meal medium, and completed at Woods Hole, Massachusetts, summer, 1938, using corn meal medium. A capital letter A, D, L, or WH, respectively after the series number indicates at which laboratory the particular experimental series was conducted. Progeny with diploid autosomes and those with triploid autosomes are listed separately in Table 1. The genotypic composition with regard to X-chromosome content of both 2A and 3A flies heads appropriate columns. For example, the third column in Table 1 contains the number of diploid females homozygous for *y²v f* (or *y² car*). Females hybrid for the translocation and a *y²v f* (or *y² car*) chromosome are in the fourth column; hyperploid females with two recessively marked X's and the left-hand fragment (L) from the translocation male are listed next; and so on. The genetic markers which show in aneuploids receiving either the left (L) or the right (R) fragment from their translocation bearing fathers depend upon the length of these left and right sections respectively in the various translocation stocks. Thus the hyperdiploid female with the L fragment from the cross

[2]If a fairly large number of progeny from a particular cross was listed, the cross was sometimes continued without counting in order to obtain hypotriploid or duplication intersexes in large numbers for fertility tests.

of m5 males x y^2v *f* triploids appears *v f;* whereas the corresponding hyperdiploid female from 4 *Bar* males x y^2v *f* is phenotypically *f*. Control intersexes, duplication intersexes, and hypotriploids are placed in groups I to VI according to the arbitrary classification of Dobzhansky and Schultz (1934). The groups may be distinguished as follows, quoting Dobzhansky and Schultz (1934):

"Class I. Extreme male type intersexes. Genitalia and coloration of the abdomen male. Penis and genital arch asymmetrical. Sex combs present. Anal tubercle of male or female type.

"Class II. As above, but penis and genital arch asymmetrical.

"Class III. Intermediate intersexes. Neither male nor female external genitalia present, or genitalia extremely rudimentary. Anal tubercle female. Coloration of the abdomen male. Sex combs present.

"Class IV. As above, but female genitalia present. Vaginal plates asymmetrical.

"Class V. Female type intersexes. Female genitalia present. Vaginal plates symmetrical. Coloration of the abdomen female (rarely intermediate). Sex combs present on at least one leg.

"Class VI. Extreme female type intersexes. As above, but sex combs absent on both legs and coloration of the abdomen female." These type VI intersexes are "practically never found in the absence of duplications." (Dobzhansky and Schultz, 1934.)

The criteria separating the six intersex types are based upon external primary and secondary sexual characteristics, but Bridges and Dobzhansky (1928) have shown that the condition of the internal genitalia closely approximates the external sex characters.

In Table 2 are recorded offspring of the relatively fertile hypotriploids m5R + 2X + 3A and 9R + 2X + 3A respectively crossed with translocation males carrying the dominant marker *Bar*. The data are arranged in form similar to that given in Table 1. Crossover individuals are indicated in the body of the table, where they occur, under columns headed by the genotypic compositions to which they probably belong. Note that the left and right fragments of the different translocations are designated by L and R, as in Table 1, but the R fragment introduced by the parent male can always be distinguished by the dominant marker *Bar* from the R fragment transmitted by the hypotriploid mother. This method, number 2, yielded duplication intersexes of interior regions of the X-chromosome if the point of breakage of the translocation carried by the male parent was not far to the right of the left end of m5R or 9R fragments carried by the female parent. Duplication intersexes 9L + m5R, 17L + m5R, w13L + m5R, and 17L + 9R plus 1X3A, respectively, appear in Table 2. The identification of the 9L + m5R + 1X3A, 17L + m5R + 1X3A, w13L + m5R + 1X3A, and 17L + 9R + 1X3A duplication intersexes is completely satisfactory in the author's opinion since there is no phenotype with which these wild type sex-combed individuals can be confused. The production of the desired duplications is very tedious for three

reasons: (1) the hypotriploid mothers are less fertile and fecund than normal; (2) a large number of progeny are inviable due to aneuploidy; (3) on account of directed disjunction in triploids making 1X2A and 2X1A eggs far exceed 1X1A and 2X2A, gametes with one $y^2v\ f$ X chromosome + m5R are more apt to be 1A than 2A, and even upon union with the correct sperm type; i.e., L fragment + 1A, give more often hyperdiploid females instead of duplication intersexes. These rare duplication intersexes were mounted in euparol for preservation.

Table 3 records the offspring from the cross of hyperploid males x recessively marked triploids described in method 3. Hyperploid male stocks w13L + 17R, 8L + w13R, 1L + 4R, and m5L/y^2 were crossed with $y^2v\ f$ triploids. This method was primarily used to obtain duplication intersexes and hypertriploids for short sections of the X chromosome. In addition, from stocks w13L + 17R, 8L + w13R, and 1L + 4R, hypotriploids or duplication intersexes resulted from the combination of either the left or right translocation fragment from the hyperploid male parent plus 2X3A. As in Table 1, but not always in Tables 2 and 4, the genotypic content of the various classes in Table 3 can be deduced from the recessive markers and the phenotypic appearance they present.

In order to obtain duplication intersexes bearing in excess of 2X3A the middle section of the X-chromosome between translocations 8 and 4, $y^2v\ f$ triploids were first crossed with 8 y^2 males. Then, the triploid daughters of the composition 8 y^2/ $y^2v\ f$/ $y^2v\ f$ were crossed to translcoation males 4 $v\ B$. Table 4 records the progeny of this second cross. The desired duplication intersex appears phenotypically v, but not B, and has the genetic composition 4L v + 8R + 1X3A. This is one of the regions which Dobzhansky and Schultz were unable to study. It is a part of this region which consistently fails to survive as a hyperploid male, according to the experiments of Patterson et al., 1937, and Patterson 1938. Not all of the other classes of individuals are phenotypically distinguishable, but an attempt has been made to group the progeny into their supposed genotypes. The table is constructed without regard to crossing over of certain markers since this usually does not affect the particular aneuploid class desired.

Progeny counts of the y^2vf triploid base stock are as follows:

y_2vf/y_2vf diploid female	y_2vf diploid male	$y^2vf/y^2vf/y^2vf$ super-female	$y_2vf/y_2vf/y_2vf$ triploid	y^2vf/y_2vf intersex					y^2vf super-male
				I	II	III	IV	V	
977	399	0	210	49	36	96	125	13	5

Description of the Aneuploids

Before describing the 3A/X-aneuploids, a few remarks are worthwhile concerning the main phenotypic differences, aside from those directly connected with sex, between triploid females (3X3A), diploid females (2X2A), intersexes (2X3A), and the supersexes (3X2A and 1X3A respectively). The triploid is distinctly larger than any of the other forms in body size. She appears bulky or stocky in comparison with the more slender diploid female. Eye facets of the triploid are larger than those of the diploid with the result that individual facets appear more distinct in the former at ordinary magnifications. Finally the hairs on the surface of the wings are very much more sparse in the triploid than in the diploid. As a consequence, triploid wings viewed from the top appear like coarse netting compared with diploid wings.

The intersex body, which possesses a characteristic squatness, is shorter than that of either triploid or diploid female and roughly comparable to that of the diploid male. The eye facets of the intersex are as large as in the triploid, but they usually appear disarranged and the eye as a whole is bulging. Hairs on the surface of the wings are sparse giving a coarse effect as in the triploid. The wing margins are often clipped; there are abnormalities in wing venation; the surface of the wing is sometimes rumpled; and the wings are occasionally held apart. Legs are frequently misshapen in intersexes, whereas this is a rarity in normal triploid and diploid females.

The triple X or super-female (3X2A) has many times been described as an undersized, weak little creature with clipped wings and rough eyes. The texture of the wings appears fine as in diploids.

The super-male (1X3A) is shorter than the ordinary diploid male. His wide abdomen possesses a peculiar flatness. The wing texture appears fine, wing veins sometimes thickened at their extremities, and the wings are often held somewhat apart. There is a disarrangement of the small eye facets and their hairs. Intersexes and the supersexes are always sterile. Triploids, on the other hand, are highly fertile, but their yield of viable offpsring is diminished because so many aneuploid eggs are produced. The reader is referred to Bridges (1921; 1922); Dobzhansky (1930b); and Gowen (1931) for detailed descriptions of these 3A forms and to Dobzhansky (1929) for detailed study of wing cell size. The latter author showed triploid and diploid females, diploid males, intersexes, and the supersexes to have different hair counts on the surface of the wing (each hair represents one cell). With 30X magnification, triploid and intersex texture of wing are readily distinguishable from the texture of the other forms.

The mutant character *forked* is manifested differently in the progeny of triploids. The supermale, with slender bristles, exhibits the most extreme expression of *forked*. That this is not due to the slenderness of the bristles is shown by the weaker manifestation of *f* in haplo-IV.

males. Triploid females appear to be slightly more *forked* than diploid females homozygous for the character, although many exceptions occur. Diploid males are generally less *forked* than diploid females, as other authors have pointed out (Muller, 1932). Finally intersexes are the least *forked* of all, as a rule. Peculiar patches of very *forked* areas occur on weakly *forked* intersexes, and occasionally, all the bristles show an extreme *forking*. The expression of *forked* in intersexes does not seem to be correlated with the sex type.

Hypotriploid Females

Table 5 gives a brief description of the various hypotriploid females the genotypic constitutions of which are certain. These observations are based upon the appearance of the animals at room temperature; i.e., 23°C. There was no difficulty in distinguishing such hypotriploids as m5R, 9R, 17R, w13R, 8L, 4L, 1L, 124L + R, and 13L + 2X3A, respectively, from the corresponding hyperdiploid female which showed the same recessive markers. In these cases, the hyperdiploid female was small; rough-eyed; with clipped, often crumpled wings; and thickened wing veins. The eyes of the hypotriploids were never rough, and the facets were always large and distinct. There were no abnormalities in the wings (except for very rare nick in the wing tips of 17R + 2X3A). The texture of the wings was always more coarse than that of the hyperdiploid.

A little confusion was experienced at first in separating the hypotriploids 17L + 2X3A and w13L + 2X3A from the corresponding hyperdiploid females. The texture of the wings in the former hypotriploids was sometimes as fine as in hyperdiploid. Moreover the eyes and wings of the w13L + 2X3A and 17L + 2X3A hyperdiploid females were usually not strongly affected by aneuploidy. Still, w13L + 2X3A and 17L + 2X3A hypotriploids were clearly shorter and more chunky than the hyperdiploids. As a result, the large evenly arranged eye facets of these hypotriploids seemed out of proportion to the small body size. Wing veins, also, were more perfectly formed in the hypotriploids.

Table 5 shows that hypotriploids varied in size from approximately the lengths of control intersexes, as in the case of 17L and w13L + 2X3A, respectively, to the large m5R + 2X3A individuals which, without detailed measurements, appeared the size of their triploid sibs. Body size was roughly proportional to the cytological length of the fragment carried in the hypotriploid. There were exceptions, however: 1L + 2X3A flies, with a length of 88 salivary gland units were decidedly smaller than 9R + 2X3A with a length of 86 units.[3] Eye facets were always larger in

[3]The drawing of the salivary X-chromosome, Frontispiece, measures 115 mm. The length of each section of the chromosome studied in the various aneuploids is expressed in millimeters of this drawing. Measurements are accurate to the nearest millimeter. A millimeter of the salivary X-chromosome drawing is used as a "cytological unit."

hypotriploids than in diploids. With but two exceptions (17L + 2X3A and w13L + 2X3A), the hairs on the surface of the wings were more sparsely scattered in hypotriploid than in diploid wings. Absence of post vertical bristles occurred in some hypotriploids, but this was also a characteristic of the corresponding hyperdiploid females as well as control intersexes. No structural abnormalities occurred in the ducts and receptacles of the internal reproductive tract of the hypotriploids listed in Table 5. Ovaries of m5R and 9R + 2X3A hypotriploid females were approximately the same size as those of normal diploid sibs. Hypotriploids with shorter fragments had ovaries reduced in size or in an immature condition.

Whereas most of the hypotriploid classes listed in Table 5 possessed female characteristics only, according to expectation, since these aneuploids are at least weakly fertile as females; especial attention should be directed to hypotriploids 17R + 2X3A and w13R + 2X3A. Some of these which developed at room temperature showed rudimentary sex combs, a secondary sexual character of males. This fact was noted by Dobzhansky and Schultz (1934) in the case of one individual of the composition w13R + 2X3A (the right hand fragment of their X-IV, 1). However, these authors did not obtain any fertile individuals of the composition w13R + 2X3A.

Intersexes from the triploid base stocks used in these experiments regularly possessed sex combs. Moreover, all the control intersex sibs of these hypotriploids had sex combs on at least one foreleg. Sex combs in type V control intersexes developing at 23° were much larger than in type V hypotriploids 17R or w13R; i.e., control intersex sex combs are commonly composed of from 8 to 11 prongs, occasionally fewer. The mean number of sex combs on the left forelegs of seventeen type I (male-like) control intersexes was 9.11 ± a standard deviation of 0.22. The mean number of sex comb prongs on the right forelegs of these seventeen type I individuals was 9.17 ± 0.23. Mean prong number of sex combs on the left foreleg of twenty type IV (female like) control intersexes was 8.60 ± the standard deviation 0.39; on the right foreleg, 8.71 ± standard deviation of 0.46. One individual of the type IV group had no sex comb on the right foreleg but 8 prongs on the left foreleg; it was left out of the calculation of mean of the prong number in the right leg. The difference between the mean prong number of right and left forelegs of type I control intersexes or of right and left forelegs of type IV intersexes is not significant. Moreover the difference between the mean prong number in type I (malelike) right or left foreleg is not significantly different from the mean number in the type IV (femalelike) group. The mean prong number in sex combs of one group of ordinary diploid males studied by Combs was 10.57 ± 0.04 (Combs, 1937). In type V hypotriploids of the composition w13R + 2X3A, on the other hand, from two to four stiff black hairs occupied the exact location of an ordinary sex comb and one or two black hairs in the case of the 17R + 2X3A type V hypotriploids.

These structures have been called sex comb rudiments. As we shall see, 8R + 2X3A duplication intersexes also occur both without sex combs and with sex combs varying from bare rudiments of a few hairs to normal-sized structures. Temperature experiments to be described shortly make more probable the present interpretation of these rudimentary sex combs.

Only two cases of fertility have been recorded of w13R + 2X3A and 17R + 2X3A hypotriploids actually bearing traces of sex combs (type V). One type V w13R + 2X3A hypotriploid produced one y^2 *v f* diploid female. A single type V 17R + 2X3A hypotriploid gave one y^2 *v f* intersex as offspring. y^2 *v f* males were the fathers in both cases.

Lowering the temperature from 23° C. to 18° C. has a pronounced effect upon the phenotype of 17R + 2X3A aneupoids, changing them from hypotriploids to duplication intersexes. At the higher temperature, these flies were fairly fertile. In body size, they were similar to their 2X2A diploid sibs. The smooth surfaced wings were nicked only occasionally. At 18° C., 17R + 2X3A aneuploids were small and squat with deeply clipped wings. Sex comb rudiments occurred more often and were larger at the lower temperature. For example, one type V 17R + 2X3A aneuploid developing at 18° C. had two prongs on its right foreleg and four on the left. A second had two on the left and none on the right. A third had two on the right and four on the left foreleg. The prongs were arranged in orderly comb-like fashion. The two 17R + 2X3A type V hypotriploids developing at 23° C. had no sex comb rudiments represented by more than two prongs. Two low temperature 17R + 2X3A aneuploids were recorded as type IV; i.e., with asymmetrical vaginal plates, but in all others examined the external genitalia were of the normal female type. Dissection of a type 1V 17R + 2X3A aneuploid developing at 18° C. revealed ovaries about one-third normal size. The few mature eggs with filaments appeared shorter and more blunt than normal eggs. Only one spermatheca was present; no parovaria. Otherwise the reproductive tract was normal. Five type V, low temperature 17R + 2X3A aneuploids were dissected. A small number of mature eggs, usually only the most posterior eggs of the ovarioles, were present in four cases, and no mature eggs in the fifth individual. All flies were five days old at the time of dissection. As in the preceding type IV 17R + 2X3A aneuploid, the mature eggs were abnormally blunt. Ovaries varied from about one-fourth to about one-third normal size. One parovarium was absent in two cases. Other parts of the reproductive tract were normal in these flies.

Table 6 gives the progeny of translocation 17, m5, and 124, 9, and w13 males X y^2 *v f* triploids respectively, developing at 18° C. At this low temperature, m5R + 2X3A, 124L + 2X3A and 9R + 2X3A aneuploids appeared the same, phenotypically, as at the higher temperature, 23° C. They maintained their hypotriploid female phenotype in spite of a drop in temperature, although the mean sex type of control

intersexes was shifted toward maleness (see Dobzhansky, 1930a for effect of temperature on 2X3A intersex types.)

Although the count of progeny from the cross of w13 males X y^2 *v f* triploids is very low, still the three type V hypotriploids of the composition w13R + 2X3A which occurred possessed larger sex combs than were found in any of the many type V hypotriploids of this composition developing at 23° C. which were examined. The sex combs in two of the w13R + 2X3A hypotriploids listed in Table 6 consisted of three prongs on one foreleg and six on the other. The third individual had from four to six prongs on both forelegs. Sex combs when present in hypotriploids of this composition developing at 23° C. are usually of one or two prongs, rarely three.

From Table 7 some idea may be had of the relative fertility of the different hypotriploid females hatching at 23° C. The figures are not strictly comparable owing to the fact that both banana and corn meal culture media have been used. A large number of each kind of hypotriploid should have been obtained at one time and set up in culture media from the same cooking in order that influences of the environment upon the fertility of these different aneuploids could at least be equalized. This scheme would however be impractical for such hypotriploids as 17L, 4L, and 1L + 2X3A, respectively, which survive so rarely.

Table 7 shows that hypotriploids with longer fragments such as 124L + R + 2X3A and m5R + 2X3A are more fertile than hypotriploids with shorter fragments such as 8L + 2X3A and w13R + 2X3A. Since a fly was classed as fertile if it produced even a larva or pupa, the question arises as to the degree of fecundity of these aneuploids. The mean number of offspring per vial was calculated, all mothers being tested individually. One must be very careful in using these figures as a measure of the ability of the respective female parents to produce functional eggs because the aneuploid progeny of different hypotriploids may possess different degrees of viability. In most cases, however, very few aneuploid offspring hatched. Therefore, a hypotriploid such as m5R + 2X3A with a mean number of 7.4 offspring per vial is clearly more fecund than 8L + 2X3A with a mean number of 3.1 per vial. At least one fly hatched per vial in these cases. However, the two fertile w13L + 2X3A hypotriploids were so by the grace of one larva and one pupa respectively, neither of which emerged as imagines. In consequence there is a difference in the degree of fecundity among the various hypotriploids, those bearing the longer fragments producing more progeny. Even the latter are distinctly less fecund than the normal triploid. In all cases except w13L; 17L; w13R, type V; and 8L + 2X3A respectively; offspring with three sets of autosomes were produced. The author is not in any doubt as to the correctness of the classifications, however.

In addition to information on fertility and fecundity of hypotriploid females, Table 7 shows differences in degree of mortality during the first three days on the part of these aneuploids. Thus of 91 individuals of

the composition 8L + 2X3A, 39 died within the first three days, a mortality percentage of 42.9 per cent. The percentage of 124L + R + 2X3A hypotriploids dying during the first three day period was only 23.3 per cent. Although mortality percentages for hypotriploids containing longer fragments are always less, these values do not seem to be directly proportional to length: w13R + 2X3A, type VI, hypotriploids, with a fragment only 5 salivary units longer than the 8L fragment, have a mortality percentage of 20.8 per cent.

Reference to Tables 1 and 3, containing the original crosses by which the hypotriploids were produced reveals a considerable range of rate of survival to the adult stage. The rare 17L + 2X3A, 4L + 2X3A and 1L + 2X3A hypotriploids are at opposite extremes from hypotriploids of composition m5R + 2X3A, 9R + 2X3A, and 17R + 2X3A, respectively. Hypotriploids with left-hand fragments (L) seem to be on the whole less viable than those with the right-hand fragment of the respective translocations.

Duplication Intersexes

(1) Duplication Intersexes Bearing Long Fragments

The duplication intersex 8R + 2X3A, carrying the longest fragment in excess of 2X; i.e., 56 salivary gland units long, closely approximates both in external appearance and condition of the reproductive tract the most intersexual hypotriploid female, w13R + 2X3A, with fragment 64 units long. A few more individuals with rudimentary sex combs occurred among w13R + 2X3A hypotriploids than among 8R + 2X3A duplication intersexes. However, sex combs were never as well developed in the w13R + 2X3A hypotriploid as they were occasionally in the 8R + 2X3A duplication intersex. At 23° C., 8R + 2X3A individuals appeared a little larger than control intersexes, but their bodies possessed the characteristic squatness of an intersex. The vaginal plates were usually perfectly formed (types V or VI); rarely there were slight irregularities and faint asymmetry (type IV). Abdominal coloration was always female in type. Wing texture was only a shade less coarse than in triploids or control intersexes. The eye as a whole was larger than in the control intersex, with individual facets intermediate in size between diploid and triploid facets. There was no disarrangement of facets corresponding to that in control intersexes.

Since an 8R + 2X3A duplication intersex with one black hair as vestige of sex comb on the right foreleg laid two eggs, it was not surprising to find the ducts of the internal reproductive tract apparently normal in the four flies dissected. In two cases, the ovaries were very small, divided into irregular egg tubes, but no mature eggs were present. A third individual showed ovaries containing four mature eggs. One egg bore filaments. Three others without filaments were seemingly in a state of degeneration and melted with the prick of a needle. Parovaria were absent in two of the four cases.

Owing to the intermediate position of 8R + 2X3A duplication intersexes between femaleness and maleness, a number were tested for fertility but without success, as follows:

	Number Tested	No. Dead at the End of Three Days	Fertile
Type V	34	8	0
Type VI	151	31	0

4R + 2X3A duplication intersexes carrying a fragment 36 salivary units long in three doses were about the same size and shape as control intersexes. Wing texture was coarse; eye facets, large. It was difficult to determine whether or not the eyes were rough owing to the presence of Bar. Often 4R + 2X3A duplication intersexes had large sex combs with no more reduction than occurs among different control intersex individuals. Sex combs in other 4R + 2X3A duplication intersexes were as rudimentary as in 8R + 2X3A, type V, duplication intersexes; i.e., composed of one or two black hairs. Only three 4R + 2X3A individuals have been obtained without any sex comb traces; i.e., belonging to class VI. In addition to the 5 type IV, 13 type V, and 1 type VI 4R + 2X3A duplication intersexes listed in Table 1, two type VI's have been recorded. The vaginal plates of the type IV individuals were sunken, only slightly rotated, with hairs disarranged. They were thus much more female-like than the vaginal plates in the majority of type IV control intersexes.

Three of the five 4R + 2X3A duplication intersexes dissected possessed undersized ovaries infested with tracheal tissue. No mature eggs were present although the ovaries were divided into ovarioles. An ovary was missing in one of these three individuals. In all three cases, the remainder of the reproductive tract appeared normal except that the parovaria were not observed in the fly with the missing ovary. Ovaries of the fourth 4R duplication intersex dissected contained, in all twelve mature eggs. An egg was nearly completely within the oviduct. The absence of one spermatheca and the parovaria prevented the remainder of the reproductive tract from being normal. In the last 4R duplication intersex dissected, there were four practically mature eggs with filaments in one ovary and a single mature egg in the other ovary. These eggs were not as white as normal mature eggs. Only one parovarium was present in this last case.

The duplication intersex carrying the next longest fragment is w13L + m5R + 1X3A. The section between m5 and w13 represented in triplicate is 35 salivary gland units long. Only one such individual has so far been found. The body size was about the same as in a 2X3A control intersex, but body shape and abdominal coloration were female. Wing texture was coarse as in control intersexes. Sex combs were composed of three and five prongs respectively, arranged in an orderly fashion. The external genitalia and anal plates were of the female type and normal. This individual showed definite signs of aneuploidy. The eyes were narrow and protruding. The facets were very rough and disarranged so that

the surface of the eye resembled an extreme *Star*. Two vertical bristles were thick and stubby. The internal reproductive tract was not investigated because it was desired to make a permanent mount of the specimen.

9L + 2X3A duplication intersexes have occurred as type V and type VI only; that is, the external genitalia were always of the normal female type, and the abdominal coloration was always female. The 9L fragment is 29 salivary gland units in length. These individuals were as small as their 2X3A control sibs. The bodies of some individuals have the squatness so characteristic of intersexes but this feature is not emphasized in others. Sex combs of type V individuals were medium-sized: composed of from three to five prongs. The eyes were very large in proportion to body size, but they were not bulging, and facets were large and distinct. In some cases the facets were disarranged; in others, smooth. The legs were frequently misshapen. Wing texture appeared as fine as that of diploids in most individuals. In only one case did wing texture seem a little more coarse than in diploids. Wing veins were often fused at their bases, and a few extra veins were present sometimes. The surface of the wings was dull, and wings were often held apart. The mutant character *forked* appeared exaggerated in some of the 9L duplication intersexes but not in others. Fertility tests of these duplication intersexes are as follows:

	Number tested	Dead at end of three days	Fertile
Type V	6	3	0
Type VI	10	5	0

Two 9L + 2X3A duplication intersexes were dissected to ascertain the condition of the internal reproductive tract. One individual was of type V; the other, type VI. In both cases one ovary was attached and one unattached to the oviduct. The ovaries were tiny, divided into ovarioles, but in an immature state. Tracheal tissue made up a large part of the bulk of both ovaries and uterus. Only one spermatheca was present in each fly dissected. Both parovaria were absent in the type V, but one was present in the type VI individual. The uterus and oviduct of the type V fly were bent abnormally.

Only three individuals of the composition 17L + m5R + 1X3A have so far been found. They all belonged to class V (i.e.. with sex combs). The section between 17 and m5 is 28 salivary gland units. The first individual was slightly larger than its control sibs and very squat. The abdomen was plump as if either bloated or full of eggs. Abdominal coloration was of the female type and vaginal and anal plates were perfectly formed and female. There were large sex combs of four and six prongs respectively on both forelegs. The eye facets were slightly smaller than in ordinary intersexes and very disarrangd, though not to the extent as in the w13L + m5R + 1X3A duplication intersex previously described. The eyes as a whole appeared bulging. Legs were normal. This individual which was hybrid for the recessive mutant *forked* showed a very weak *forked* in half a dozen bristles. It was tested for fertility but died before the end

of the first three days of life. The second individual of this composition was also weak *forked;* but the third 17L + m5R + 2X3A duplication intersex did not show any weak *forked* bristles.

(2) Short Duplication Intersexes

Duplication intersexes with fragments as long as or shorter cytologically than 1R, 27 salivary gland units long, showed variations in external genitalia and presence of sex combs sufficiently to be grouped in types II-VI. 4L + 8R + 1X3A with a section in triplicate 20 units long, and w13L + 17R + 1X3A with triplicate section 7 units sometimes occurred as type I, the extreme male type intersex form. Hence, whereas the long duplication intersexes 4R + 2X3A and 8R + 2X3A were obviously more femalelike than their sib control intersexes from a consideration of qualitative characteristics, it becomes necessary to calculate quantitatively, whether or not the sex type of shorter duplication intersexes has been shifted in a more female direction.

In most cases there are two genetically different control intersexes for each duplication intersex. For example, from the cross 1 B^s males x y^2 *v f* triploid females, 2X3A intersexes result of the composition y^2 *v f*/y^2 *v f* + 3A and y^2 *v f*/1 B^s + 3A. Therefore homogeneity tests must be made to see if the two kinds of control intersexes may be regarded as samples from the same population in respect to their sex types. Any differences between 2X3A intersexes of these two genotypes, hatching in the same bottles, under the same environmental conditions, must be due to modifiers in the translocated X-chromosome dominant over their allels in the y^2 *v f* chromosome in the 2X3A condition. The X-chromosome of all translocation stocks without the marker *Bar* have been preserved intact (except for a rare crossingover with the Y) since these stocks are balanced with attached X females. The original translocation stocks were produced by X-raying wild type sperm.

Tests of homogeniety between similar control intersexes from different experimental series of the same cross have also been made. Differences in sex type could occur in these comparisons because of change in temperature and perhaps culture media in the two series. The results of homogeniety tests among control intersexes are given in Table 8.

The first ten rows of Table 8 are devoted to comparisons of genetically different control intersexes, sibs, derived from the same experimental series. In six cases of these ten comparisons, the two groups are homogeneous with respect to their distribution among the five arbitrary sex types. The probability that 17 *B*/y^2 *v f* + 3A intersexes are similar to their y^2 *v f* / y^2 *v f* + 3A sibs is between 0.02 and 0.01. On the other hand, 17 / y^2 *v f* + 3A and y^2 *v f* / y^2 *v f* + 3A sibs are clearly homogeneous. However, the 17 and 17*Bar* translocated X-chromosomes may well differ in gene content in the R fragment since *Bar* has been introduced into the latter by crossing over. 8 y^2 / y^2 *v f* + 3A and y^2 *v f* / y^2

v f + 3A intersexes from series 2 are definitely heterogeneous and the probability is as low as between 0.05 and 0.02 that 8 y^2 / y^2 *v f* + 3A and y^2 *v f* / y^2 *v f* + 3A, series 1, may be regarded as belonging to the same population. In comparing 8 y^2 / y^2 *v f* + 3A, series 1, with 8 y^2 / y^2 *v f* + 3A, series 2, where a combination of genetic and environmental differences may affect the sex type distribution, we find clear homogeneity between the two series. However, y^2 *v f* / y^2 *v f* + 3A, series 1, and y^2 *v f* / y^2 *v f* + 3A, series 2, from the cross 8 y^2 males x y^2 *v f* triploids have a low probability of 0.1 and 0.5 of being homogeneous. In two cases in Table 8 intersexes of experimental series 1 were definitely homogeneous with those of like X-chromosome content in experimental series, 2, the parents being of the same genetic composition.

Comparison of the sex types of control intersexes with those of short duplication intersexes may be found in Table 9. In cases where two kinds of control intersexes differing either in experimental series number or genotype were found to be homogeneous, their numbers were pooled and then compared with the duplication intersexes. Similarly the numbers of duplication intersexes from two different but homogeneous experimental series were pooled. Two methods of comparison between control and duplication intersexes were used: (1) a homogeniety test of X^2 (chi square) as in Table 8; (2) a test of the significance of the difference between the respective mean sex types by reference to the t-distribution if the numbers of duplication intersexes were very low; to the deviation of the normal distribution in terms of its standard deviation in cases where high numbers of both control and duplication intersexes were obtained. It should be noted that these tests of the significance of the difference between means should be used only if the distributions in question may be regarded as samples from a normal distribution. Tests for normality can be made only when the numbers are very much higher than in the present case. Hence the normality of these distributions is assumed but not proven. If it is fair to compare the mean sex types of control and duplication intersexes, a significantly higher value of the latter indicates a shift in the female direction. Heterogeneity calculated by the X^2 method between the sets of control and duplication intersex types respectively only allows one to say that the two groups of values are probably not drawn from the same population in respect of their sex types.

According to Table 9, the mean intersex type of 1R B^s + 2X3A individuals is significantly higher than that of their control sibs. The fragment in triplicate is 27 salivary gland units long. This calculation is supported by the fact that whereas some 1 R B^s + 2X3A flies were as squat in body shape as control intersexes; others definitely appeared slender and long, like females. The wings of these duplication intersexes resembled those of control intersexes; their margins were clipped and texture coarse. Eye shape and facets were obscurd owing to the presence of the extreme B^s. The mutant character *forked* was pronounced but not

exaggerated in some of the 1R B^s + 2X3A duplication intersexes but as weak as in homozygous *forked* 2X3A control intersexes in other 1R B^s + 2X3A duplication intersexes. All of these duplication intersexes with the exception of the three type VI's possessed large sex combs with little more variation in size than was found in intersexes free from the duplication. (Number of prongs was not actually counted.) None of these duplication intersexes was fertile. Fertility tests are as follows:

Type	Number tested	Dead after three days	Fertile
IV	3	2	0
V	7	2	0
VI	3	1	0

Two type V 1R B^s + 2X3A duplication intersexes were dissected. In one individual, the two small, largely tracheal ovaries were attached to the oviduct. In the other fly there was no oviduct: both ovaries were lying free in the body cavity. The ventral receptacle was smallish and irregularly coiled in each case. In the first fly with oviduct present, there was a normal sized and a small spermatheca. In the second fly without oviduct there was only one spermatheca. No parovaria were observed in either case. The uterus in both individuals contained a large amount of tracheal tissue.

Duplication intersexes bearing the fragment 124M, 18 salivary gland units long, do not appear to have a higher mean sex type than sib intersexes without the duplication. However only 19 of these duplication intersexes survived among the 5189 progeny of 124 males x y^2 *v f* triploids in the two experimental series. In comparing the mean duplication intersex type with mean control intersex type of which several hundred individuals were obtained, the number of degrees of freedom used in referring to the t-distribution is limited by the lower number of duplication intersexes;[4] i.e., it is one less than 19. From Table 9, we see that the probability is between 0.8 and 0.7 that the mean sex type (3.4211) of the pooled numbers of homogeneous 124M + 2X3A duplication intersexes, series 1 and series 2 do not differ significantly from the mean sex type (3.3363) of pooled numbers of homogeneous control intersexes of the composition y^2 *v f* / y^2 *v f* + 3A, series 1 and series 2 plus 124 / y^2 *v f* + 3A, series 2. In comparing the mean sex type (3.4211) of the 124M + 2X3A duplication intersex with the mean sex type (3.0478) of 124 / y^2 *v f*, series 1, control intersexes, we find by referring to the t-distribution that the probability of the two means differing is between 0.2 and 0.1; i.e., the difference is not significant. The means of the two groups of control intersexes here in question; i.e., 3.3363 and 3.0478 do not differ significantly.

The 124M + 2X3A duplication intersexes were of a stocky build and larger than control intersexes. They were about the same size as 124L

[4]For this information I wish to thank Dr. B. L. Welch, of the Department of Statistics, University College, London.

+ R + 2X3A hypotriploids. The eyes as a whole were large and smooth-surfaced with facets approximately as distinct as those of triploids. Legs were often misshapen. Wing texture was variable: in some individuals the distribution of hairs on the surface of the wings was as dense as in diploids; in others, more sparse.

4L + 8R + 1X3A duplication intersexes with a fragment 20 salivary gland units long, have occurred as types I, III, IV, and V individuals, as reference to Table 4 (2) will show. Owing to the low number of duplication and control intersexes so far obtained, no attempt has been made to determine if their respective mean sex types differ. 4L + 8R + 1X3A duplication intersexes resembled their control sibs closely in regard to body size and shape, eyes, and wing texture. It is to be noted from Fig. 1 that 124M and the overlapped region of 4L + 8R include 13 of their respective 18 and 20 salivary gland units in common. A type IV duplication intersex of the composition 4L + 8R + 1X3A was dissected and showed internal reproductive tract typically intersexual.

According to Table 9, m5L + 2X3A duplication intersexes, which have been found as types II–VI, are very much more female like than their control sibs without the duplication. The m5L fragment is 16 salivary gland units in length. These duplication intersexes were either the same size or smaller than the control intersexes. Their eyes were more or less bulging as in 2X3A flies. Individual eye facets were in most cases obviously larger than in diploid flies. Wing texture, however, was not appreciably more coarse than in diploids. The sex combs of types IV and V intersexes were sometimes as large as in control intersexes, but occasionally they appeared in various stages of reduction. Legs were frequently misshapen. The mutant character *f* was not exaggerated. Twenty-two type V duplication intersexes bearing the m5L fragment were found to be sterile.

One duplication intersex of the composition 17L + 9R + 1X3A has been recorded in Table 2. It was the size and shape of an ordinary 2X3A intersex. The vaginal plates were rotated and protruding; coloration of the abdomen, male; and the right foreleg possessed a sex comb of four prongs. In other words, this duplication intersex looked like a typical type IV intersex. The wing texture was as coarse as in a control intersex. The bulging eyes with large facets of this creature were also similar to those of a 2X3A control intersex. The specimen was not dissected but mounted in euparol for preservation. The section in triplicate in this duplication intersex is 15 salivary units long.

Seven duplication intersexes of the composition 9L + m5R + 1X3A have been recorded in Table 2. Of these, 2 were of type III; 4, type IV; and 1, type V. The section in triplicate is 13 salivary gland units long. The eye facets of these duplication intersexes were more disarranged than is usual in 2X3A intersexes. Body size and shape of duplication and control intersexes were indistinguishable. In most of the 9L + m5R + 1X3A duplication intersexes, wing texture was very coarse; in one type III individual, however, wing texture was finer than in the control. The wings were sometimes deeply clipped. The vaginal plates of the type V

were of the normal female type; type IV duplication intersexes had definitely rotated and protruding vaginal plates just as in type IV control intersexes. Variation in size of sex combs was the same as in control intersexes.

One 9L + m5R + 1X3A duplication intersex was dissected. Seven apparently mature eggs were present in each ovary. One ovary was not attached to the oviduct. The uterus was largely tracheal. The ventral receptacle and seminal receptacles were normal; the parovaria, not observed.

Two intersexes of the composition 13R + 2X3A have been found. One was classified as type IV; the other, as type VI. The type IV individual had an eight pronged sex comb on each foreleg. The genitalia were of the female type, slightly protruding and definitely rotated. Eyes were bulging and individual facets, large. Since the body color of the fly was yellow, the sex type of the abdomen was difficult to determine, but it appeared to be of the male type. In short, this duplication intersex looked like a typical type IV control intersex. It was mounted in euparol for preservation. The second 13R + 2X3A duplication intersex appeared bodily like the first except that no sex combs were present, and the genitalia were normal. It was therefore classified as type VI. The 13R section is 13 salivary units in length.

1L + 4R + 1X3A intersexes occurred as types I–V. With the exception of excessively large eyes, they appeared like typical 2X3A control intersexes. This very large eye was also characteristic of 1L + 4R + 2A hyperploid males. Sex combs of the duplication intersex were always large and showed about the same size variations as in the control intersexes. Dissection of one type V duplication intersex revealed very small tracheal ovaries, one attached and one unattached to the oviduct. The oviduct was somewhat twisted as well as the uterus which was also infested with trachea. The ventral receptacle was irregularly coiled, but parovaria and seminal receptacles appeared normal. The section in triplicate in 1L + 4R + 1X3A duplication intersexes is 9 salivary units in length.

Only sixteen 8L + w13R + 1X3A duplication intersexes have been obtained, because 8L + w13R + 2A hyperploid males cross very badly with triploid females. Of these duplication intersexes, 2 were type III; 8, type IV, and 6, Type V. Phenotypically, these duplication intersexes appeared the same as their control sibs except for recessive markers in the latter. The section in common with 8L and w13R is 8 salivary units long.

Duplication intersexes of the composition w13L + 17R + 1X3A, occurring as types I–V, also resembled their control sibs. Nevertheless, Table 9 shows that the mean sex type of the former is slightly but significantly higher than that of sib intersexes without the duplication. The section of w13L overlapping 17R is 7 salivary units long. This is the shortest fragment combined with 2X3A in these experiments.

HYPOINTERSEXES

Hypointersexes, possessing 1X + fragment of an X + 3A must be very inviable. Among the numerous possibilities in the progenies of the different translocation base stock males X triploid females, at most only four distinguishable combinations survive. These are 124L + R + 1X3A, m5R + 1X3A, and possibly, 9L + 1X3A, and 124M + 1X3A. It may be that some of the numerous malelike individuals possessing m5L, 1R, or 4R really have three sets of autosomes and are indistinguishable from hyperploid males with these fragments and two sets of autosomes. However, these aneuploids hatch relatively early as compared with the late hatching super-males (1X3A) and intersexes. Furthermore, their abdomens are long and slender, not broad and flattened as in the super-male.

The five 124L + R / y^2 *v f* + 3A hypointersexes found (Table 1) were slightly smaller than control intersexes. Their bulging eyes contained large distinct facets and showed the recessive mutant, *vermilion*. Wing margins were deeply clipped; wing texture, as fine as in diploids or super-male. The external genitalia and anal plates were of the male type in all individuals and sex combs were unreduced in size. These aneuploids were sterile.

A single y^2, apparently male individual occurred in the progeny from the cross of m5 males x y^2 *v f* triploids, series 1, Table 1. The eyes were normal. Wing texture was fine. The fly must have been a hypointersex of the composition m5R + 1X3A. Evidently these hypointersexes hatch more frequently when they develop at low temperatures because five of them are recorded in the small, low temperature series of m5 males x y^2 *v f* triploids. Five more hatched in some bottles the progeny of which were not counted. All except one of these m5R + 1X3A hypointersexes had normal, maletype genitalia and were therefore classed as type I. The genitalia of the one exception were rotated male type but otherwise normal appearing; it was classed as type II. The coloration of the abdomen was in each case male. Some of the sex combs were decreased in size as compared with normal, but none were composed of less than five prongs. The wing texture in these hypointersexes developing at low temperature was as coarse as in 2X3A intersexes. The eyes were bulging but with facets smaller than those in control intersexes. Body size and shape was intersexual rather than similar to that of the super-male. None of the m5R + 1X3A hypointersexes was fertile.

Two male-like creatures thought to be 9L + 1X3A were obtained from a cross of m5R + 2X3A hypotriploid x 9B males, Table 2, and from 9 males x y^2 *v f* triploids, series 2L, Table 1, respectively. Only one 9L + 1X2A hyperploid male has been recorded in the numerous experiments of Patterson et al., and it was sterile. These two individuals were also sterile. The wings were outstretched; wing texture fine; regions on vein V roughened in one individual. The eyes were slightly bulging and the small facets disarranged. Coloration of the abdomen was male; sex

combs, well developed. Genitalia were of the normal male type. The mutant character f was not exaggerated.

A y^2 *f* male-like individual resulted from a cross of 124 males x y^2 *v f* triploids, series 2, Table 1. The eyes appeared diploid; i.e., the facets were small and regularly arranged. The character f was more weakly expressed than in ordinary diploid males. Patterson and Stone have recently obtained a few hyperdiploid males carrying in excess the fragment 124M (Patterson, 1938). The male-like creature reported in the present experiments carried 124M, a y^2 *v f* X-chromosome, and either two or three sets of autosomes.

Hypertriploid Females

Females bearing fragments in excess of the 3X3A complement were produced regularly by the cross of hyperploid males x triploid females. In this way m5L + 3X3A; w13L + 17R + 2X3A; 8L + w13R + 2X3A; and 1L + 4R + 2X3A were obtained (Table 3). By nondisjunction, single females of the composition 4R B, 1R B^s, and 124M plus 3X3A respectively in the cross of 4*B*, 1B^s, and 124 translocation base stock males x y^2 *v f* triploids (Table 1). In cases where *Bar* was present, it was as wide as in triploids. Breeding tests of certain hypertriploid females are presented in Table 10. As Table 10 shows, females with m5L or the section of the

Table 10.

Hypertriploid Females	Number Individuals Tested for Fertility	Number dead at end 3 days	Number dead but fertile	Number alive and fertile	Per cent alive after 3 days	Per cent fertile
m5L + 3X3A	330	6	1	324	98.2	100.0
w13L + 17R + 2X3A	296	3	2	293	99.0	100.0

X-chromosome in common with w13L and 17R in excess of 3X3A are highly fertile. They are highly viable also, according to Table 3, though of course their wild type phenotype gives them the advantage over sibs carrying recessive genes.

Discussion

Dobzhansky (1930a) found genes in 3 capable of shifting the mean sex type of intersexes when present in only one dose. It is therefore important to determine how homogeneous the X-chromosomes of the different translocation stocks used are with respect to genes capable of modifying intersex sex type. In all crosses of translocation base stock males x y^2 *v f* triploids, two kinds of control 2X3A intersexes occurred: one received two y^2 *v f* X-chromosomes from the mother and a Y from the father; the other received the translocated X from the father and a y^2 *v f* chromosome from the mother. The Y-chromosome has been shown to have no effect upon the intersex sex type (Dobzhansky and Schultz,

1934). Intersexes receive two of each autosome from the triploid mother and one of each from the father. These two kinds of control intersexes, however, have equal chances of receiving a particular autosome of a pair from the parent male and of receiving any two of the three 2 and 3 chromosomes respectively from the triploid mother. Therefore, if their sex types differ in frequency, the translocated X-chromosome must contain modifiers which affect the intersex sex type when present in only one dose. Although the y^2 *v f* triploid stock has not been pure lined, it has been inbred for about three years. Translocation stocks arose from X-raying wild type flies (not a pure line). Modifiers within the translocation have been preserved intact, unless *Bar* has been crossed in, by balancing with attached X females. X^2 (chi square) tests in Table 9 show 5 of 8 cases homogeneous; one borderline case; and two heterogeneous. (y^2 8 x y^2 *v f* triploids gives the two control series heterogeneous in series 2 and a borderline case in series 1. We have therefore counted this translocated chromosome (8 y^2) as being different with respect to modifiers of the sex type of the control intersex from the y^2 *v f* chromosome). One concludes from these comparisons that there are not many modifiers capable of affecting the sex type of intersexes in the heterozygous state which are different in the various translocation stocks and the y^2 *v f* chromosomes of the triploid stock. There can thus be no doubt that shifts toward females in duplication intersexes and hypotriploids are due to the presence of certain X-chromosome genes in three rather than two doses.

In the foregoing discussion the phenotypic appearances of duplication intersexes and hypotriploids developing at 23° C. have been considered. It has long been known that fall in temperature shifts the mean sex type of ordinary 2X3A *Drosophila* intersexes toward maleness. Thus Dobzhansky found that at 20° C. most 2X3A intersexes were of class III; at 24° C., class IV; at 28° C., class V. Dobzhansky (1930a) and Dobzhansky and Bridges (1928) showed that embryologically nearly all the male organs begin differentiation in intersexes first. Then a turning point occurs at which male development ceases and that of female organs (with one exception) begins. Thus only rarely do external male and female genitalia complete development in the same individual. Hence the arbitrary classification into sex types is clearcut. High temperature causes an early turning point with production of predominantly female type intersexes. These results and interpretation follow those of Goldschmidt on the effect of temperature on intersexes in *Lymantria*. Goldschmidt emphasizes that the time of the turning point is not precisely the same in intersexes of the same genotypic constitution. Hence intersexes show different gradations of maleness and femaleness. Even in highly inbred lines, Dobzhansky found the sex type to range from 1.743 to 1.009 in intersexes of the triploid line selected 23 generations for malelike intersexes and from 2.635 to 4.350 in the line selected 23 generations for femalelike intersexes. (Dobzhansky, 1930a.)

Owing to the instability of the turning point in intersexes and its dependence upon temperature the technique has been criticized of adding

fragments of the X-chromosome to the 2X3A intersex complement in measuring the female potency of these fragments. The results of the present author's low temperature experiments upon the hypotriploids 17R + 2X3A, m5R + 2X3A, and 124L + R + 2X3A, 9R + 2X3A and w13R + 2X3A are fully to be expected in the light of previous work. They justify the use of triploid aneuploidy experiments in studying sex balance. At 23° C., 17R + 2X3A individuals appeared like hypotriploids. They were female in body size and shape. Only two individuals showed rudimentary sex combs (one or two hairs). These aneuploids were from 19 to 35 per cent fertile. A slight nick in the wings was seldom observed. At 18° C., 17R + 2X3A aneuploids looked like duplication intersexes. The ovaries were greatly reduced in size; wings were warped and deeply nicked; body size was stunted; and sex combs were present and of medium size (up to 4 or 5 prongs). On the other hand, m5R + 2X3A, 124L + R + 2X3A and 9R + 2X3A hypotriploids appeared the same whether they developed at 23° C. or at 18° C. Evidently the turning point has been stabilized in these aneuploids with the longer fragments. It occurs too early for any male character to appear even though temperature is decreased.

No one of the eight short sections of the X-chromosome of *Drosophila* when added to the 2X3A intersex complement produces a marked shift in the direction of femaleness, much less a functional triploid female. Therefore a single primary sex gene cannot exist which acts regardless of the dosage of any lesser modifying genes present in the X-chromosome. This conclusion was reached by Patterson, Stone, and Bedichek (1937) and Patterson (1938) from a study of diploid aneuploidy of the X-chromosome with the reservation that such a gene might conceivably be located in a very short section in the *g–pl* region of the X-chromosome (bands 13A2 to 13A6 of Bridges's 1935 salivary gland map). Hyperploid males and hypoploid females carrying this region in double and single dose respectively have consistently failed to survive. The region in question is included between translocations 8 and 4. Intersexes of the composition 4L + 8R + 1X3A appear phenotypically like their control 2X3A sibs. The sex types of this duplication intersex range from I to V; i.e., they are all sex combed individuals.

The most striking feature of sex balance in *Drosophila* brought out by the present studies is the graded effect in the female direction of adding longer and longer adjacent pieces of the X-chromosome to the intersex chromosome constitution, 2X3A, at 23° C. For example, duplication intersexes of the composition m5L + 2X3A range from male sex type II to a few extreme female type individuals, type VI. 9L + m5R + 1X3A duplication intersexes appear like typical intersexes and occur as sex types III, IV, and V. The fragment 9L consists of m5L plus the region between m5 and 9. All 9L + 2X3A duplication intersexes belong either to type V or type VI: their genitalia, anal plates, and abdominal coloration have always been of the female type, and sex combs may be present (V) or absent (VI). There has thus been a qualitative shift toward

femaleness in the duplication intersex bearing the 9L fragment as compared with duplication intersexes containing either of the two component sections in triplicate.

Only one 17L + 9R + 1X3A duplication intersex has been recorded. It belonged to type IV and looked like a typical control intersex of that class. Nevertheless, 17L + 2X3A individuals never have sex combs and one bred as a female. The 17L fragment consists of 9L + the section between 9 and 17. Obviously 17L plus 2X3A accomplishes the shift to femaleness although it differs from 9L only by the 9–17 section.

Considering the right-hand end of the chromosome, the progression toward femaleness with longer and longer adjacent pieces is even more evident. 1R + 2X3A duplication intersexes occur as types III–VI. With the exception of three type VI individuals, these duplication intersexes possess large sex combs but appear more female like in body shape than their control sibs. 4R + 2X3A flies look still more like females. Sex combs are reduced sometimes more than is ordinarily the case among control intersexes. An egg was found almost completely within the oviduct of one individual. The 4R fragment and 1R fragment differ by a short section which may be studied alone in the duplication intersex of the composition 1L + 4R + 1X3A. The sex type of these individuals varies from I to V; i.e., they look very much like control intersexes. 8R + 2X3A duplication intersexes occur only as types V and VI, the latter type being the most numerous. Sex combs are definitely reduced in size below the normal range of variation which characterizes the control intersexes. One type V 8R + 2X3A duplication intersex laid two eggs, but none of the 185 individuals tested have proven fertile. The 8R fragment and the 4R fragment differ by the section studied alone in 4L + 8R + 1X3A duplication intersexes. These individuals appear like typical 2X3A intersexes free from the duplication. The sex types of this short duplication intersex 4L + 8R + 1X3A vary from I to V. w13R + 2X3A hypotripolids have been found as types V and VI; i.e., with and without sex combs. Beyond the possible presence of rudimentary sex combs, these flies appear completely female. Five w13R + 2X3A hypotriploids including one with sex comb rudiments produced progeny. The w13R fragment and 8R fragment differ by the short section present in triplicate in 8L + w13R + 1X3A duplication intersexes. In this case, the sex types vary from III–V, although only a low count was made. 17R + 2X3A hypotriploids are fairly fertile. Among those developing at 23° C., only two of these 17R + 2X3A hypotriploids have shown rudiments of sex combs. As in the preceding cases, the 17R fragment differs from w13R by a short section studied in the duplication intersexes of the composition w13L + 17R + 1X3A. The sex types of these short duplication intersexes range from I–V. However the mean sex type of these short duplication intersexes was significantly higher than the mean sex type of the control. In this case, but not in the preceding short duplication intersexes described, there was a sufficiently high count made to

justify a comparison between the mean sex type of the short duplication intersex and that of its control intersex sibs.

Thus in general, adjacent segments of the X-chromosome which have been used in these experiments show an additive potency in shifting the intersex toward femaleness. There is, however, a slight difference in regional strength. Sex comb rudiments persist longer in individuals with 2X3A and a right-hand fragment (R) than with a left-hand fragment (L). This fact may be seen by comparing w13L with w13R, and 17L with 17R + 2X3A, respectively. 9L is the longest fragment to the left of 17 which when added to 2X3A gives sex combed individuals. The section between m5 and w13 is the longest section to the left of w13 which will allow the formation of sex combs when added to 2X3A. Nevertheless, weak fertility occurs in w13R + 2X3A hypotriploids before the disappearance of sex combs. Furthermore, 17R + 2X3A hypotriploids in which, though developing at 23° C., sex comb rudiments have twice been observed are fairly fertile. Fertility in left-hand fragments begins with 17L + 2X3A hypotriploids. Thus, in as much as sex comb traces remain longer in flies with 2X3A plus a right-hand fragment and fertility begins earlier in hypotriploids with left-hand fragments, we can say that in the part of the X-chromosome to the left of 17, the female sex genes are stronger than in the portion of the X-chromosome to the right of 17. Of course the degree of fertility in 17R + 2X3A is vastly higher than in 17L + 2X3A.

Patterson et al. (1937) stated that it is possible that a primary female sex factor might exist in the X-chromosome the dominance strength of which was altered when the proper ratio of other genes in the X-chromosome (modifying genes) was upset. Such a primary gene might not be detected by diploid aneuploidy experiments. 1X2A plus a very short or a very long section of the X-chromosome usually live but medium-sized sections plus 1X2A die. Hence we observe only hyperploid males or hypoploid females. Neither of these forms shows the characteristics of the other sex. If a primary female sex factor of this kind were present; i.e., a gene with dominance strength subject to the dosage of its modifying genes; then we should expect that it would be necessary for the main gene to attain a certain dosage before alteration of the remainder of the X-chromosome genes would affect sex differentiation. In the intersex the main gene would be represented twice. This dosage might be sufficient so that adding extra fragments to the 2X3A complement would shift differentiation toward femaleness regardless of whether or not the main gene were included in the fragment. However, in adding medium-sized pieces we might anticipate that the fragment with the sex gene would be distinctly more effective than others without it. As we have seen, this expectation is not realized in the present author's experiments. We should probably not expect either the L(left) or R(right) fragment of a single translocation to produce the complete shift to femaleness when added

to 2X3A as in the case of 17L and 17R; w13L and w13R, respectively[5] (also perhaps m5L + 8R + 2X3A). The rather uniform female strength in sections of equal cytological length and the additive effects in combinations of the adjacent sections studied agree with Dobzhansky and Schultz's theory that there is no main gene a great deal stronger than any other in the X-chromosome.

In the two hypointersexes studied, 124 L + R + 1X3A and m5R + 1X3A, sexual differentiation was shifted almost completely in the direction of maleness. These sterile individuals showed no female characters and very few signs of intersexuality. This extreme shift toward maleness in the hypointersexes studied confirms the finding of Dobzhansky and Schultz that the sex type of hypointersexes bearing a *y–sc* deficiency X-chromosome plus a complete X and three autosomes was strongly shifted in the male direction. (Dobzhansky and Schultz, 1934.) Presumably subtraction of female sex genes from the intersex complement is responsible for the shift toward maleness in these three hypointersex cases studied.

However, the fact that hypointersexes appear to show a more violent shift toward maleness than do duplication intersexes, on the other hand toward femaleness, brings us to some generalizations regarding subtraction vs. addition of short sections of a chromosome from balanced chromosome complements (i.e., those containing whole chromosomes). Subtraction of a given section of a chromosome seems to have a stronger effect than addition of that section to the chromosome complement in question. Thus hypoploid females (1X + fragment of an X +2A) survive less well, if at all, are less fertile, and show a more severe phaenotypic change than hyperploid females (2X + fragment of an X + 2A). (Patterson et al., 1937.) This same relation holds in the case of 2X females hypoploid or hyperploid for a section of one of the autosomes (Patterson, Brown and Stone, 1940). This rule holds in a milder way for hypotriploids in comparison with hypertriploids, in all the cases studied. Apparently hypointersexes which represent subtractions from the intersex complement (2X3A) are more affected in sex differentiation among other things, than are duplication intersexes, which represent additions of short sections to the intersex complement. Nevertheless the degree of change among various kinds of aneuploids with respect to their balanced forms is different. Thus the degree to which hypotriploids and hypertriploids differ from triploids in viability, and sex differentiation is less than the degree to which the viability and sex differentiation of hypointersexes and duplication intersexes differ from control intersexes. The alteration of proportionships of the total chromosome set is greater when a short section of the X-chromosome is added to or subtracted from the set 2X3A than from the set 3X3A. These facts should make us cautious about fixing the exact

[5]Since the fertility of 17L + 2X3A and w13L + 2X3A is based on one and two cases, respectively, it is realized that caution should be exercised in accepting this evidence. The author, however, has no doubt that her identification of these aneuploids was correct.

quantitative sex potency of any region of the X-chromosome when considering evidence from more than one kind of aneuploid class. The strength of a particular section of the X-chromosome should be compared with strengths of other sections in aneuploids of the same general composition. For example, the potency of m5L in duplication intersexes (m5L + 2X3A) should not be compared with the potency of 124M in the hypointersex which has this section represented only once (124L + R + 1X3A). Rather the potency of m5L in the duplication intersex, m5L + 2X3A, should be compared with the potency of 124M in the duplication intersex, 124M + 2X3A.

Even if the strengths of different X-chromosome sections are estimated from a consideration of similar aneuploid types, it appears that the shift in sex type does not furnish a strictly quantitative measurement of the female potency of the different short regions of the X-chromosome. For example, Dobzhansky and Schultz find the difference[6] between the mean sex type of duplication intersex 112 and control sibs to be 4.15 ± 0.06 minus 2.59 ± 0.08 or 1.56 ± 0.09; between duplication 107 intersexes and the control, 4.43 ± 0.04 minus 2.43 ± 0.06 or 2.00 ± 0.07; between duplication 118 intersexes and control, 4.53 ± 0.05 minus 1.97 ± 0.07 or 0.56 ± 0.09; between duplication 134 duplication intersexes and the control, 4.70 ± 0.07 minus 2.50 ± 0.05 or 2.20 ± 0.09; between duplication 136 intersexes and their control, 5.00 ± 0.09 minus 1.62 ± 0.07 or 3.38 ± 0.09. These duplications are arranged in ascending order of their length. The longest, 136, contains from y^+ through pn^+ plus bb^+ and a little inert region. The other duplications contain shorter fragments from the left end of the X plus small amounts of inert region. In the present author's experiments, the difference between the mean sex type of a duplication intersex carrying the left end of the X-chromosome including the locus of *white;* i.e., m5L, and the mean sex type of its control sibs is 4.69 ± 0.05 minus 3.49 ± 0.04 or only 1.20 ± 0.07[7]. In other words the mean sex type of duplication intersexes carrying m5L is raised significantly above that of the control but only as much as or less so than the shortest left-hand duplication intersex studied by Dobzhansky and Schultz. The discrepancy might be due to subjective differences in classification of the sex types on the part of the investigators. However, classification of the sex combless type VI must be fairly consistent. The ratio of the numbers of individuals of type VI to the total number of duplication intersexes counted is for duplication intersex 112, 107, 118, 134, and 136; 0.0618 ± 0.006, 0.0395 ± 0.013, 0.0739 ± 0.018, 0.1146 ± 0.032, 0.2373 ± 0.055, respectively. The ratio of type VI's to total duplication intersexes carrying m5L is only 0.0387 ± 0.017, i.e., about the radio found for duplication 107, carrying the loci of y^+ sc^+ svr^+ and a little inert region. Therefore we should be careful in deducing the quantitative strength of a particular short fragment from the small shift in sex type of the duplication intersex

[6]The errors here given are standard deviations.

[7]The errors here given are standard deviations.

that it produces; i.e., the extent of this shift may be different in different experiments owing presumably to a different genetic milieu or different environmental conditions in the different experiments.

Another method of attack upon the problem of sex determination consists of studying mutations which affect sex differentiation. Mutants such as *sex combless* in the X, degenerate (black seminal receptacles) in the 2, and *rotated penis* in the 3 chromosome have been found from time to time by various Drosophila investigators. The X-chromosome has been most thoroughly studied though the autosomes are larger and consequently have more potentially mutating genes. As a result the actual numbers of these genes per chromosome is biased, and we cannot tell whether more have occurred in any one chromosome in relation to its length than in any other chromosome.

Berg (1937) has undertaken a systematic production of sterility and lethal mutations in the X and in the autosomes. She found more sterility mutations per cytological unit arising under the influence of X-radiation in the X-chromosome than in either of the autosomes. Lethal mutations on the contrary occurred more often per cytological unit in the II and III than in the X. More semilethals occurred in the X than in the autosomes.[8] To explain these facts Berg suggested that sterility and lethality are extreme members of allelomorphic series of sex and viability genes respectively. On this hypothesis she explains the scarcity of lethal mutations in the X-chromosome as being due to a redifferentiation of viability genes into sex genes. The commonness of semilethal mutations in the X supports this view according to Berg. A rough parallel exists between the mutability of sterility and lethal mutations in X-chromosome and autosomes, studied by Berg, and the effect on hyperdiploid fertility and viability, according to studies by Patterson, Stone, and Bedichek (1937) on X-hyperdiploid females; Patterson, Brown and Stone (1940) on 2 and 3 hyperdiploid males and females; and Burdette (1938) on X and autosomal reduplication females. These conclusions, although suggestive, do not warrant our considering the fertility effects observed in aneuploid females as being due to upsets in sex genes.

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[8]The author states that "the impossibility of distinguishing by the methods used, lethal gene mutations from lethals due to loss of genes does not by any means decrease the value of the experiment."

SUMMARY

Males from eight X-4 translocation stocks were crossed to triploids with recessive markers, producing (1) sterile intersexual "duplication intersexes" with a short fragment of the X plus two complete X's and three autosomes, and (2) weakly fertile hypotriploid females with longer X-fragments plus 2X3A. Duplication intersexes bearing interior regions of the X-chromosome in triplicate were studied by combining right- and left hand fragments from two different translocations. Of eight duplicationintersexes with very short sections, covering successively the entire X-chromosome, in triplicate; none showed a marked shift in the female direction. Therefore, a single primary sex gene cannot exist, capable of producing a functional female when represented three times with 3A regardless of the dosage of the remainder of the X-chromosome. There was a graded shift toward femaleness in the phenotypic appearance of individuals of the composition X-fragment plus 2X3A with increasingly longer fragments. Individuals were hypotriploid (weakly functional as females) with either right or left hand section of two translocations with *t-lz* (17) *and lz-v* (w13) breaks. Furthermore, fertility of certain aneuploids of composition X-fragment plus 2X3A begins before the last trace of intersexuality vanishes, since some hypotriploids with the right hand fragment of the *t-lz* and *lz-v* breaks possess very rudimentary sex combs. For this reason and the fact that shorter sections from the left than from the right hand region of the X-chromosome plus 2X3A result in weakly functional females, we may conclude that the portion of the X to the left of the *t-lz* break seems a little more female potent in relation to its cytological length than the portion to the right of this break. A drop in temperature from 23° C. to 18° C. produces intersexuality in those individuals carrying the right hand fragment of the *t-lz* break but does not alter the female appearance and function of hypotriploids bearing longer fragments. These results, which confirm the work of Dobzhansky and Schultz, make more plausible the multiple sex gene theory of Bridges.

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Table 1.

Translocation Males Mated to *y²vf* (or *y² car*) Triploids. Composition of X-chromosome Indicated

		2 A Progeny										3 A Progeny																															
												L/y²vf/y²vf duplication intersex or hypo-triploid ♀					R/y²vf/y²vf duplication intersex or hypo-triploid ♀					y²vf/y²vf control intersex					L + R/y²vf control intersex																	
P₁ T ♂	Experiment Number	y²vf/ y²vf diploid ♀	L + R/ y²vf diploid ♀	L/y²vf/ y²vf hyper-diploid ♀	R/y²vf/ y²vf hyper-diploid ♀	L + R/ y²vf/ y²vf super-female	y²vf diploid ♂	L/y²vf hyper-ploid ♂	R/y²vf hyper-ploid ♂	L + R nondis-junction ♂	L + R/ y²vf/ y²vf triploid ♀	II	III	IV	V	VI	II	III	IV	V	VI	I	II	III	IV	V	I	II	III	IV	V	y²vf super-male	L or R/ y²vf hypo-intersex		L or R/ y²vf/ y²vf/ y²vf hyper-triploid
m5	1D	74	51	15	1	0	50	6	0	0	17	0	0	1	0	0	0	0	0	0	*12*	0	0	3	4	1	0	3	12	7	3	0	R 1		0
m5	2L	177	93	52	5	4	107	9	0	1	73	0	1	1	0	0	0	0	0	0	*98*	4	7	13	16	4	7	16	30	42	2	4	0		0
9	1D	136	47	79	0	0	59	0	0	0	29	0	0	0	0	0	0	0	0	0	*26*	0	4	4	5	1	12	48	6	7	0	2	0		0
9	2L	95	45	61	0	0	70	2(?)	0	0	53	0	0	0	5	2	0	0	0	0	*35*	4	4	8	10	0	12	16	15	7	0	6	0		0
9B	3L	168	95	96	3	0	122	0	0	0	82	0	0	0	1	7	0	0	0	0	*69*	10	16	17	39	2	17	49	40	33	3	13	0		0
17	1L	137	122	59	77	3	106	0	0	1	79	0	0	0	0	*4*	0	0	0	*2*	*33*	3	9	14	20	2	8	20	25	51	10	4	0		0
17B	2D	258	100	140	30	0	168	0	0	0	116	0	0	0	0	*12*	0	0	0	0	*78*	0	4	26	10	7	2	10	45	69	27	1	0		0
w13B	1D	489	366	222	212	0	284	1	0	0	305	0	0	0	0	*55*	0	0	0	*20*	*45*	2	16	56	81	7	10	68	166	152	19	9	0		0
8y²	1A	351	175	139	124	0	199	0	0	0	138	0	0	0	0	*18*	0	0	0	15	51	5	11	11	29	3	6	25	37	96	38	9	0		0
8y²	2D	1458	736	462	783	0	667	0	0	9	535	0	0	0	0	*91*	0	0	3	43	153	6	52	68	141	33	14	125	130	407	211	42	0		0
8y²	3L	122	42	23	47	1	56	0	0	0	49	0	0	0	0	*7*	0	0	1	2	18	1	2	6	17	1	1	5	7	40	17	4	0		0
4B	1L	176	104	11	188	0	73	0	3	0	64	0	0	0	0	*1*	0	0	5	13	1	1	10	26	7	0	6	12	54	20	2	8	0	R 1	
1B⁸	1D	400	235	6	501	1	153	0	117	0	122	0	0	0	0	*6*	2	3	7	2	0	1	21	44	5	0	14	56	96	22	0	4	0	R 1	
1B⁸	2L	145	122	5	229	10	114	0	80	0	79	0	0	0	0	*5*	0	6	12	10	3	3	5	11	15	1	12	11	24	39	6	6	0		0
13	1WH	96	18	2	130	0	24	0	24	0	18	0	0	0	0	*7*	0	0	1	0	1	0	1	0	3	0	3	6	7	25	7	1	0		0
			L+M+R	M	L+R					L+M+R		M					L+R										L+M+R						L+R	M	M
124	1A	367	173	236	82	12	151	0	0	0	142	2	3	2	0	0	0	0	0	0	*128*	2	11	18	25	7	17	41	75	68	8	3	2	0	1
124	2D	767	462	519	165	8	435	0	0	3	324	2	3	4	3	0	0	0	0	0	*290*	6	27	58	97	19	24	72	117	157	35	15	3	1?	0
124	3L	92	109	50	16	1	102	0	0	2	51	0	0	1	1	0	0	0	0	0	*44*	2	3	9	24	1	6	5	14	32	0	4	3	0	0

TABLE 2.

Translocation Males Crossed to Hypotriploid Females. Most Probable X-chromosome Composition Indicated

	2 A Progeny									3 A Progeny														
											L + R/y^2vf desired duplication intersex			y^2vf/y^2vf control intersex				L + R Bar/ y^2vf control intersex						
P_1 T ♂	P_1 hypotriploid ♀	y^2vf/y^2vf diploid ♀	L + R Bar/ y^2vf diploid ♀	L/y^2vf/ y^2vf hyper-diploid ♀	R Bar/ y^2vf/ y^2vf hyper-diploid ♀	L + R/y^2vf hyperdiploid overlap ♀	L + R Bar/ y^2vf/y^2vf super-female	y^2vf diploid ♂	L/y^2vf hyper-ploid ♂ or L + R hyper-ploid overlap ♂	L + R Bar/ y^2vf/y^2vf triploid ♀	III	IV	V	I	II	III	IV	I	II	III	IV	R Bar/ y^2vf/ y^2vf hypo-triploid or duplication intersex	y^2vf super-male	R/y^2vf hypo-intersex	R/L + R Bar/ y^2vf hypo-triploid
9L + 9R Bar	m5R/y^2vf/ y^2vf	14 + $1y^2v$	6	4	0	30 + 1v + 2f	1	5 + $2y^2$ + $2y^2v$ + 1f	L/y^2vf 1	7	2	4	1	1	2	0	0	6	20	8	2	1	9	1	0
17L + 17R Bar	m5R/y^2vf/ y^2vf	7 + $3y^2$ + $2y^2v$	10	7	1	19 + 2f	0	4	0	5	0	0	3	1 y^2v	0	1	0	4	4	2	13	2	2	0	0
w13L + w13R Bar	m5R/y^2vf/ y^2vf	15 + $4y^2v$ + $6y^2$	14	9	6	63 + 4f + 1v	0	13 + $2y^2v$ + $1y^2$	0	11	0	0	1	1 y^2v	2 + $1y^2$ v	0	$1y^2$ + $1y_2v$	7	12	12	11	V 2 VI 2	10	0	2(?)
17L + 17R Bar	9R/y^2vf/y^2vf	13 + $3y^2$ + $7y^2v$	18	6	0	46	0	12 + $1y^2$ + $1y^2v$	1	4	0	1	0	0	0	0	0	27	9	4	1	2 weak Bar	4	0	0

TABLE 3

Progeny of Hyperploid Males Crossed to y^2vf Triploids

2 A Progeny

P_1 ♂	Series Number	Diploid ♀		Hyperdiploid ♀				Superfemale		Diploid ♂	Nondisjunction ♂			Hyperploid ♂	
		y^2vf/y^2vf	y^2vf/y^2	$L+R/y^2vf$	$L/y^2vf/y^2vf$	$R/y^2vf/y^2vf$	$L/y^2/y^2vf$	$L+R/y^2vf/y^2vf$	$y^2/y^2vf/y^2vf$	y^2vf	y^2	Hyperploids L/y^2	Hyperploids $L+R$	R/y^2vf	L/y^2vf
m5L+y^2	1A	815	500	----	843	----	445	—	4	473	2	2	----	----	200
w13L+17R	1A	1041	----	457	589	147	----	1	----	523	----	----	1	0	0
8L+w13R	1D	17	----	6	7	16	----	0	----	9	----	----	0	0	0
8L+w13R	2L	9	----	5	2	1	----	0	----	0	----	----	0	0	0
1L+4R	2L	55	----	23	0	55	----	0	----	13	----	----	0	7	0

3 A Progeny

P_1 ♂	Series Number	Triploid ♀	Hypertriploid ♀		Hypotriploid ♀ or Duplication Intersex														Control Intersex												Supermale
		$y^2/y^2vf/y^2vf$	$L/y^2/y^2vf/y^2vf$	$L+R/y^2vf/y^2vf$	$L/y^2vf/y^2vf$							$L/y^2/y^2vf$						$R/y^2vf/y^2vf$		$L+R/y^2vf$					y^2vf/y^2vf					y^2/y^2vf					y^2vf
					I	II	III	IV	V	VI	I	II	III	IV	V	VI	V	VI	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	
m5L+y^2	1A	178	330	----	0	0	2	5	3	1	0	1	2	38	83	5	----	----	----	----	----	----	----	12	23	40	81	20	48	87	100	300	102	13
w13L+17R	1A	----	----	296	0	0	0	0	0	39*	----	----	----	----	----	----	0	207	19	63	131	244	130	16	25	37	56	17	----	----	----	----	----	8
8L+w13R	1D	----	----	5	0	0	0	0	0	2	----	----	----	----	----	----	0	1	0	0	1	6	3	0	0	1	0	0	----	----	----	----	----	1
8L+w13R	2L	----	----	1	0	0	0	0	0	0	----	----	----	----	----	----	0	3	0	0	1	2	3	0	1	0	0	0	----	----	----	----	----	0
1L+4R	2L	----	----	21	0	0	0	0	0	1	----	----	----	----	----	----	2	2	1	1	3	8	6	0	3	5	8	0	----	----	----	----	----	3

*This class is too small. The writer first recognized it when there were already 112 of the corresponding 17R hypotriploids listed.

TABLE 4.

Production of 4L + 8R + 1X3A Duplication Intersexes from the Cross of 4L *v*, 4R *B* Males by 8Ly^2, 8R/y^2vf/y^2vf *Triploid Females*

2A Progeny

Phenotype	Supposed genotype	Series 1D	Series 2L
y^2vf ♀	y^2vf/y^2vf	19	9
y^2 ♀	$8y^2/y^2vf$	122	72
vB ♀	$4vB/y^2vf$	77	12
B ♀	$8y^2/4vB$	42	13
y^2 ♂	$8y^2$	12	9
y^2vf ♂	y^2vf	13	7
+ aneuploid ♀	$4Lv/y^2vf/8y^2$	0	4
y^2vB ♀	$4RB/y^2vf/y^2vf$	10	1
y^2B ♀	$4RB/y^2vf/8y^2$	126	57
v ♀	$4Lv + 8R/y^2vf$	83	54
y^2 wide B ♀	$8Ly^2 + 4RB/y^2vf/y^2vf$	0	6
v wide B ♀	$8R/y^2vf/4vB$	lumped with vB	34
y^2v ♀	$8R/y^2vf/y^2vf$	8	11
B aneuploid ♀	$8Ly^2/y^2vf/4vB$	8	11
y^2f aneuploid ♀	$8Ly^2/y^2vf/y^2vf$	3	2
vf aneuploid ♀	$4Lv/y^2vf/y^2vf$	4	3
vB super-female	$y^2vf/y^2vf/4vB$	1	0
v super-female	$y^2vf/y^2vf/4Lv + 8R$	4	0

3A Progeny

Phenotype	Supposed genotype	Intersex type	Series 1D	Series 2L
B triploid	$8y^2/y^2vf/4vB$		17	10
vB triploid	$4vB/y^2vf/y^2vf$		32	9
v wide B dupli-	$8R/y^2vf/4vB$	V	4	1
cation intersex		VI	0	1
B weak f hypo-triploid	$8Ly^2/y^2vf/4vB$		27	14
y^2f hypo-triploid	$8Ly^2/y^2vf/y^2vf$		0	1
vf hypo-triploid	$4Lv/y^2vf/y^2vf$		2	0
y^2vB duplication	$4RB/y^2vf/y^2vf$	V	1	0
intersex		VI	2	1
y^2B duplication	$4RB/y^2vf/8y^2$	IV	1	0
intersex		V	1	0
Desired aneu-	$4Lv + 8R/y^2vf$	I	0	1
ploid class:v		II	0	0
duplication in-		III	1	1
tersex		IV	5	4
		V	1	0
		VI	0	0
y^2B hypo-tri-	$8Ly^2 + 4RB/y^2vf/y^2vf$		2	4
ploid				
B control inter-	$4vB/8y^2$	I	1	0
sex		II	2	0
		III	1	0
		IV	3	5
		V	2	1
v B control	$4vB/y^2vf$	I	1	2
intersex		II	9	1
		III	19	7
		IV	30	25
		V	6	0
y^2 control inter-	$8y^2/y^2vf$	I	0	1
sex		II	1	0
		III	4	0
		IV	1	2
		V	1	0
y^2vf control	y^2vf/y^2vf	I	0	0
intersex		II	0	0
		III	3	1
		IV	3 + 1y^2v	0
		V	0	1
y^2vf super-male	y^2vf		1	2

TABLE 5

Description of Hypotriploids Developing at 23° C.

Fragment in excess of 2X	Length in mm. of sal. gl. drawing	Table No.	Body size	Wing texture (sparseness of hairs)	Eye facets	Sex combs	Reproductive tract	Other abnormalities
m5R	99	1	size of a triploid	as coarse as wing of triploid	as large as those of a triploid	no traces	normal	sometimes post scutellar bristles curved medially
9R	86	1	size of a diploid female	as coarse as wing of triploid	as large as those of a triploid	no traces	normal	post scutellars curved medially; sometimes very weak forked
17L	44	1	size of a control intersex	usually intermediate between diploid and triploid; but variable	very large in proportion to body size	no traces	ovaries in a very immature condition; divided into irregular ovarioles; no yolk formation; rest of tract normal; large amount tracheal tissue present	
17R	71	1 and 3	size of a diploid female	slightly less course than triploid wing	large and distinct but not so large as those of 17L in proportion to body size	rarely, sex comb rudiment present (type V)	normal	inner wing margin very occasionally clipped; post vertical bristles sometimes missing
w13L	51	1 and 3	size of a control intersex	usually slightly more coarse than diploid wing but variable	very large in proportion to body size	no traces		wings held a little apart; wing veins more perfectly formed than in the hyperdiploid; legs sometimes misshapen
w13R	64	1	size of a diploid female	slightly less coarse than triploid wings	intermediate between triploid and diploid eye facets	sometimes rudiments of sex combs on one or both of forelegs (type V)		wing margin occasionally clipped longitudinally so as to give a narrow wing; post vertical bristles absent sometimes
8L	59	1	intermediate between control intersex and diploid female	almost as coarse as triploid wings	intermediate	no traces	ovaries reduced to about 1/8 normal size; no mature eggs but ovarioles normally arranged; early yolk segmentation stage in largest oocyte; rest of tract normal	forked exaggerated; legs misshapen sometimes
4L	79	1	size of control intersex	slightly more coarse than diploid wing	intermediate	no traces		
1L	88	1	size of control intersex	intermediate between wing of triploid and diploid female	intermediate	no traces	ovaries about one fourth normal size; one ovary with 3 oocytes in last stage of yolk formation; filaments not formed; rest of tract normal	body rather squat; one specimen had deep gash down middle of thorax
13L	102	1	size of a diploid female	intermediate between triploid and diploid wing	facets large	no traces	ovaries full of mature eggs nearly normal size; all ducts normal	
124L + R	97	1	size of a diploid female	as coarse as that of a triploid	facets large	no traces	ovaries slightly smaller than normal	

TABLE 6.

Progeny of Translocation Males Mated to *y²vf* Triploids, Developing at 18° C.

		2 A Progeny							3 A Progeny																
P_1 T male	Series Number	y^2vf/y^2vf diploid female	L + R/y^2vf diploid female	L/y^2vf/y^2vf hyper-diploid female	R/y^2vf/y^2vf hyper-diploid female	L + R/y^2vf/y^2vf super-female	y^2vf diploid male	L + R diploid n. d. male	L + R/y^2vf/y^2vf triploid	L/y^2vf/y^2vf hypo-triploid or duplication intersex	R/y^2vf/y^2vf hypotriploid or duplication intersex			y^2vf^2/y^2vf control intersex					L + R/y^2vf control intersex					y^2vf super-male	R/y^2vf hypo-intersex
											IV	V	VI	I	II	III	IV	V	I	II	III	IV	V		
17	3L	111	73	37	40	1	54	1	42	1	2	8	8	1	9	17	2	0	1	19	54	7	1	4	0
17	4L	19	22	4	5	0	21	1	11	0	0	4	3	0	1	7	0	0	0	3	10	2	0	2	0
17	5WH	45	52	17	17	0	52	1	35	0	0	11	13	3	8	8	0	0	1	24	31	1	0	2	0
17B	6WH	19	8	5	0	0	6	0	3	0	1	1	0	1	2	2	0	0	0	5	6	0	0	0	0
m5	3L	44	28	12	3	0	32	L/y^2vf 3	23	0	0	0	25	0	0	4	0	1	0	0	16	7	1	5	5
9	4WH	25	23	14	0	0	17	0	13	IV VI 1 1	0	0	12	1	1	1	0	0	6	4	3	0	0	1	0
w13B	2WH	40	46	7	9	0	39	0	44	3	0	3	2	0	4	4	0	0	1	15	11	0	0	3	0
			L+M+R	M	L+R	L+M+R				M	L	+	R							L+	M+	R			
124	4L	49	26	22	12	4	18	0	16	IV 1	0	0	9	0	0	2	5	0	1	2	16	5	0	5	0

TABLE 7

Fertility Tests of Hypotriploid Females

Composition of Hypotriploid Female	Number Individuals Tested	Number Dead After 3 Days	Number Dead But Fertile	Number Alive and Fertile	Mortality During First 3 Days	Per Cent Fertility	Mean Number Progeny Per Vial	Number Vials Counted for Mean Number Per Vial
m5R + 2X3A	29	5	1	18	20.7	72.0	7.4	12
9R + 2X3A	24	8	1	9	37.5	52.9	5.8	7
17L + 2X3A	13	6	0	1				
17R + 2X3A; Type V	2	0	0	1				
17R + 2X3A; Type VI	75	7	0	13	9.0	19.1		
17R Bar + 2X3A; Type VI	78	17	1	22	23.1	35.5		
W13L + 2X3A	72	38	0	2	52.7	5.8		
W13R Bar + 2X3A; Type V	21	8	0	1	38.1	7.7		
W13R Bar + 2X3A; Type VI	48	9	1	4	20.8	10.0	1.8	5
8L + 2X3A	91	39	1	9	42.9	17.0	3.1	8
4L + 2X3A	1	0	0	0				
1L + 2X3A	2	1	0	1				
13L + 2X3A	1	0	0	1				
124L + R + 2X3A	180	24	18	153	23.3	98.2		

TABLE 8

Homogeneity Tests of Sex Types Among Control Intersexes (2X3A)

Male parent	Composition of x-chromosome	Experiment series number	Sex types of intersex					Total	Number degrees freedom	Chi square	Probability of homogeneity
			I	II	III	IV	V				
m5	y^2vf/y^2vf	2	4	7	13	16	4	44	4	4.030	$0.5<P<0.3$
	$m5/y^2vf$	2	7	16	30	42	2	97			
17	y^2vf/y^2vf	1	3	9	14	20	2	48	4	1.831	$0.8<P<0.7$
	$17/y^2vf$	1	8	20	25	51	10	114			
17B	y^2vf/y^2vf	2	0	4	26	10	7	47	4	12.938	$0.02<P<0.01$
	$17B/y^2vf$	2	2	10	45	69	27	153			
w13B	y^2vf/y^2vf	1	2	16	56	81	7	162	4	10.236	$0.05<P<0.02$
	$w13B/y^2vf$	1	10	68	166	152	19	415			
$8y^2$	y^2vf/y^2vf	1	5	11	11	29	3	59	4	10.089	$0.05<P<0.02$
	$8y^2/y^2vf$	1	6	25	37	96	38	202			
$8y^2$	y^2vf/y^2vf	2	6	52	68	141	33	300	4	29.722	$P<0.01$
	$8y^2/y^2vf$	2	14	125	130	407	211	887			
4B	y^2vf/y^2vf	1	1	10	26	7	0	44	4	4.255	$0.5<P<0.3$
	$4B/y^2vf$	1	6	12	54	20	2	94			
$1B^s$	y^2vf/y^2vf	1	1	21	44	5	0	71	3	5.453	$0.2<P<0.1$
	$1B^s/y^2vf$	1	14	56	96	22	0	188			
$1B^s$	y^2vf/y^2vf	2	3	5	11	15	1	35	4	1.420	$0.9<P<0.8$
	$1B^s/y^2vf$	2	12	11	24	39	6	92			
$m5L/y_2$	y^2vf/y^2vf	1	12	23	40	81	20	176	4	6.108	$0.2<P<0.1$
	y^2/y^2vf	1	48	87	100	300	102	637			
124	y^2vf/y^2vf	1	2	11	18	25	7	63	4	1.341	$0.9<P<0.8$
	y^2vf/y^2vf	2	6	27	58	97	19	207			
$8y^2$	y^2vf/y^2vf	1	5	11	11	29	3	59	4	8.925	$0.1<P<0.05$
	y^2vf/y^2vf	2	6	52	68	141	33	300			
$8y^2$	$8y^2/y^2vf$	1	6	25	37	96	38	202	4	5.466	$0.5<P<0.3$
	$8y^2/y^2vf$	2	14	125	130	407	211	887			
124	$124/y^2vf$	1	17	41	75	68	8	209	4	9.660	$0.05<P<0.02$
	$124/y^2vf$	2	24	72	117	157	35	405			
124	y^2vf/y^2vf	1+2 pooled	8	38	76	122	26	270	4	20.900	$P<0.01$
	$124/y^2vf$	1	17	41	75	68	8	209			
124	y^2vf/y^2vf	1+2 pooled	8	38	76	122	26	270	4	6.130	$0.2<P<0.1$
	$124/y^2vf$	2	24	72	117	157	35	405			
9	$9/y^2vf$	1	12	48	6	7	0	73	3	16.119	$P<0.01$
	$9/y^2vf$	2	12	16	15	7	0	50			
$1B^s$	y^2vf/y^2vf and $1B^s/y^2vf$	1+1 pooled	15	77	140	27	0	259	4	83.600	$P<0.01$
	y^2vf/y^2vf and $1B^s/y^2vf$	2+2 pooled	15	16	35	54	7	127			

TABLE 9

Homogeneity Tests of Sex Types of Control and Duplication Intersexes

X-chromosome Composition (1) Duplication Intersex and (2) Control Intersex	Series Number	Sex Types						Total	Number Degrees Freedom	Chi. Square	Probability of Homogeneity	Mean Sex Type of Duplication Intersex $\bar{x}_2$	Mean Sex Type of Control $\bar{x}_1$	$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$	Number Degrees of Freedom	Probability that 2 Means Are Drawn from Same Population
		I	II	III	IV	V	VI									
m5L/y^2/y^2vf	1	0	1	2	38	83	5	129	5	221.9090	P<0.01	4.6900	3.4859	18.0795	940	P<0.01
pooled y^2/y^2vf and y^2vf/y^2vf	1; 1	60	110	140	381	122	0	813								
w13L + 17R/y^2vf	1	19	63	131	244	130	0	587	4	25.1299	P<0.01	3.6865	3.2185	4.4784	736	P<0.01
y^2vf/y^2vf	1	16	25	37	56	17	0	151								
124M/y^2vf/y^2vf	1 + 2	0	4	6	6	3	0	19	4	6.9462	0.2<P<0.1	3.4211	3.0431	1.5460	18	0.2<P<0.1
124/y^2vf	1	17	41	75	68	8	0	209								
124M/y^2vf/y^2vf	1 + 2	0	4	6	6	3	0	19	4	2.5534	0.7<P<0.5	3.4211	3.3363	0.3569	18	0.8<P<0.7
pooled y^2vf/y^2vf and 124/y^2vf	1 + 2; 2	32	110	193	279	61	0	675								
1RB^a/y^2vf/y^2vf	1 + 2	0	2	9	19	12	3	45	5	132.9000	P<0.01	4.1111	2.6911	9.4666	302	P<0.01
pooled y^2vf/y^2vf and y^2vf/1B^a	1; 1	15	77	140	27	0	0	259								

V. THE EFFECT OF ARTIFICALLY PRODUCED TETRAPLOID REGIONS OF THE CHROMOSOMES OF DROSOPHILA MELANOGASTER

WALTER J. BURDETTE[1]

The Department of Zoology, The University of Texas, Austin, Texas.

This work is part of a series of studies carried out in this laboratory to determine the effect of aneuploidy of various regions of the chromosomes of *D. melanogaster*. These experiments determine the effect of a section of a chromosome present in the tetraploid condition, with the remainder of the chromosome complement diploid. The variables measured were the viability, fertility and phenotype.

EXPERIMENTAL METHODS

The methods used to obtain duplications has been described elsewhere (Patterson, Stone and Bedichek 1937; and especially, Patterson, Stone and Brown 1940). Males and females were mated that had the duplication to be tested carried heterozygous with an inversion. By the use of suitable markers, the normal, heterozygous duplication and homozygous duplication classes could be sorted and checked for abnormalities in the F_1. As an additional precaution, all cases were checked cytologically on the salivary gland chromosome.

In the fertility tests one female was mated to three unrelated normal males (Stephenville, Texas, stock which showed 98 to 100 per cent fertile). Only those cases where the fly tested was alive three days after mating were considered in calculating fertility. The number of matings required to obtain one hundred matings tested for fertility is recorded in column three of Table 2. In both viability and fertility tests, the parents were removed at the end of the ninth day and the progeny counted on the sixteenth day.

Each base stock used was a translocation involving the fourth and some other chromosome. The frontispiece shows by number the position of the breaks in the X, 2 or 3, as the case might be. The symbols L and R used in Tables 1, 2 and 3 indicate the left and right hand fragment of the translocated chromosome, respectively. The segment in duplicate is defined by the numbers of the translocations used in the production of the duplication. For example, w13L/17R is a duplication of the sector between the points of breakage of translocation w13 and 17 (see Figure 1). The technical nomenclature of the translocations (e.g., T(1–4)A) is omitted from the text.

The origin, genetic and cytological location of the translocation may be found in Patterson et al. (1934), Stone (1934), Painter (1934a, 1934b, 1935), Bolen (1931) and Patterson (1938).

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[1]Now at Yale University, New Haven, Connecticut.

It would have been desirable to test each section of each chromosome, but some stocks were not available and inviability or infertility prevents the use of some others. For example, the complete inviability of males hyperploid for the 4/8 section of the X-chromosome excludes the possibility of obtaining this region in the tetraploid condition. Regions at the ends of the chromosomes were excluded because suitable markers could not be included in the available time. The one exception is the right end of the X-chromosome, or the duplication of 13R. A duplication male was obtained in the progeny of *y v f car* XX-females hyperploid for 13R, which had replaced one arm of the attached X-chromosomes with 13R by crossingover. This was balanced with the inversion in AM. Both heterozygous and homozygous females occurred and were positively identified in this stock.

The autosomal aneuploid stocks tested consisted of five second chromosome duplication stocks balanced over the *Cy* inversions and four third chromosome duplication stocks balanced over *Me' ca.* These stocks with their marker genes and balances are listed in Table 3 (see Frontispiece for loci of breaks in the translocations). If a lethal was known to be present in the base translocation stocks (Patterson, Stone, Bedichek and Suche 1934), from which these duplications were synthesized, the duplication was tested back to the translocation (Table 4). This was done in order to determine if the lethal in the original translocation was present, and so located (i.e., not covered by the duplication) that it would be lethal to the homozygous duplication.

Experimental Results

Homozygous duplication females were obtained for six regions of the X, but failed to survive for one, 13L/1R, Table 1. Of the stocks tested for the autosomes, only 31L/36R survived (Table 3). This is a very short region, 79F3 through 80C5 on Bridges' 1935 salivary chromosome map.

It is difficult to obtain satisfactory tests as the heterozygous duplication stocks are so often poorly viable and fertile. Also, it was not possible to predict the fertility of these when inbred from a knowledge of their fertility when outcrossed.

Phenotypic variation must be used with caution, as it is well known that differences in environment modify it considerably. The consistent modification of the homozygous duplication females is the small size of the abdomen. However the abdomen of some individuals appears to be quite normal. The size of the flies varies so much with changes in culture conditions that no attempt was made to correlate body size with the aneuploid condition. The body size of the homozygous overlap females in some cases is quite large or, as is usual in the case of *17L + 9R,* smaller than normal. All of the homozygous *9L + M5R* females which were examined had rough eyes. The wings of all homozygous *13R* hyperploid

females were slightly curled. Wings of this type were also occasionally encountered in the *13R* males and heterozygous females. Dissections of the abdomen showed that the homozygous overlap females have small ovaries although none were found so rudimentary as those usually encountered in triple-X females. There is no disturbance in the accessory sex organs or the reproductive tract. Sections of ovaries from heterozygous hyperploid females made previously show no histological abnormalties to account for the sterility of some of the individuals. The small size of the abdomen of the homozygous overlap females and the reduced ovary size seem to be due to the small number of mature eggs present in the ovary. This conclusion was reached from unpublished work on X-hyperploid females.

The method for obtaining and controlling the hyperploid *13R* region has already been explained. The homozygous stock was obtained and checked in larval ganglion and salivary gland preparations with acetocarmine. The metaphase plate clearly shows two J-shaped chromosomes instead of the ordinary X-chromosome configuration of rods. The salivary gland chromosome presents a much thickened appearance in the region of the homozygous duplication, *13R*. The portion of the fourth chromosome translocated is found in the region of 18C on Bridges' map (1935 and 1938) of the X-chromosome. The definition of the bands in the distal heterochromatic region of the X-chromosome is greater, and the organization of elements is easier to distinguish than in the normal diploid or in triploid salivary gland chromosomes. In making comparisons the attached *13R* stock is used since there is obviously so much difference in the viability of *13R* heterozygous attached and not attached in both the male and female (Table 1). The heterozygous females of the attached *13R* stock were tested for fertility, and it is these tests which are used for comparison with the fertility of females homozygous for attached *13R*. The attached *13R* heterozygous females were found to be 99 per cent fertile, while the non-attached *13R* heterozygous females are 97 per cent fertile. There is no significant difference in the fertility.

In the attached *13R* stock overlap males were found to be 54.5 per cent viable and heterozygous females 86.9 per cent viable, whereas the corresponding males and females from the nonattached stock were found by Patterson, Stone and Bedichek (1937) in another test to be 139.4 per cent and 160.6 per cent viable respectively. These percentages were obtained by dividing the number of overlap males or females by the number of the complementary class of normal individuals in the same population. There is definitely a very wide discrepancy in viability in the two cases.

The results obtained with respect to viability are very striking (Table 1). The females homozygous for the X-chromosome duplications are in every case very much less viable than the heterozygous and the normal individuals. In the cases where the viability of heterozygous individuals is lower than the normal, the reduction in viability of the homozygous

compared to the heterozygous females is relatively greater. Although in some cases the heterozygous females are more viable than the normal, in no case are the homozygous females nearly so viable as the normal diploid females. The basis for these comparisons is the occurrence of inversion males in the same populations in which the heterozygous females occur in one cross and the homozygous females occur in another. These inversion males are the complementary class of both types of females. Thus by the use of a count of a population of these males and heterozygous females on the one hand and these males and the homozygous females on the other, it is possible to compare the two types of females from the different populations. Significance tests were applied to the viability and fertility data (Fisher (1936)). There is no uniformity or general relationship which holds with respect to the variations in viability of the heterozygous and homozygous overlap females in the different stocks with the exception that all homozygous females are less viable than both the normal and heterozygous females.

The matings designed to produce homozygous autosomal overlaps recorded in Table 3 yielded the individuals desired for only one overlap, *31L* + *36R* in the chromocentral region of the third chromosome. For the other stocks tested, the number of flies examined is sufficiently large to conclude that homozygous hyperploid individuals for the overlaps used are either not viable or negligibly so as not a single individual tetraploid for any of the regions used was found among a total of 16,603 progeny. No conclusion as to the effect of aneuploidy may be drawn from the non-occurrence of 29L + 27R homozygous since they do not occur heterozygous with either translocation involved. Likewise, *40*L + *29*R is inviable with the *29* translocation, and no conclusion may be drawn from this case as to the effect of the aneuploidy although *40L* + *29R* heterozygous with translocation *40* is viable. Translocation *27* is inviable homozygous, but *27L* + *53R* over *27* is viable. The failure of the homozygous overlap to occur is due to the hyperploidy, since the lethal in *27* evidently is either in the overlap region or that portion of the *27* translocation to the right of the break. Also, *27L* + *53R* heterozygous with translocation *53* is viable. In summarizing these results it may be said that *27L* + *53R*, *30L* + *40R*, *34L* + *30R*, *8L* + *12R*, *39L* + *5R* and *27L* + *14R* are inviable in the homozygous condition due to the hyperploidy of the overlap region.

Due to the necessity of using small samples in the fertility tests the results may not be considered as reliably characteristic of the population from which the sample was drawn as they were in the case of the viability tests. In every case the fertility of the homozygous overlap females is lower than that of the heterozygous. The amount of reduction in fertility seems to be greater in comparing heterozygous to homozygous than in comparing the normal to the heterozygous. There is a significant difference in the fertility of *8L* + *W13R*, *1L* + *4R* and *13R* heterozygous and homozygous. The difference in fertility of *17L* + *9R* and *W13L* + *17R* in the homozygous and heterozygous condition is possibly significant.

As only four homozygous individuals of the *9L + M5R* stock lived long enough to be tested, this case is hardly to be considered, although the test shows a significant difference in fertility between females diploid and tetraploid for this region and between females diploid and triploid for this region. All of the differences is fertility of homozygous individuals compared to normal individuals are significant. In any case if there be a significant difference in fertility coincident with a difference in dosage of a region of the X-chromosome studied, fertility is reduced in the individual with the largest unbalance. In the comparisons which have been made the data for heterozygous overlaps is taken from that of Patterson *et al.* (1937) and Patterson, Stone and Brown (1940).

CONCLUSIONS

The data presented here and other phases of this work (Patterson, Stone and Bedichek 1937; Cumley 1940; Patterson 1938) are discussed by Patterson, Stone and Brown (1940) in an article in this bulletin. These data show certain facts which may be summarized as follows: There is decreased fertility and viability of homozygous duplications over the corresponding heterozygous duplication, and these in turn are usually less viable than normal. The effect of a homozygous duplication is not proportional to its length, nor are duplications of similar size necessarily similar in their effect.

The X-chromosome seems to differ from autosomes in its tolerance to change in proportion between the genes. Although some regions do not decrease viability and fertility if present as a heterozygous duplication, yet all regions tested do effect viability and fertility to some degree if present as homozygous duplications.

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TABLE 1

Tetraploid Regions in the X Chromosome. Viability

Replication	Relative Length	Per cent Fertility		Number Mated		PROGENY			Inversion Overlap	Per Cent Viable
						Overlap		Inversion		
		♀	♂	♂	♀	♂	♀	♂	♀	
9L + M5R	.114	41.6	48.0	10	5	89	12	114	235	10.5
17L + 9R	.150	1.1	96.3	10	2	407	17	302	972	5.6
W13L + 17R	.060	89.0	89.7	1	1	1078	227	352	1303	64.5
8L + W13R	.076	89.0	22.0	5	10	411	186	249	961	74.7
1L + 4R	.084	91.0	100.0	1	1	501	348	380	875	91.6
13L + 1R	.117	42.0	92.0	10	10	136	000	56	205	00.0
13R	.109	20.0	97.0	10	10	495	293	909	790	32.2

TABLE 2

Tetraploid Regions in the X and 3 Chromosome. Fertility

Replication	Relative Length	No. of Matings	Number Fertile	Number Sterile	Per Cent Fertile	Per Cent Parent Fertility		PROGENY		Avg. F_1 Vial
						♂	♀	♂	♀	
y ec ct 9L + M5R/ In–A99bNCO$_2$sn^{31f}	.114	10	1	3	25	41.6	48.0	0	4	4.0
y ct 17L + 9R v wy^2 f car/ In–A99bNCO$_2$sn^{31f}	.150	181	90	10	90	1.1	96.3	1412	2043	38.4
ec W13L + 17R car/ In–A99bNCO$_2$sn^{31f}	.060	169	81	19	81	89.0	89.7	623	537	14.4
y^2 8L + W13R B/ In–A99bNCO$_2$sn^{31f}	.076	105	9	91	9	89.0	22.0	1 (4 pupae)	6	1.2
y1L + 4R B/ In–A99bNCO$_2$sn^{31f}	.084	109	83	17	83	91.0	100.0	672	585	15.2
y v f(car) 13 R/ In–AM	.109	164	86	14	86	20.0	97.0	1218	1880	36.0
ve 31L + 36 R ♂ homozygous ♀		100 100	90 97	10 3	90 97			3500 2770	3660 2910	79.6 58.6

TABLE 3

Autosomal Overlaps
(pair matings)

	P_1 Inbred	F_1				Total
	Duplication	Heterozygous		Homozygous		
		♂	♀	♂	♀	
Chromosome 2	al 27L + 53R/Cy pr	1112	1068	0000	0000	2180
	29L + 27Rsp/Cy pr	1182	1213	0000	0000	2395
	al 40L + 29R/Cy pr	1156	1157	0000	0000	2313
	30L + 40Rsp/Cy pr	1284	1080	0000	0000	2364
	34L + 30Rsp/Cy pr	1108	1036	0000	0000	2144
	Total	5842	5554	0000	0000	11396
Chromosome 3	ve 8L + 12R/Mé ca	1142	880	0000	0000	2022
	ve 27L + 14R/Mé ca	1060	787	0000	0000	1847
	ve 39L+ 5R/Mé ca	1765	1139	0000	0000	2904
	ve 31L + 36R/Mé ca	1017	924	351	288	2580
	Total	4984	3730	0351	0288	9353
	Total	10826	9284	0351	0288	30033

TABLE 4

Heterozygous Autosomal Overlap x Translocation

	P_1	F_1			
	Duplication x Translocation	Trans. or Inv./Overlap		Trans./Overlap	
		♂	♀	♂	♀
Chromosome 2	$\frac{\text{al 27L + 53R}}{\text{Cy pr}} \times \frac{\text{al sp 27}}{\text{Cy pr}}$	viable		viable	
	$\frac{\text{29L + 27Rsp}}{\text{Cy pr}} \times \frac{\text{al sp 27}}{\text{Cy pr}}$	1200	1169	0000	0000
	$\frac{\text{29L + 27Rsp}}{\text{Cy pr}} \times \frac{\text{al sp 29}}{\text{Cy pr}}$	1036	1092	0000	0000
	$\frac{\text{al 40L + 29R}}{\text{Cy pr}} \times \frac{\text{al sp 29}}{\text{Cy pr}}$	740	679	0000	0000
Chromosome 3	$\frac{\text{ve39L + 5R}}{\text{Mé ca}} \times \frac{\text{ve ca 5}}{\text{ve Dcx ca}}$	160	140	25	43
	$\frac{\text{ve39L + 5R}}{\text{Mé ca}} \times \frac{\text{ve ca 39}}{\text{ve Dcx ca}}$	147	143	2	12

VI. EFFECT OF TEMPERATURE ON FERTILITY OF HYPERPLOID MALES OF *DROSOPHILA MELANOGASTER*

RUSSELL W. CUMLEY[1]

Department of Zoology, The University of Texas, Austin, Texas.

Numerous investigations have been carried out wherein the effects of temperature on the expression of various mutant genes of Drosophila have been observed (Harnley 1936, Ekar 1939, and others). Other workers have studied the effects of temperature upon the fertility and viability of Drosophila (Dobzhansky 1935, and others). In these studies changes in temperature have been shown to exert a considerable influence upon the manifestation and action of mutant genes, and upon the fecundity of the flies. The purpose of the present work has been to observe the effects of temperature changes upon the viability and fertility of hyperploid males of *D. melanogaster* which possess normal genes in duplication. Three different hyperploid types were studied, with duplications as follows: First, $w^{M5}L$, the *y–w* interval from 0–3C2 on Bridges' marked map of the X-chromosome; second, W 13L–17R, the *t–lz* region from 8A through 9A of this map; and third, 13R, the *car-bb* region from 18D through 20D of the map (Bridges 1935). The genetic and cytological location of the regions studied may be seen on the maps shown on frontispiece.

These three particuar hyperploid stocks were selected because at normal temperatures they have quite different fertility and viability.

The hyperploid males were allowed to develop at the following temperatures, 13.5, 17.5, 21, 26, 29, each plus or minus 1 degree Centigrade. This was accomplished by mating young *yvf* attached-X females with hyperploid males which had been developed at room temperature. The mated flies were kept at room temperature for twenty-four hours and then transferred to the constant temperature incubators. Thus the males to be tested were carried through the larval and pupal stages at constant temperatures. Immediately after emerging the test males were mated with *yvf* attached-X females and kept at about 21 degrees Centigrade. In these test matings one male was placed in a food vial with three females. A marked control (*yellow*[2]) was carried at the same temperatures and handled in the same way as were the hyperploids.

About three hundred vials were made up for each of the types tested, including the controls. Four days after the matings had been made the vials were examined, and if the male or two of the females were dead, the culture was discarded. The vials were emptied of the parents from six to eight days after the matings had been made. On the sixteenth day after the matings, the progeny in each vial were counted. In this way

[1]Now at The University of Wisconsin, Madison, Wisconsin.

the number of fertile and sterile males were determined for each type raised at each of the five temperatures.

From the data thus collected, it was possible to compute the percentage of males which were fertile after having spent their pre-imaginal life at a given temperature. Also, it was possible to determine the relative fertility of the fertile males raised at different temperatures. This was accomplished by computing the average number of offspring per male parent. These two values are incorporated in the accompanying table. From this table the following features may be noted.

The y^2 males were more fertile than any of the hyperploid males, at any given temperature. This stock possessed a high degree of tolerance to differences in temperature during growth in so far as the percentage of fertile males is concerned, but a distinctly reduced tolerance is so far as the number of offspring per fertile male parent is concerned. At the optimum temperature, which lies in the vicinity of 17.5 Centrigrade, the males were 98.6 per cent fertile and produced 88.6 offspring per male parent. At 21 degrees Centigrade and 26 degrees Centigrade there were likewise high percentages of fertile males, but they produced appreciably fewer offspring.

The W 13L–17R duplication males were nearly as fertile as the y^2 controls, when the measure of fertility is the percentage of males that were fertile. Likewise, the percentage of fertile males in this stock was nearly the same at 17.5 degrees and 21 degrees Centigrade. However, these males produced only 47.4 offspring per fertile male at the optimum temperature of 17.5 degrees. Part of the reduced yield from the hyperploid males must be due to the viability of some of the aneuploid combinations formed by them, and differential viability at different temperatures also contributes to the difference. They were very intolerant to the 13.5 degrees Centigrade temperature, being only 14.2 per cent fertile and producing only 16.3 offspring per fertile male.

The w^{M5}L duplication males were most fertile and produced the most offspring per fertile male at 21 degrees Centigrade. The flies could not live at 13.5 degrees Centigrade and were completely sterile at 29 degrees Centigrade. Not only is this stock the least fertile of the group studied, but also it has an optimum temperature significantly different from that of the others.

These experiments have a bearing on certain phases of gene activity. It has been claimed that twice the frequency of the gene should have twice the activity, for example, that 1N, 2N, and 3N individuals should be alike (Bridges 1938, and others). The results obtained in these experiments raise this question, "At what temperature are those genes present in hyperploids in such activity that their products are twice normal?" Obviously, at different temperatures the genic balance is diffierent as seen in its effects on the individual. Consequently, it seems a fallacy to argue that as long as genes stay in the same relative frequency they will have the same effect.

From these experiments we may conclude that temperature affects the function of normal genes, just as it exerts its effect upon mutant genes; and that different types of genic unbalance are influenced differentially at different temperatures as the effect is measured with respect to viability and to fertility.

TABLE 1

Data Regarding Fertility of Hyperploid Males of *D. melanogaster*

Test	Duplication	Developmental Temperatures				
		13.5°	17.5°	21°	26°	29°
Per cent fertile	y^2 Control	82.2	98.6	98.1	96.0	79.1
	$w^{M5}L$	—	83.3	70.0	62.6	41.9
	W 13 L–17R	14.2	92.7	92.9	82.5	60.5
	13R	—	15.2	38.6	21.7	0.0
Average hatch	y^2 Control	48.2	88.6	61.2	56.2	42.0
	$w^{M5}L$	—	60.8	25.5	31.5	28.2
	W 13 L–17R	16.3	47.4	25.2	30.1	27.3
	13R	—	19.5	21.2	10.6	—

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VII. EXPERIMENTALLY PRODUCED ANEUPLOIDY INVOLVING THE AUTOSOMES OF *DROSOPHILA MELANOGASTER*

J. T. PATTERSON, META SUCHE BROWN AND WILSON STONE
The Department of Zoology, The University of Texas, Austin, Texas

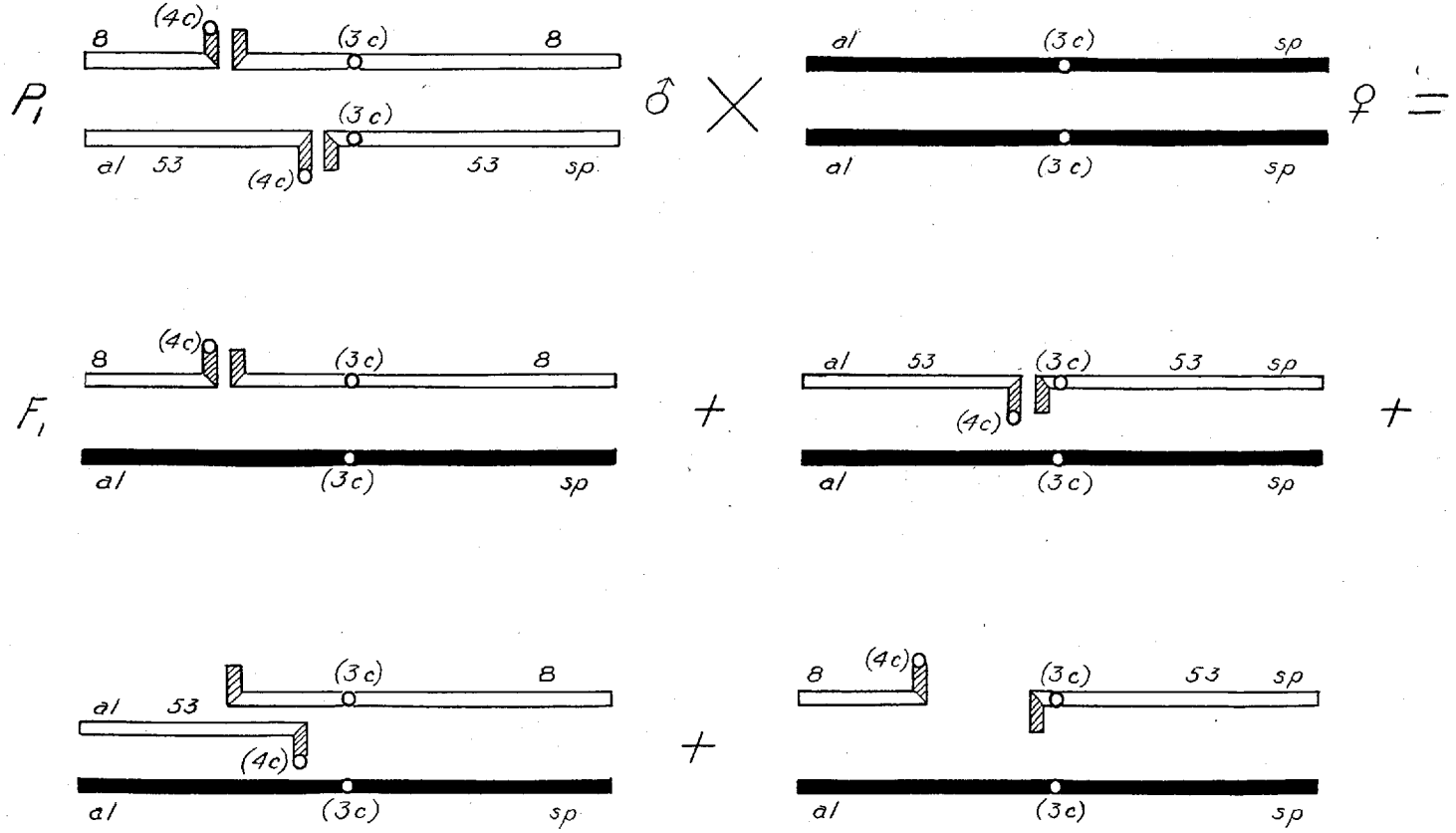
A series of related studies has been carried out to determine the effect of genic unbalance, as shown by aneuploid combinations of genes in diploid D. melanogaster (Patterson, Stone and Bedichek, 1935, 1937; Cumley, 1940; Burdette, 1940). Other studies of a similar nature have been carried out with this species, for example, Mohr (1932) on the 4th chromosome, Bridges (see 1939), Dobzhansky and Schulz (1934), and Pipkin (1940) on triploid intersexes. In addition there is a large amount of literature, particularly in plants, related to various phases of ploidy. This paper will present the new evidence available in *D. melanogaster.*

MATERIAL AND METHODS

Aneuploids for various sectors of the second chromosome were produced in the manner comparable to that used in the production of X-chromosome aneuploids (Patterson, et al., 1937). In this case the situation is somewhat simpler. Individual males heterozygous for two translocations, one marked with *aristaless (al)* and *speck (sp)* that had been introduced into the translocation by crossing over, were crossed to females without translocation but homozygous for *aristaless* and *speck.* One male and three females were placed in each food vial. As there is no crossing over in the male of *D. melanogaster* under ordinary conditions, the complementary hyper- and hypoploid F_1 combinations, wherever viable, could be detected as *aristaless* or *speck* individuals. Euploid individuals were phaenotypically normal or *aristaless speck.* To guard against contamination all vials were discarded unless both phenotypically normal and *aristaless speck* flies appeared.

There were nine translocations involving the second and fourth chromosomes used. Their numbers are: 43, 8, 53, 27, 29, 40, 30, 34, 6. All represent mutual translocations in which the distal part of the right or left arm of the second chromosome is exchanged for a segment of the fourth chromosome, thus becoming attached to the fourth chromosome centromere. The point of breakage of each of these translocations is shown on the cytological and genetic maps, on frontispiece. These translocations should be designated T 2, 4-A, but for brevity we use only the number as shown in the diagram.

In this case the hyperploid segregant is *aristaless,* and the hypoploid is *speck;* if 8 instead of 53 had carried the marker genes, the converse would have been true. In addition to the aneuploid segregants from two adjacent translocations, 43 and 6 were used to determine the effect of



Aneuploid Production by Segregation

hyper-and hypoploidy of the two end sectors. In this case 43/*aristaless speck* was backcrossed to *aristaless speck* females. Aneuploid progeny again would be *aristaless* or *speck*. A similar experiment was performed with 6.

Aneuploids involving the third chromosome were produced in a similar manner. In these translocations, which should be designated T 3, 4-A, the recessive genes used to follow segregation were *veinlet (ve)* and *claret (ca)*. Both the genetic and cytological position of the breaks in the several cases are indicated on the frontispiece. The results with the third chromosome are much more extensive due to the fact that many more translocations were available for use in experimentation. The numbers of the translocations used are: 52, 23, 13, 12, 8, 56, 14, 36, 31, 27, 37, 9, 1, 5, 28, 2, 39, 30.

The aneuploids are indicated by the numbers from the component translocations, thus: 40/29 would indicate that the hyper- or hypoploid, as the case might be, involved the region between the breakage point of the translocation 40 and that of translocation 29. The effect of the 4 chromosome components is ignored, although this must sometimes introduce an unavoidable error.

Results

With the use of nine 2–4 translocations, hyperploids were obtained for all loci of the second chromosome. These included hyperploids for seven adjacent overlap sections and three sectors involving end regions of the chromosome. At the left end no hyperploids for 43L were recovered in over 3600 F_1 flies. From the combination 43/8 only one sterile hyperploid was obtained in 1114 F_1 flies, and several thousand additional flies yielded no aneuploids. However, the two regions are included in the hyperploid 8L, which is both viable and fertile. At the right end, the combination 34/6 remains untested, since both translocations are unmarked. The region common to the two translocations is included in the hyperploid 34R, which is viable though sterile. In this case there were 26 females and 12 males among 7230 flies. No hypoploid was obtained for any region of the second chromosome. All hyperploid stocks were verified cytologically by an examination of the salivary gland chromosomes.

The use of thirteen 3–4 translocations yielded hyperploids for eleven regions of the third chromosome. For five of these regions hypoploids also proved to be viable. In addition, by using translocations with intermediate breakage loci, both hypo- and hyperploids were obtained for five shorter regions included in the longer regions above. At the left end of the third chromosome hypoploids only were obtained for the region between 23 and 13 (only 193 females and 91 males were secured from about 20,000 flies). No hyperploid was obtained for 23L, in some 12,000 F_1 flies; but in cultures of 52, a translocation lost before these tests were made, viable and fertile hyperploids for a shorter region to the left were

detected. In the right arm, only the combination 37/9 failed to yield aneuploids although some 20,000 flies were examined for this combination.

Stocks of all fertile aneuploids were carried by backcrossing males to *al sp ey* and *ve ca ey* virgins, respectively, for the second and third chromosomes in each generation. The following description of phenotypic variations of aneuploid forms are based on a comparison of aneuploid males and females with their normal siblings in these cultures.

Among flies hyperploid for sections of the second chromosome wing modifications are the most common. Classified as to position, one or both wings may be widespread, as in 8L and 30/34; slightly spread, as in 8/53, 53/27, 29/40, 40/30, 34R and 6R; or normal, as is usual in 27/29. In addition, wings may be slightly raised in 29/40 and 30/34, or bent down in 8L. In three hyperploid stocks, 53/27, 30/34 and 8L, the wing is frequently concave ventrally, similar to *curved*. In 8/53 and 30/34 the size of wing cells is slightly increased, and in 30/34 and 8L the wing margin is sometimes folded. The wing surface is slightly uneven in all hyperploids except 27/29, but rough or disarranged eye facets were found only in 30/34, and here rarely. The proboscis is frequently extended in hyperploids of five stocks: 53/27, 27/29, 29/40, 40/30, and 30/34. Rotated genitialia were found in a small per cent of hyperploids of 8/53, 53/27 and 30/34. In two cases, 8/53 and 29/40, the body including the wings is shorter and broader than normal. The change in body form is more marked than the shortening and broadening of the thoracic region, including the scutellum, which accompanies homozygous *aristaless*. In 6R the hyperploid is appreciably larger than non-hyperploid flies of the same culture. The hyperploids of 8L and 34R are reduced in size, with shorter antennae, smaller wings and short, pointed abdomens. Flies hyperploid for 34R are apparently lightly chitinized, the body wall appearing fragile and translucent.

All of the stocks hyperploid for short regions of the third chromosome are phenotypically normal (36/31, 31/27, 5/28, 39/2). Three stocks hyperploid for longer regions 8/56, 14/27 and 5/39, are likewise normal. In four others, 13/12, 12/8, 9/1, and 39/30, the only phenotypic distinction from normal is the occasional slight spreading of the wings. In 1/5 the wings are sometimes coarse as well as spread. In 27/37 the wings are usually widespread and sometimes cloudy. In 56/14 the proboscis is frequently extended, and the rim of the eye is so constricted that the eyes bulge at the sides and the head is flattened in the anterior-posterior direction. In 30R one or both wings are usually spread or raised; the wings are sometimes cloudy, less iridescent and more rounded at the tip. The head is often extremely misshapen when *eyeless*, the antennae and proboscis are distorted or imperfect, and the maxillary palps reduced, misplaced or reduplicated. The entire facial region is sometimes collapsed. The abdomen is either short and broad or else long and narrow.

The male genitalia are infrequently rotated or imperfect. The body size is frequently greatly reduced in both sexes, but the phenotypic distortion in this stock is more marked in the female than in the male.

Among the stocks hypoploid for sections of the third chromosome four are phenotypically normal: 14/27, 36/31, 28/2 and 2/39. In one, 31/27, the proboscis is rarely extended, and in two, 5/39 and 5/28, the proboscis is frequently extended and the wings are sometimes slightly spread. The male recovered in 9/1 had slightly rough eyes and spread wings. The genitalia were normal. Three stocks are sufficiently abnormal to merit separate descriptions. In 1/5 the wings are spread, slightly curved down and cloudy. The eyes are rough, the antennae short, and the body color darker than normal. In the female the abdomen is pointed. In 12/8 the wings are again slightly spread and curved down, and often twisted or crinkled at the edges. Extra joints in the legs are found rarely. Bristles are slightly finer and shorter than normal. The head, thorax and abdomen are broader than in the *ve ca* flies in the same culture. In 23/13 the wings are usually spread, shorter and more rounded at the tip, and cloudy. The head is broad, flat antero-posteriorly and the maxillary palps are frequently imperfect. The abdomen is short, broad and pointed. The genitalia are sometimes imperfect, often rotated in the male. The whole body is dark in color, fragile looking, and frequently reduced in size, especially in the male.

With the few exceptions noted, there is no marked difference in phenotype between the sexes in aneuploids of the second and third chromosomes.

The relative vigor or survival rate of the adult aneuploids can be judged roughly from individual matings made to test fertility. Three hundred or more males and females from each hyperploid stock for chromosome 2 were backcrossed individually to three *al sp ey* females or males, respectively. From the aneuploid stocks of chromosome 3 one hundred or more males and females were backbrossed to *ve ca ey*. After the third day each vial was examined to determine if the test fly, male or female, was still alive. In the tests of 2 chromosome hyperploids, the per cent of females surviving the third day ranges from 52.6 for 8L to 96.3 for 6R (Table 1). The per cent of males surviving ranges from 67.0 for 8L to 98.3 for 30/34. In the third chromosome, the per cent of hyperploid females surviving the third day ranges from 60.9 for 27/37 to 99.2 for 39/2 (Table 2). The per cent of surviving males ranges from 86.2 for 5/39 to 100 for 14/27, 28/2 and 39/2. With one exception in the second chromosome and three in the third, of which two are scarcely significant, the per cent of surviving males of each hyperploid stock exceeds that of the females.

Among the fertile hypoploids of the third chromosome, the per cent of females surviving ranges from 74.2 for 12/8 to 100 for 2/39 (Table 3). The per cent of males surviving ranges from 90.5 for 12/8 to 99.5 for 5/39. Among the hypoploids from 23/13, which were completely sterile, only 82.4 per cent of the males and 60.8 per cent of the females survived three

days. With three insignificant exceptions, males again survive the first three days after emergence to a greater degree than females of the corresponding stocks.

Fertility in aneuploid stocks can be measured by the per cent of flies which produce offspring, and by the relative number of offspring which each fertile fly produces. Of the hyperploids of the second chromosome, one was completely sterile (34R). In the established stocks the per cent of fertile females ranges from 30.6 for 8L to 99.7 for 6R (Table 1). These figures were calculated from the number of females which produced offspring that were alive three days after mating. The average number of F_1 per vial for the same two stocks is 7.2 and 59.0, respectively. The maximum average number of F_1 is 91.8, obtained from 27/29. These figures were calculated from a count of the number of F_1 from a representative number of individual matings for each stock. For males the per cent of fertile matings ranges from 50.3 for 8/53 to 98.7 for 27/29. The average number of F_1 per vial for these two stocks is 16.3 and 79.1, respectively. Values for other stocks can be seen in Table 1. As the table indicates, the per cent of fertile males and females does not necessarily correspond, nor is one sex uniformly more fertile than the other. Neither is there a direct correlation between the per cent of fertile hyperploids and the number of offspring per vial. The latter can not be compared directly with reference to the sex of the parent flies, since each hyperploid male was mated to three females to insure an adequate test of the male's fertility.

Among the hyperploids of the third chromosome the per cent of fertile females ranges from 73.1 for 13/12 to 100 for 2/39. The average number of F_1 per vial for these stocks is 11.0 and 64.1, respectively. The minimum and maximum average numbers per vial for hyperploid females are 5.7 for 39/30 and 69.1 for 8/56. This variation in the number of offspring per female from stock to stock is not proportional to the per cent of fertile matings. For males, the per cent of fertile matings ranges from 77.3 for 27/37 to 100 for 2/39. The average number of F_1 per vial for these two stocks is 63.6 and 74.4 respectively. The minimum and maximum average numbers per vial for hyperploid males are 47.1 for 5/39 and 75.4 for 13/12. In two cases 27/37 and 5/39, the per cent of fertile males is significantly less than that of fertile females. In all other cases the per cent of fertile males equals or exceeds that of fertile females.

Among hypoploids of the third chromosome one, 23/13, was completely sterile. Two, 9/1 and 1/5, produced hypoploid F_1 so infrequently that the stocks could not be maintained. To make sure that 9/1 *ve* and 1/5 *ve* were not hyperploid for the right end of the third chromosome covering *ca*, a test was made with translocation 1. Among 9000 or more F_1 no hyperploids were found. However, 2R hyperploids are both viable and fertile as determined elsewhere (Brown, 1940). In the remaining hypoploid stocks the per cent of fertile females is above ninety for all except 12/8.

For the males the rate is above ninety in every case. The average number of offspring per vial ranges from 22.4 for 5/28 females to 67.2 for 28/2 females. The average number of offspring from hyperploid males ranges from 50.8 for 5/39 to 83.8 for 31/27.

Comparison of hypoploid with hyperploid stocks shows that a stock hypoploid for a heterochromatic area or a short euchromatic area may be equally, greater or less fertile than the corresponding hyperploid stock, judged either by the per cent of fertile matings of either sex, or by the number of offspring per mating.

A measure of the relative viability of aneuploids and their diploid siblings can be obtained from a count of the offspring of individual males. In all such counts the two sexes are approximately equal in number (Tables 1 and 2), both among aneuploids and their normal siblings. Considering the number of diploids as 100 per cent, the per cent of viability of hyperploids ranges from 39.6 for 8L to 101.8 for 6R, in the second chromosome. In the third chromosome the relative viability of hyperploids ranges from 36.9 for 30R to 155.5 for 1/5. The minimum and maximum average values for hypoploids are 55.3 for 12/8 and 122.1 for 36/31.

More aneuploid types were recovered for the third chromosome because this chromosome was divided into more and smaller sections. Apparently also the markers *al* and *sp* had a greater effect on phenotype and viability than *ve* and *ca*. According to egg and hatch counts the *al sp ey* stock contained more lethals, or genes reducing viability. The hatch from 723 eggs was 88.3 per cent, with 1.6 per cent dead pupae, for *ve ca ey*. For *al sp ey* the hatch from 893 eggs was 75.1 per cent with 8.7 per cent dead pupae.

The rate of recovery of aneuploids is affected, not alone by their viability, but by the type of segregation which must occur to produce the desired gamete. When the points of breakage of two translocations are close together, random segregation producing hypo- and hyperploids is more frequent (e.g., 5/28, 28/2, 2/39).

Another factor which influences the recovery of aneuploids is segregation of the fourth chromosome. The infrequent occurrence of the hyperploid 27/29, and the greater frequency of hypoploids in 14/27 and 31/27 (in this case there were only two hyperploids, but 323 hypoploids in 1882 F_1 flies), compared with the equal frequency of hyper- and hypoploids in 36/31, suggests that the two 2 or 3 spindle fiber attachments rarely pass to the same pole, or that aneuploids with an additional 2 or 3 spindle fibre attachment occur less frequently than when the number of spindle fiber attachments is unchanged. However, a similar excess of hypoploids was found in 5/39, and a closer analysis of the data revealed that certain hyperploids were found in only some vials of a cross, whereas hypoploids when frequent were found in all vials; and furthermore, these hyperploids were always *eyeless*. Hence it became apparent that when the fragments

of the third chromosome carried little or none of the fourth chromosome the aneuploid, being haplo 4 eyeless, failed to survive. Only when an additional, free fourth chromosome was carried by the parent fly with two translocations were both kinds of aneuploids recovered.

The effect on phenotype, viability and fertility is a function, not only of the length of the chromosome which is duplicated or missing, but also of the particular genes in the region involved. The addition or loss of heterochromatin has a lesser effect on phenotype than a change in amount of euchromatin (e.g., 27/29, in 2; 14/27, 36/31, 31/27 in 3). Hyperploidy of heterochromatin may increase viability (36/31), but a decrease in the amount present is not accompanied by decreased viability or fertility (27/29 in 2; 36/31, 31/27 in 3). When the euchromatin area added is short, the viability may be stimulated (12/8, 1/5, 5/28, 28/2), but the loss of the same region in the hypoploid does not necessarily decrease viability or fertility.

Considering all aneuploids, the viability is decreased in fourteen hyperploids, normal in four (27/29, 6R in 2; 14/27, 2/39 in 3) and increased in five (12/8, 1/5, 36/31, 5/28, 28/2 in 3). Among the hypoploids the viability is decreased in two (12/8, 5/39), normal in four (14/27, 31/27, 28/2, 2/39) and increased in two (36/31, 5/28).

The per cent of fertile hyperploids is noticeably decreased in both sexes in four cases (8L, 8/53, 30/34, 30R in 2), in one sex only in nine cases, and is ninety or above in ten. Among fertile tested hypoploids the per cent of fertile individuals is noticeably reduced in only one case (12/8).

Discussion

This set of experiments produced a number of aneuploid genotypes. These represented all the regions in a contiguous series for both the second and third chromosomes. In these experiments the effect of part or all of the fourth chromosome in the aneuploid condition could not be determined. Aneuploids for some sectors failed to survive while others survived but perhaps with abnormal viability and/or fertility. The size of the various regions depended on the translocations available for analysis. Certain regions in the third chromosome were studied both as a single block, then several component parts were studied separately. As the whole haploid genome of *D. melanogaster* consists of four chromosomes, many of these blocks must be as complex and necessary genetically here as whole chromosomes are in those forms where the whole genome is divided among many chromosomes. Consequently aneuploidy here must often be as extensive in gene unbalance as are trisomics and monosomics in many cases. These experiments allowed us to measure the effect of autosomal aneuploidy on sex determination, genic balance and the present condition of activity of blocks of genes following mutation and selection. The effects studied must have been due to unbalance of normal genes as the translocations used had little or no effect when heterozygous with normal.

Sex Determination

At the present time sex in simple bisexual XX:2A, XY (or XO):2A forms is thought to be determined by the balance between the active products of the genes which are cumulative both as alleles and as multiple factors. The genes for femaleness are concentrated in the X-chromosome in forms where XX:2A is female; and those for maleness are concentrated in the autosomes (see Bridges, 1939). The relative frequency of these two sets of genes determines the sex of the individual—male, intersex or female. It should not be assumed that the genes are of equal potency in their effect. From a study of the sex of individuals from the progeny of triploids with three sets of autosomes and 2X + or — a fragment of the X, Dobzhansky and Schultz (1934) and Pipkin (1940), have shown that several parts of the X-chromosome shift sex in the female direction. Not all regions (genes) are equally effective but no one region by itself dominates the reaction in these intersexes. In addition to these differences several regions tested had much more effect in shifting the sex type in the 2X — : 3A than the 2X + : 3A condition.

This X-autosome balance relation is not the only method of sex determination in forms with the XX–XY mechanism.

Warmke and Blakeslee (1939) have shown that in *Melandrium dioicum* the mechanism is of a different sort. This difference is most important because it allows the production of a fertile functional dioecious tetraploid form which breeds true. Here the Y-chromosome carries the factors far maleness and the X-chromosome those for femaleness and the role of the autosomes is unknown.

This type of mechanism where 3X + Y : 4A is male and 4X : 4A is female allows a very important step in evolution in forms that have differentiated into two sexes, namely, increase in chromosome number and therefore the increased lability of the genotype so important in evolution. It is now no longer necessary to assume that all increases in total gene number have been accomplished by slight changes after the differentiation of forms into the two sexes.

Our experiments with diploid aneuploids (Patterson, Stone, Bedichek, 1935 and 1937) and gynandromorphs and mosaics (Patterson, Stone, 1938) have failed to show any small sectors (one region is not sufficiently tested) of the X-chromosome which dominates the female reaction. Up to the present no study has been made of autosomal aneuploidy with triploid + or — autosomes.

The failure to find genes or regions that strikingly modify sex determination in the X or autosomes of *D. melanogaster* does not imply that they are not present. In *D. virilis,* Lebedeff (1939) has shown that the recessive *ix* gene if homozygous can completely reverse sex; 2X : 2A individuals with *ix* homozygous are males although they are sterile. Also with one or two modifers, there are developed hermaphrodites with both male and female genitalia although these are also sterile. This production of hermaphrodites is of especial interest as it indicates that at most a few genes

can determine the expression or suppression of both male and female type genitalia. If there are numerous plus and minus modifiers as Bridges (1939) claims, they are subsidiary to these few main reactions.

Whether sex in Habrobracon is determined by multiple factors, Snell (1935) or by multiple alleles, Whiting (1935, 1940) and Bostian (1939), the mechanism is of considerable interest in terms of the effectiveness of two sets of genes in controlling development. In this case the male determiner genes are not as cumulatively effective as the sum of two (or more) different female factors. The dominance of the female reaction seems to depend on the summed action of at least two different genes; neither by itself can dominate the reaction. Here dominance of the reaction is conditioned by a more complex reaction than a sum of the reaction of identical alleles. It is of course only in those stocks that give diploid males, or a female area at the meeting line in a binucleated mosaic male, that it is necessary to assume different female factors. Possibly the female factor often mutates to a hypomorphic allele, which becomes homozygous on inbreeding, giving diploid males. In the stocks giving diploid males on inbreeding, the absence of clear cut intersexes might be taken as an argument against the multiple factor theory although inviability, or difficulty in detection may explain their absence.

The experiments here presented fail to show that any small sector of an autosome has a marked effect on the sex of diploid individuals. This follows from the fact that in all cases adequately tested the males and females hyperploid for the same region of either the second or third chromosome were about equally fertile where fertility is measured as the relative number to produce offspring. At least there is no marked and consistent difference with one sex more fertile than the other. This varied from region to region, as sometimes males would be more fertile than their sisters, while for other regions the reverse would be true. Hypoploid males were as fertile as, or more fertile than females hypoploid for the same sector. There was no marked and consistent difference in the viability of comparable males and females—either hyper- or hypoploid, here measured as the relative number to survive to the adult stage (see Tables 1, 2, and 3). In some cases the aneuploid females were more viable and/or fertile than the corresponding aneuploid males but there were no consistent relations between viability and fertility nor reciprocal relations between hyper- and hypoploids. Therefore although certain regions might be considered to have a particular effect on the two sexes, no general trend could be noted. Only the absolute number of females or males to produce offspring could be compared as necessarily the fecundity can be compared only between members of the same sex. The fact that hyperploid males survived the measured three day interval better than their hyperploid sisters might be taken as indirect evidence for genes affecting maleness in the autosomes were it not for the fact that hypoploid males also possess the same survival advantage over their hypoploid sisters. To stress the more important classes, the hypoploid males are in all cases as fertile as their hypoploid sisters and are in most cases more

viable than their hypoploid sisters, in contrast to the relation in their non-hypoploid sibs.

These data allow us to draw no positive conclusions concerning the role and number of genes for maleness in the autosomes. Certainly these results allow us to locate no very important male determiner gene(s) in the autosomes. Nor do they show that the male determiner genes are numerous and widely distributed. In fact, these experiments do not give us much reason to believe that there is an accumulation of male factors in the autosomes. Sex determination is a peculiar mechanism and sex differentiation in such forms as these is determined by a genic balance reaction which ordinarily alternates with the sex chromosomes. If we disregard the location of the female and male sex determining factors, single or multiple, we have a system which has two alternate balanced phases, 1X : 2A and 2X : 2A. If we consider the activity of these other genes as they affect the internal environment we see that there are two different internal environments. Gene activity is obviously correlated intimately with the internal environment including the activities, past and present, of the other genes. Therefore regardless of where the male and female sex determining factors—they might even be the same gene—are located, either on the X or in the autosomes, they must react in one or another way as the environment is changed. Considered this way, the internal environment of 1X : 2A, 2X : 2A, 3X : 2A and 2X : 3A are all different and wherever the sex factors are located, they must determine sex in each of these several environments. The fact that the sex determiners might be in either X or autosomes may be illustrated from *D. virilis*. In the case of the mutation *ix* and its normal allele, 2X : 2A individuals will be females or males depending on whether the recessive *ix* is heterozygous or homozygous in the third chromosome (Lebedeff, 1939).

In that case the sex determining genes are an allelic pair. In *D. melanogaster* the work of Dobzhansky and Schultz (1934), Patterson, Stone and Bedichek (1935, 1937), and Pipkin (1940) have shown that changing the relative amount of parts of the X-chromosome may shift the sex of the individual in some cases, in others it causes marked deviation from the normal phenotype, viability, and fertility. Also Patterson, Stone and Bedichek showed there was a marked differential effect of X-chromosome unbalance in the two sexes. There has been no such marked differential effect of aneuploidy of the autosomes between the males and females. The data for the X were consistent with the hypothesis that there are numerous factors in the X which effected femaleness (and maleness). These data for the autosomes give no evidence for the assertion that there are many genes for maleness located in the autosomes. Obviously sex determination is a genic balance phenomenon but it does not necessarily follow that the genes which determine sex either changes or do not change frequency to shift the sex determining mechanism.

Genic Balance

There are certain facts which should be remembered in a discussion of genic balance or sex determination as illustrated in *D. melanogaster*. One such fact is that most genes in this form act in place independent of whether or not other alleles or other multiple factors are present in the same individual. In mosaics as has been shown repeatedly and in transplants (see review by Ephrussi, 1938), most of the genes act in place so that the character of the tissue is determined by its immediate internal environment.

There are very few instances of diffusable substances entering and there are no indications of hormone actions such as are present in certain other forms studied (see the discussion by Danforth, 1939). The fact that there is differentiation in relation to changes in gene balance also implies that these genes are the limiting factor in the system. If they were not the limiting factor, change in their frequency would not effect the reaction. This must be the relation wherever changing relative or absolute gene frequencies produces a different end result. This is therefore true for the difference in male-female reaction, intersexes, triploids and polyploids in general as well as aneuploids.

The conditions found in a number of other forms are pertinent for analysis of the situation in *Drosophila*. Blakeslee (1934) and Satina, Blakeslee and Avery (1937) have shown that in *Datura* aneuploidy of both whole and half chromosomes varies in its effect with the chromosome segment concerned. Anderson and Sax (1936) however report that the presence of extra fragments of chromosomes in *Tradescantia* apparently has no effect. Numerous investigators have reported that in polyploid forms, one or more chromosomes over or under the multiple of the N number has little effect. This is not universally so. In *Primula kewensis* ($4N = 36$) Newton and Pellewe (1929) and Upcott (1939) have shown that in addition to the 36 chromosome plants, numerous 35 and 37 chromosome plants were fertile, although a number of 34 chromosome plants were sterile. It was impossible to tell if the different phenotypes encountered there were determind only by aneuploidy.

Olmo (1935) has shown that monosomics are usually subnormal in *Nicotiana*. Goodspeed and Avery (1939) have studied trisomic and other types of *Nicotiana sylvestris*. In this form the trisomics investigated have all differed morphologically from one another as well as from the normal. Double trisomics and tetrasomics also differ. Most forms are partly fertile. Both triploid and autotetraploid forms differed from the diploid and each other sufficiently to show that the genic balance relation changed with change in frequency of the N number without changing the relative frequency. Thus Bridges's (1939) statement that "In all other cases a true doubling of a set of chromosomes gives no change in characteristic beyond that attributable to the changed size relations" does not fit this case nor for that matter others also, as Muntzing (1936), Lindstrom (1936), and

Kostoff (1938) show that many characters, especially physiological ones are different in diploids and autopolyploids.

Goldschmidt (1938) from the work on *Datura* and Goodspeed and Avery (1939) from the work on *Nicotiana* reach the conclusion that the genes affecting size and shape are not distributed at random in the several chromosomes, as different trisomics have different phenotypes. Goodspeed and Avery present a discussion in which they speak of "a physiological action of a chromosome as a whole" and attempt to explain this as some sort of "position effect"—association effect reaction. In the first place, different trisomics would be expected to differ as they do if the genes controlling size and shape are distributed in the chromosomes at random. The only way either Goldschmidt or Goodspeed and Avery could have expected the several trisomics to be alike would be to have known beforehand that the genes controlling size and shape were very numerous, cumulative and equal in effect and nondominant. If they had known that and found the trisomics differed, their conclusions might have been warranted. As it is, a relatively small number of genes which differ in their effect, scattered at random will explain their results without recourse to "the chromosome as a whole." Furthermore, their conclusions ignore the fact that abnormal concentration of gene products of a particular group of genes may influence reactions not affected by the normal concentration of these substances. There is no differential specificity of various aneuploid regions as measured in phenotype or viability or fertility in our data. Certain regions have certain specific effects but often several regions have the same general effect on viability, fertility or phenotype.

Certain inferences may be drawn concerning genic balance from the effect of aneuploidy on the phenotype, viability and fertility from these studies with *Drosophila*. There has been found no *a priori* predictability that the effect of aneuploidy for any particular region will produce either upon or between any of the variables measured. The aneuploids may be normal in so far as these experiments could test that condition, or there may be any combination of effects on viability and phenotype. In general there was a correlation between phenotypic abnormality, inviability and infertility but this is not always the case. There seems to be somewhat more correlation between the sexes for the presence of phenotypic abnormalities than for viability and for survival for the three day test period and less with fertility as this variable is often different.

There are several generalizations about gene activity and genic balance that can be drawn from this and related works. These experiments were carried out on uniform food and at a temperature of 22° ± 1° C, for Cumley has shown that a change in temperature has marked though different effects on several different aneuploid stocks. This was also found to be the case with aneuploid intersexes by Dobzhansky and Pipkin. Furthermore, Burdette has shown that there is no consistent relation between heterozygous and homozygous hyperploidy, i.e., a particular group of genes duplicated and reduplicated. The permanent addition of genes to the genome by the production of homozygous hyperploids must

ordinarily involve small blocks of genes as homozygous hyperploids are usually so reduced in viability and fertility. In general, it may be said that the viability relations are in the following order: 3 N > 3 N + fragment > 3 N — fragment; 2 N > 2 N + fragment > 2 N — fragment; 2 N + fragment > 2 N + 2 (fragment). Also the experimental values obtained will vary under different environmental conditions. In addition, just as with the X-chromosome (Patterson, Stone, Bedichek, 1937), it is impossible to predict the effect of a long duplication from a knowledge of the effect of the short component sectors and vice versa. We can therefore conclude that the threshold of gene activity which will cause a departure from the normal condition, in so far as this can be measured by the summed activities of genes in a block, varies with the frequency. It also varies with various environment agencies such as temperature, crowding (this last was shown by the fact that aneuploids were recovered most frequently in a particular optimum hatch) the gene itself and its associates with the effects of the genes not only in the aneuploid sector but elsewhere.

Of the several autosomal combinations tested, only the third chromosome hyperploid 36/31 lived homozygous (see Burdette). One second chromosome aneuploid stock, 53/27, was sterile over the *Curly* inversions and seven third chromosome aneuploid stocks were sterile over the complex inversion *Dcx*. This implies homozygous recessive sterility factors of independent origin or a conditioned dominance summation effect of factors in the inversions and translocations producing sterility with the additional effect of the aneuploidy. This last seems more probable. As Burdette has shown, the effect of reduplicating a certain part of a chromosome cannot be predicted from the knowledge of the effect of the heterozygous duplication. However to hold that the relations should be predictable, e.g., that the reduplication should be twice as inviable as the duplication, would be to imply far too much knowledge of the threshold and summation activities of the genes.

The activity of a given gene must depend on the concentration of available substances in the cytoplasm, including the products from the previous action of other genes. Even so there still is no reason to assume that twice the normal concentration of gene products would cause a departure from normal predictable from a knowledge of the effect of 3/2 the normal amount.

There must be a number of scattered genes which produce their products in relation to their frequency although not necessarily in a concentration directly proportional to their frequency. Further there is a limited range of tolerance to differential (abnormal) concentration of the gene products as both hypoploidy and hyperploidy causes measurable abnormalties in development and function of these organisms.

We shall not attempt to review the many works on related subjects including much work on heteroploidy and polyploidy. Allopolyploids are in some respects similar to aneuploids. They have numerous genes

present in different frequencies due to mutation to different alleles which may have completely different functions in the different parent stocks. The differences between the gene complex connected with the same functions demonstrated in cotton by Harland (1936) amply proves this. Although the genes in each distinct N set represented in the allopolyploid —as long as they retain simple multiples of all parts of all N sets—have been brought into balance by selection in the parent strains, the combined sets may or may not be in balance. Nor can we expect that an allopolyploid will be equivalent to the simple sum of the component parts. Consequently various polyploids would be expected to differ from their parent strains in their viability, fertility, and phenotypic reactions. Nor in our opinion should autopolyploids be expected to be the same as diploids even though they are simple multiples of the N number of chromosomes.

Gene Selection

Berg (1937a, b, c) has claimed that the difference in the rate with the same dosage of X-rays of lethals and sterility factors in X and autosomes shows effect of the difference in selection, involving some sort of selection for sex factors. Prabhu (1939), however, showed that most of the male sterility genes in the X were localized in one region, perhaps being alleles. Therefore there is less indication of a gene difference between X-chromosome and autosome. Certainly if most sterility factors are alleles, there is evidence for a labile gene giving male sterility, but none for a different rate of sterility mutation between X and autosome.

Perhaps this difference is valid, but our data show a difference between the second and third chromosome, at least in the survival of hypoploids. A number survived in 3, but none in 2. How much of this is due to the fact that most, but not all, of these regions in 3 were shorter regions than those tested in 2 cannot be determined. If this is a real rather than apparent difference, there is, or was a difference in selection between 2 and 3. The obvious difference in selection in the X and autosome has resulted in a difference in the genetic effect of changing gene balance from the XX of the female to the X or XY in the male sex. However, it does not follow that the results will necessarily lead to the multiplication of sex determiner genes in the X. At the present time in selection in *D. melanogaster*, X chromosome genes are normally present once in the male developing system and twice in the female developing system. Selection has occurred which will insure a balanced activity of the genes present in the particular frequencies of these developing systems. This, of course, is necessary but the accumulation by mutation and selection of many further additional genes as a margin of safety that support the determination of sex, is not necessary to the organism even if it might be useful. It remains an open question to us why it should be considered useful to the organism to accumulate mutations only to reinforce a single, already

satisfactory, system in development. Also, it is difficult to see how they would be retained against mutation pressure. It seems of highly questionable value for an organism to specialize more and more of its genes to a particular function—if the genes are selected for a different function, that is entirely another matter.

The difference between the responses of males and females to different aneuploid conditions can be explained without recourse to the limited role of genes in sex determination. Let us consider the several aneuploid classes. If we have each autosome set constant at 2N, the variations in the X for any particular sector may be: X + Fragment; 2X — F; 2X + F; 2X + 2F. The general order of viability and fertility, with certain exceptions, going from least normal toward normal is: 2X — F; 2X + 2F; X + F; 2X + F. The fect that 2X — F and X + F are less viable and fertile than 2X + F is explainable in terms of genes determining sex—at least fertility, and to a less extent, viability. However, this does not explain the inviability and infertility of the 2X + 2F aneuploids. We are of the opinion that the relations here are simply gene balance phenomena, and not sex determination alone, although it may contribute. The genes in the X have been selected for balance and dominance relations to function in the male developing system haploid, and in the female developing system diploid. Therefore lack of some genes in the female is a decided departure from the normal condition. The male hyperploid, X + F, is more of a departure from the selected balance system than the corresponding female hyperploid as there is some margin of safety and tolerance to some excess of gene products. For the X itself, and the fragment, the 2:1 abnormal balance of the fragment is farther from normal than the 3:2 ratio in the hyperploid female. The homozygous hyperploid with its 4:2 ratio is the hyperploid farthest from normal. Here, apparently, the reduplication of genes has gone past the "margin of safety," and the concentration of their products has become so high as to become a decided disadvantage to the developing organism.

If sex is determined by a large number of cumulative factors, X + F:3A and 2X — F:3A should be more nearly the 1:2 ratio of normal males than X:3A or 2X:3A. However, these last are much more viable than the ± aneuploids despite the fact that 2X — F:3A has a very decided shift toward maleness (Pipkin, 1940). Therefore it is not sex unbalance but general genic unbalance related in part to other systems that must so reduce the viability of these 2X — :3A and X + :3A aneuploids.

These facts together with the presence of sex sterile forms, gynanders, and intersex and hermaphrodite forms in *D. melanogaster, D. simulans* and *D. virilis* show that absence or presence of one or both secondary sex apparatus is not the primary cause of the inviability of these unbalanced forms. These facts stress the delicate and specific nature of the balance here—both 1X and 2X forms survive but 1X + and 2X — forms are abnormal.

Gene Action

In order to discuss gene action it is necessary to define certain concepts such as the normal gene and dominance. The normal gene is, at any particular time, the allele most frequent in all the population of a species. It is an autocatalyst and produces some substance or controls some reaction perhaps as an enzyme with certain definite limitations. We know that aneuploids, both hyperploids and hypoploids, are often abnormal, due to their abnormal genic internal environment. This shows that the *normal* gene produces its substances in a certain concentration relative to the concentration of the products of the other reactions which are occurring in development. The genic balance relation demands that a gene to be "normal" or "beneficial" must not only produce a certain substance but also produce it in a certain relative concentration at a particular time. Therefore a mutation beneficial in a certain genotype might well be very detrimental in another.

Wright (1934) has discussed the physiological and evolutionary theories of dominance and his article gives an account of the necessary theories. The present work shows that there are many genes or at least some genes in each region where aneuploids differed from normal individuals, that were not dominant in the sense that one allele was equal to two which in turn were equal to three. Burdette has added that they are not equal to four and Cumley has shown that their action varied to some extent with the temperature. We can describe dominance in the diploid as the condition of a gene when it gives a detectable effect in the heterozygous. A number of the dominant mutations in *D. melanogaster* then, are to be explained as the lack of the sufficient concentration of products from the haploid normal gene to produce the normal phenotype in the otherwise diploid system, viz., *Ly, N* and *M,* and haplo 4. Some normal genes, dominant in the sense that they are haplo-sufficient in the diploid which is another possible definition of dominance, often have alleles which are haplo-insufficient. Examples of this are the w^a — ^{R} and f^{34f} genes which are often phenotypically normal in the male and homozygous female but which give intermediate reactions with mutant alleles. Also we have the cases such as the normal Russian and normal American alleles of *white* which are not "dominant" in the triploid to two *white* genes (Muller 1935). Under similar cimcumstances, one normal allele of *singed* is not completely dominant to two mutant alleles. Consequently, dominance must be in part, at least, the result of the production of genic products in a certain concentration, and at a certain time, in relation to the concentration of other products in the cell. The gene *A* in *Ephestia* is dominant in the sense that *A/a* has the same effect as *A/A*. Yet the amount of hormone produced varies under different conditions (from Euphrusi, 1938). Here a gene can be said to be dominant because it is not the limiting factor in the reaction under these circumstances.

Necessarily, the gene must act in a cell with the components at hand which will include the effects of previous activity by other genes, and so all these must contribute to the internal environment when the genes act. Genes have a "margin of safety" in their activity in either or both directions in as far as the products of gene activity are sufficient to produce a normal reaction when this gene is not in the same frequency with the other genes. The "margin of safety" must vary quite widely with different genes as can be seen from the differences between different aneuploid combinations. Any effect of aneuploidy must result from these genes without a sufficient "margin of safety," but it would be a mistake to locate them in the region in the abnormal frequency. They might well, and probably often are, located elsewhere, and their reaction is effected by the abnormal concentration of products of the genes in the aneuploid sector. There is no way to determine their frequency relative to genes unaffected, nor can we know how often we did not detect a slight disability or increased viability.

With these facts, it can be seen that a mutation can well act as a dominant either by too little or too much activity in a certain direction, which in the right amount would be normal. Furthermore, we see no *a priori* reason why the normal gene should be completely dominant or equivalent in action in all relative frequencies as long as it is usually present in the diploid. It can do so through selection of modifiers or of stronger alleles due to a decided mutation pressure in order to have a margin of safety for the heterozygous. Certainly in the actual case of *D. melanogaster,* at least some autosomal genes are not dominant, as so few hypoploids survive except for short regions; also we find the decided effect of reduplication in the absence of such classes (Burdette). This is also true in the *X,* for both hypoploid ♀ and hyperploid ♂, and in addition, reduplication ♀ ♀ are decidedly affected by aneuploidy.

Silow (1939) is of the opinion that many of the normal genes of diploid cotton, as well as the mutants, are not completely dominant. He points out that a number of cases in other forms are similar. This agrees with the situation in *D. melanogaster.*

Cumley's work with temperature and hyperploid males shows that in the gene relations there, in part due to dominance relations, the same gene frequency gives different reactions under different conditions. Euphrusi (1938) speaks of the autonomous or nonautononomous action of *vermilion* and *cinnibar* which is determined by environment. Selection between alleles would be influenced in the same way. One mutation would have the advantage over an allele if conditions, the external and internal environment, were right for it. Undoubtedly, this non-dominant condition of the gene, especially where the alleles have differet functions, even when the same or similar functions are carried out by different gene pairs, contributes to the unbalance of the hybrids in species crosses, often leading to abnormality and sterility.

This has been shown for cotton by Harland (1936) and some of the theoretical implications have been discussed by Muller (1939) and Patterson, Stone and Griffen (1940). This must, in part, explain why amphidiploids are fertile where the hybrid diploid was sterile, even when this is not the result of the upset in chromosome mechanics. This must contribute decidedly to the abnormalities encountered in the *pseudoobscura-miranda* crosses (Dobzhansky, 1935, 1937) and Macknight (1939).

Primula kewinsis, Newton and Pellewe (1929), Upcott (1939), where the diploid hybrid is sterile, although the pairing seems normal, but the amphidiploid is fertile seems a good example of some such phenomenon. It seems to apply to polyploids in general. (Lindstrom, 1936; Muntzing, 1936; Anderson, 1937; and Kostoff 1938.) The decided difference especially in characters which have been called physiological in autotetraploids and their diploid parents must be due to difference in the genic balance resulting from the fact that reduplication could not be equivalent for genes with different kinds and degrees of dominance.

We have stressed the departure from normal encountered in these several aneuploid combinations. Yet probably the most remarkable fact is that so many of the aneuploids were sufficiently near normal to survive and function. This illustrates the remarkable lability and flexibility of the system which still functions to produce an individual. This flexibility must be due in part to the fact that many genes have been selected to work toward the normal phenotype and function.

We wish to acknowledge our indebtedness to Dr. T. S. Painter who allowed us to use a modified copy of his salivary gland chromosome map and to Dr. A. B. Griffen who checked cytologically several of the translocations and the aneuploid stocks.

SUMMARY

(1) Nine translocations involving the second and fourth chromosomes divided the second chromosome into ten sectors. The effect of the aneuploidy of any one of these sectors, produced by segregation from the heterozygous translocation or translocations was measured in its effect on viability, fertility and phenotype. Eighteen translocations involving the third and fourth chromosomes divided the third chromosome into many more regions. A number of them were tested in aneuploid combinations.

(2) No hypoploid for any region of the second chromosome was obtained. Hypoploids were obtained for a number of the short sectors of the third chromosome.

(3) Hyperploids were obtained for most of the sectors of both the second and third chromosomes studied.

(4) Some sectors had one effect on viability, fertility and phenotype while another region of about equal size might have a quite different effect. It is difficult to generalize more than to say that in terms of the variables measured aneuploidy was detrimental.

(5) The results prove that there are genes in many regions that are not haplo-sufficient in that they are not equivalent in effect when present once and twice.

(6) There are genes in many sectors which have a different effect when present twice and three times.

(7) The amount of gene products are therefore detectably different with change in gene frequency. The reactions in the developing systems are determined by these gene products which are related to gene frequencies. This of course is another way of saying that there is a genic balance system.

(8) There is no consistent difference in the effect of autosomal aneuploidy on the two sexes such as has been demonstrated for the X-chromosome.

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TABLE 1

Aneuploid Recovery and Hyperploid Tests—Chromosome 2

2 Chromosome Combination	8L	8/53 al sp	53/27 al sp	27/29 al sp	29/40 al sp	40/30 al sp	30al sp/34	6R
Number Counted *al sp* ♂ x al ∓ ∓ sp ey ♀	1491	2196	4318	4420	4195	497	802	2709
Number Hyperploids Recovered	15 sp	248 al	439 al	12 al	625 al	13 al	200 sp	9 al
Per cent Hyperploids Recovered	1.0	11.2	10.1	0.2	14.8	2.6	24.9	0.33
Number Hyperploid ♀ ♀ crossed 1 ♀ x 3 ♂ ♂	594	434	533	379	366	495 sp*	327	333
Number viable after three days	313	307	367	341	300	471	308	321
Per cent viable after three days	52.6	77.3	68.8	90.0	81.9	95.1	94.1	96.3
Number fertile	96	131	345	335	284	405	266	320
Per cent of viable ♀ ♀ fertile	30.6	42.8	94.0	98.2	94.6	90.2	86.3	99.7
Number of vials from which F_1 counted	10	45	44	20	40	20	111	20
Average number of F_1 per vial	7.2	4.8	21.1	91.8	15.9	25.4	26.0	59.0
Number hyperploid ♂ ♂ crossed 1 ♂ x 3 ♀ ♀	428	334	407	321	353	337	412	352
Number viable after three days	287	300	359	313	329	328	405	316
Per cent viable	67.0	90.0	88.2	97.5	93.2	97.3	98.3	89.7
Number fertile	223	151	282	309	264	300	334	291
Per cent of viable ♂ ♂ fertile	83.2	50.3	78.5	98.7	80.2	91.1	82.4	92.0
Number of vials from which F_1 counted	16	30	146	30	50	20	201	20
Average number of F_1 per vial	68.3	16.3	44.5	79.1	28.0	50.5	42.4	56.5
F_1 al sp ey ♀	407	173	1908	672	542	343	2676	273
F_1 al sp ey ♂	383	148	1720	509	449	303	2576	287
F_1 Hyperploid ♀	144	90	1482	639	236	183	1801	297
F_1 Hyperploid ♂	159	79	1390	555	207	182	1481	273
Per cent viability of hyperploids (al sp = 100 per cent)	39.6	42.2	79.2	96.1	44.7	63.7	62.5	101.8

*From $\frac{40 \text{ al sp}}{30}$

TABLE 2

Aneuploid Recovery and Hyperploid Tests Chromosome 3

3 Chromosome Combination	13 ve ca / 12	12 ve ca / 8	8 ve ca / 56	56 ve ca / 14	14 / 27 ve ca	27 ve ca / 37	9 ve ca / 1	1 ve ca / 5	5 / 39 ve ca	39 ve ca / 30	30 R	36 / 31 ve ca	5 / 28 ve ca	28 / 2 ve ca	2 / 39 ve ca
Number counted (*ve ca* ♂ xve ca ey ♀) + +	503	351	576	637	387	1448	536	1021	522	1276	1084	939	666	550	893
Number hyperploids recove ed	114(ca)	108(ca)	162(ca)	81(ca)	33(ve)	32(ca)	117(ca)	224(ca)	43(ve)	103(ca)	104(ve)	262(ve)	151(ve)	139(ve)	112(ve)
Per cent hyperploids recovered	22.7	37.7	28.1	12.7	8.5	2.2	21.8	11.9	8.3	8.1	9.6	27.9	22.7	25.3	12.5
Number hypoploids recovered	0	44(ve)	0	0	93(ca)	0	1(ve)	5(ve)	103(ca)	0	0	211(ca)	146(ca)	124(ca)	222(ca)
Per cent hypoploids recovered	0.0	12.5	0.0	0.0	24.0	0.0	0.2	0.5	19.7	0.0	0.0	22.5	21.9	22.5	24.9
Number hyperploid ♀ ♀ crossed 1 ♀ x 3 ♂ ♂	256	282	255	255	223	341	260	255	210	212	313	248	213	119	128
Number viable after three days	246	260	250	217	213	208	234	230	200	203	230	217	210	116	128
Per cent viable after three days	96.1	92.2	98.0	85.1	95.5	61.0	90.0	90.2	95.2	95.8	76.7	87.5	98.6	97.5	100.0
Number fertile	180	254	243	198	206	190	197	206	195	166	188	208	208	115	126
Per cent of viable ♀ ♀ fertile	73.2	97.7	97.2	91.2	96.7	91.3	84.2	89.6	97.5	81.8	81.7	95.9	99.0	99.1	98.4
Number of vials from which F_1 counted	20	10	16	20	10	10	34	10	10	28	25	20	10	10	10
Average number of F_1 per vial	11.0	63.0	69.1	17.0	56.1	25.9	21.7	32.0	64.8	5.7	10.4	21.3	28.9	62.5	44.8
Number hyperploid ♂ ♂ crossed 1 ♂ x 3 ♀ ♀	222	239	264	206	245	243	211	209	254	248	299	323	223	140	149
Number viable	221	234	255	202	245	212	207	200	219	242	283	311	216	140	148
Per cent viable	99.5	97.9	96.6	99.0	100.0	87.2	98.1	95.7	86.2	97.6	94.6	96.3	96.9	100.0	99.3
Number fertile	213	228	247	199	240	164	199	190	174	239	243	307	207	139	147
Per cent of viable ♂ ♂ fertile	96.3	97.4	96.9	98.5	98.0	77.4	96.1	95.0	79.5	98.8	85.9	98.7	95.8	99.3	99.3
Number of vials from which F_1 counted	5	15	10	10	10	10	10	10	10	10	16	10	10	20	10
Average number F_1 per vial	75.4	48.3	59.1	56.0	66.8	63.6	51.6	55.7	47.1	64.2	60.8	55.1	64.8	59.7	68.8
F_1 ve ca ey ♀	115	171	164	151	159	226	169	116	122	182	367	115	150	278	193
F_1 ve ca ey ♂	105	148	145	163	165	118	113	102	123	172	342	121	138	241	156
F_1 hyperploids ♀	77	205	127	126	153	104	116	179	109	141	125	147	185	345	152
F_1 hyperploids ♂	80	201	158	120	191	118	118	160	117	147	137	168	175	331	187
Per cent viability of hyperploids (ve ca = 100%)	71.4	127.3	92.2	78.3	106.2	64.6	83.0	155.5	92.2	81.4	37.0	133.5	125.0	130.3	97.1

TABLE 3

Hypoploid Tests Chromosome 3

3 Chromosome Combination	12 ve ca/8	14/27 ve ca	5/39 ve ca	36/31 ve ca	31/27 ve ca	5/28 ve ca	28/2 ve ca	2/39 ve ca
Number hypoploid ♀♀ crossed 1♀ x 3♂♂	276	258	266	237	220	250	117	126
Number viable after three days	205	249	233	229	213	214	101	125
Per cent viable	74.2	96.9	87.6	96.6	96.8	85.6	86.3	99.2
Number fertile	158	245	217	229	211	202	101	125
Per cent of viable ♀♀ fertile	77.1	98.0	92.7	100.0	99.1	94.4	100.0	100.0
Number of vials from which F_1 counted	20	10	10	10	5	20	10	10
Average number F_1 per vial	29.2	47.7	36.0	52.1	50.0	22.4	67.2	64.1
Number hypoploid ♂♂ crossed 1♂ x 3♀♀	212	283	208	216	213	212	156	110
Number viable	192	281	207	202	202	207	151	110
Per cent viable	90.6	99.3	99.5	93.5	94.8	97.6	96.8	100.0
Number fertile	187	266	201	200	199	204	150	110
Per cent of viable ♂♂ fertile	97.4	94.7	97.1	99.0	98.5	98.6	99.3	100.0
Number of vials from which F_1 counted	10	10	10	10	10	10	20	10
Average number F_1 per vial	59.0	66.5	50.8	60.3	83.8	66.2	70.2	74.4
F_1 ♀ ve ca ey	196	181	127	157	213	164	357	200
F_1 ♂ ve ca ey	184	149	135	155	197	145	334	162
F_1 ♀ hypoploids	79	158	111	184	205	176	361	188
F_1 ♂ hypoploids	131	177	135	197	223	177	352	194
Per cent viability of hypoploids (ve ca = 100 per cent)	55.3	101.5	93.9	122.1	104.4	114.2	103.2	105.5

VIII. THE w^{m5} AND ITS DERIVATIVES

A. B. GRIFFEN AND WILSON S. STONE

The phenomenon of "position effect" in *Drosophila* was first demonstrated and proved conclusively by Sturtevant (1925) with *Bar*. Since that time many changes of phenotype or effect accompanying change in association of genes have been ascribed to position effect. Only a few have been proven to be association effects by the critical tests employed by Sturtevant.

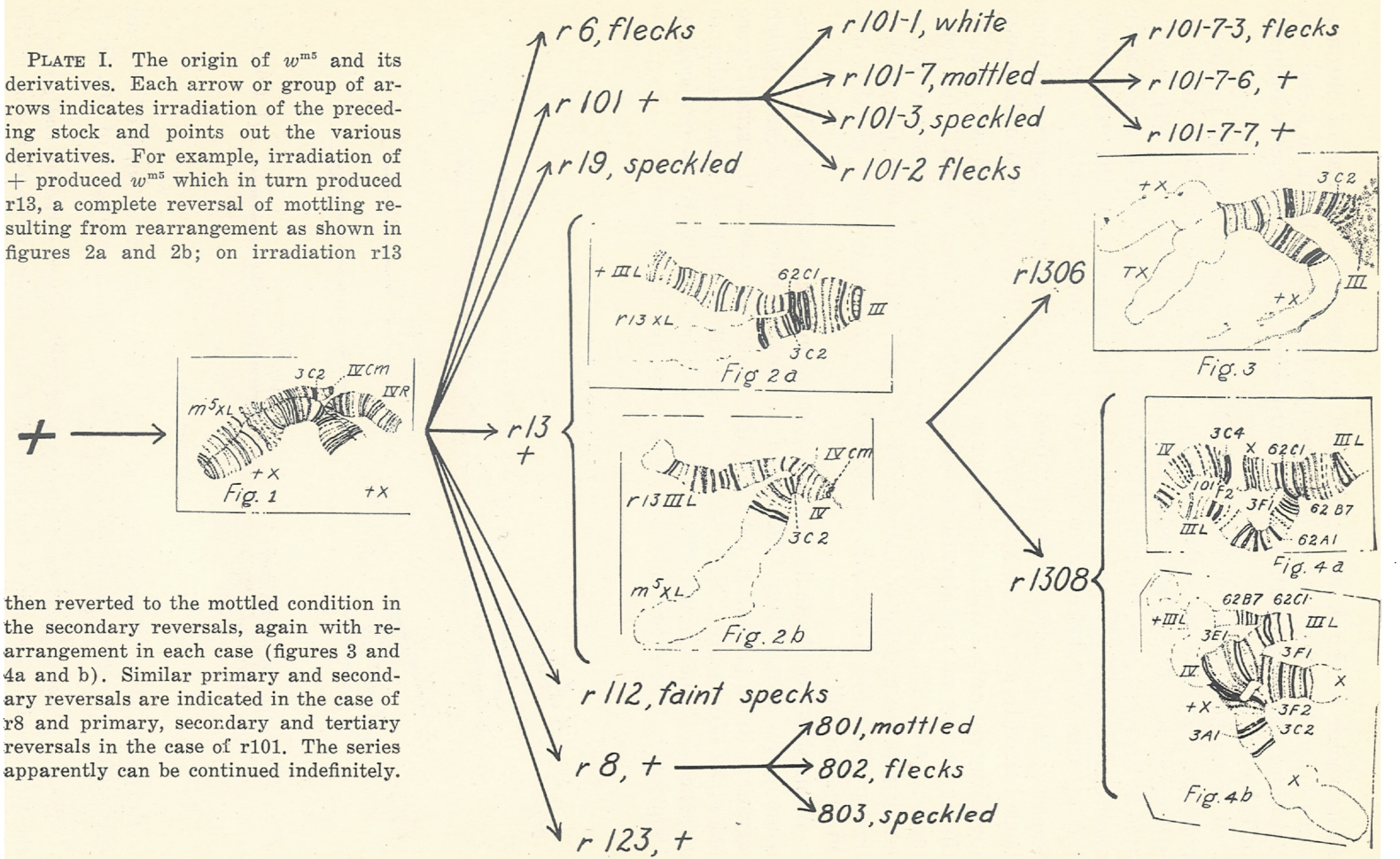
The term position effect implies that the activity of the gene is influenced by its position in the chromosome. When the association of a gene has been modified through the formation of deletions, insertions, inversions or translocations, changes may result. The alteration of the phenotype, etc., in such cases may be caused by three general types of change within the chromosomes; these are: (1) the loss of a gene or of several genes; (2) a mutation, which is a change within the gene itself; the mutation may be either dependent on, or entirely independent of the break in the chromosome; (3) a change in the result of the gene's function caused by a change in its association—an indirect effect. There may also be combinations of these types. It is difficult, if not impossible, to differentiate between these possible types of change for the majority of the cases that so glibly have been called position effects. As the situation rests at present, under the head of position effect have been lumped together all phenomena in which change of activity is related to some change in position of genes, regardless of the nature of the change; we shall therefore reserve the term association effect for those cases in which the effect is proven to be due to the immediate association of genes. The most important and best analyzed of these association effects is the case of *Bar* referred to above.

Sturtevant proved that the *Bar* effect was due to the association of the genes involved. If he separated the two sets of *Bar* genes from the same to homologous chromosomes by crossing over, he changed their effect; and if he reversed the process, placing two sets of *Bar* genes in the same chromosome, he regained the effect. In other words, effect depended on position and was altered with change in position. The cases of *hairy*, Panshin (1935) and *curled*, Dubinin and Sidorov (1935) were tested in the same manner. These three are the only cases which have been studied by such adequate tests.

MATERIALS AND METHODS

The material used in this study consists of w^{m5} and its derivatives. Other mottles will be described elsewhere. w^{m5} is a mutual translocation between chromosomes X and 4. As described in a preliminary report (Griffen and Stone, 1937), XL from O through 3C2 (Bridges' map, 1935) is exchanged for 4R through 101F2; see Figure 1, Plate 1. This case of variegation was described by Bolen (1931) who showed that genetically the *yellow*

PLATE I. The origin of w^{m5} and its derivatives. Each arrow or group of arrows indicates irradiation of the preceding stock and points out the various derivatives. For example, irradiation of + produced w^{m5} which in turn produced r13, a complete reversal of mottling resulting from rearrangement as shown in figures 2a and 2b; on irradiation r13 then reverted to the mottled condition in the secondary reversals, again with rearrangement in each case (figures 3 and 4a and b). Similar primary and secondary reversals are indicated in the case of r8 and primary, secondary and tertiary reversals in the case of r101. The series apparently can be continued indefinitely.

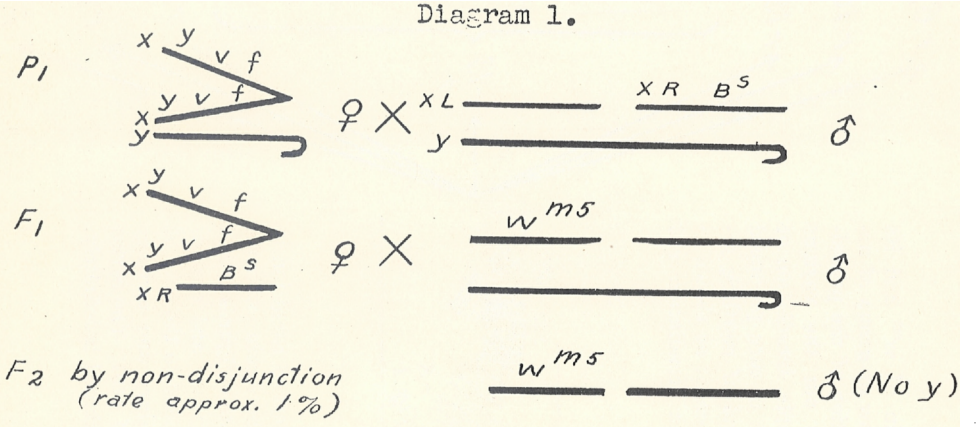


white sector of the X was exchanged for the *eyeless* sector of chromosome 4, but that the locus of *bent* was proximal to the break in 4 and was therefore associated with XL. A description of the phenotype is included in Table 3.

It is not possible by crossing over to return this XL segment to its old position; consequently it is necessary to use less diagnostic methods in attempted to relate effects with position in this system. w^{m5} was irradiated in the adult male and mated to attached X females. The F_1 males were examined for changes in phenotype. In another part of the experiment the test was extended by crossing the irradiated males to *y v f; bw; e; ey** females; the F_1 males were examined for phenotypic changes and then backcrossed to *y v f; bw; e; ey* females for the detection of translocations involving the w^{m5} XL–4L chromosome. In this case a record was kept of the frequency of translocations between chromosomes 2 and 3 for comparison with the 2–3 frequency of other experiments. Subsequently, a number of secondary translocations in which XL had been moved to a new position were X-rayed to study the relations between position and effect more fully.

As mottling in the male is much more pronounced without a Y-chromosome (Gowen and Gay, 1933, and others), a number of cases which appeared to be partial or complete w^{m5} reversals with a Y present were tested as XO males. This was accomplished by mating these males to attached-X females hyperploid for the XR of T(1;4)B^s as outlined in Diagram 1. The results of these tests are given in Table 3.

Diagram 1.



Results

Reversals of mottling to normal red in this case of w^{m5} and of w^{m4}, an inversion, were first produced in this laboratory by students of Dr. H. J.

*The *y v f; bw; e; ey* is an attached-X stock with *yellow, vermilion* and *forked* in the X, *brown* in 2, *ebony* in 3, and *eyeless* in 4. By crossing and backcrossing to this stock all linkages between these several chromosomes can be established.

Muller in 1929–1930; these reversals were not analyzed. Later, in 1935, Dr. George Mickey ran an experiment on the frequency of reversals in w^{m5} and w^{m4}. The results are given in Table 1 with Dr. Mickey's permission. It can be seen that both mottles revert to the normal red eye color with w^{m4} reversals more frequent than those of w^{m5}.

TABLE 1

Males Irradiated with Approximately 4200 r Units and Mated to *y v f* Females

Males Irradiated	Total F_1 Males	Apparent Reversals		
		Fertile	Sterile	True
w^{m4}	1950	45	9	35
w^{m5}	7015	22	0	20

The present work concerns only w^{m5} and its derivatives. A very large number of partial and complete reversals to red eye color was induced without attention to their frequency. The cases in which the frequency was measured are recorded in Table 2. In Table 3 are recorded some of the w^{m5} derivatives together with their phenotypes in the X–Y and X–O males, their linkages and their cytological analyses. The relations between position and effect may be seen here. It is evident that there are numerous positions at which the *white* locus, 3C2, will show mottling and that there also numerous positions at which it produces the normal red pigmentation.

TABLE 2

Changes in Phenotype and/or Linkage of XL–4

w^{m5}

No Change Linkage (XL–4)	Frequency of 2–3 Translocations	XL–4–2 Linkage	XL–4–3 Linkage	XL–4–2–3 Linkage	Total
1203 with no phenotypic change	115	7 reversals	4 reversals	1 reversal	1390
6 reversals		5 partial reversals	5 partial reversals		
27 partial resersals		1 speckled	3 no change	1 change in eye color	
2 speckled		1 change in eye color	1 whitish color		
1 = partial with no hyperploid females present		4 no change	1 light color	2 no change	
sub-total: 1239	sub-total: 115 = 8.27 per cent	sub-total: 18 = 1.3 per cent	sub-total: 14 = 1.0 per cent	sub-total: 4 = 0.3 per cent	

Furthermore, complete reversals to normal red (see r8, r13 and their derivatives) were returned to the mottled condition as secondary reversals, and the secondaries were in turn reversed to normal red as tertiary reversals; the tertiaries will not be discussed in detail here but have been illustrated in one portion of Plate 1 (the r101 series).

TABLE 3

Base Stock	Derivative	Phenotype of ♂		Linkage	Cytology
		X-Y	X-O		
+	w^{m5}	brownish with dark mottling	pinkish with carnation mottling	X–4	3C2/101F1
w^{m5}	r4	red, with very faint mottling	red; mottling unchanged	X–4—3	3C2/67D4
w^{m5}	r6	red, faint flecks	red, dark surface spots	X–4—3	3C2/89B1
w^{m5}	r8	+	+	X–4—2	3C2/58D3
r^{8}	801	white with red mottling	inviable	X–4—2—4L	3C2/101A
r^{8}	802	red, small dark flecks	red, speckled with black	X–4—2—3	3C2/90F5
r^{8}	803	red, speckled with black	red, more speckled	X–4—2—4L	3C2/101D
w^{m5}	r13	+	+	X–4—3	3C2/62C1
r^{13}	1306	red, mottled (less than w^{m5})	red, dark patches and flecks	X–4—3—2	3C2/41C1
r^{13}	1308	red, speckled	red, very pronounced mottling	X–4—3—X	3C2/3F2
w^{m5}	r15	+	faint flecks	X–4—2	3C2/55F4
w^{m5}	r16	white (no mottling)	white	X–4—2	3C2/33B1
w^{m5}	r18	+	speckled	X–4—2	3C2/36D3
w^{m5}	r19	red, speckled	red, dark mottling and patches	X–4—3	3C2/70A4–B1
w^{m5}	r20	red, faint flecks	red, distinct mottling and surface patches	X–4—2	3C2/35A2
w^{m5}	r21	red, faint mottling	red, mottled, dark patches	X–4—3	3C2/91A3
w^{m5}	r22	+	red, speckled	X–4—2	3C2/48B3
w^{m5}	r101	+	red, faint specks	X–4—2	3C2/23B2
w^{m5}	r109	+	red, faint specks	X–4—3	3C2/97B1
w^{m5}	r112	red, faint specks	red, speckled	X–4—2	3C2/47A4
w^{m5}	r113	+	+	X–4—2	3C2/22A1
w^{m5}	r118	red, heavily mottled	red, dense mottling	X–4—2	3C2/44A1
w^{m5}	r123	+	+	X–4—4R on X	3C2/102F1
w^{m5}	r136	red, heavily mottled	red, dense mottling	X–4—2	3C2/42B2

Discussion

The data on the rate of translocation of the XL component of w^{m5} (Table 2) can be compared to the rate for normal (+) chromosomes as presented by Patterson, Stone, Bedichek and Suche (1934) in their Table 1. The dosage used was not the same but the frequency of 2–3 translocations in the two experiments allows an accurate comparison. As there were only two-thirds as many 2–3 translocations in the experiments with w^{m5}, two-thirds as many cases involving comparable breaks in the X or 4 chromosomes would be expected. In the + series, 4.29 per cent of the cultures had breaks in the X. Only a small part of these involved the X to the left of *white* locus and none between 3C2 and 3C3 at the w^{m5} breakage point. 1.52 per cent of the cultures had translocations involving chromosome 4, but only part of these coincided with the breakage point of the w^{m5} translocation. 12.36 per cent involved chromosomes 2 and 3 only. In the case of w^{m5} 8.27 per cent of the cultures involved 2 and 3 only.

The XL component of w^{m5} was involved in 2.6 per cent of the translocations; 2.6 x 3/2 or 3.9 per cent of the cultures therefore involved this XL component in contrast to 4.29 per cent involving the complete X-chromosome and 1.52 per cent involving the complete chromosome 4 in the normal series. Furthermore, 1.95 per cent involved XL–4–2 in the w^{m5} experiment while 0.5 per cent involved 4 and 2 in the + experiment; and 1.5 per cent involved XL–4–3 in w^{m5} as opposed 0.55 per cent involving 4 and 3 in the + experiment.

Most of the w^{m5} breaks were between 3C2 and 101F1 at the point of the original breakage and union that produced w^{m5}, while only part of the translocations involved anything like the same locus in either the X or chromosome 4 in the + series. There is therefore no doubt that the point of union of XL and 4 in w^{m5} is a "weak attachment" in comparison to either attachment in the normal condition. In this case the new attachment after irradiation (w^{m5}) is less stable than the normal attachments.

We may now consider the relation of the w^{m5} series to the other valid cases of association effect. Sturtevant showed that crossing over between two *Bar* chromosomes could give double *Bar* and normal and that, by crossing over, double *Bar* could give *Bar* again. This proved that the difference in effect between *B/B* and *+/BB* was due to the association effect of the two *Bar* genes in the same chromosome. Muller, Prokofjeva-Belgovskaja and Kossikov (1936), and Bridges (1936) have shown cytologically that *Bar* is associated with a duplication; this does not modify or detract from its importance as a demonstration of association effect, but enhances its value in that the visible rearrangement in the chromosome permits detailed cytological study of the *Bar* case.

The *Bar* effect is not due to hyperploidy since other *Bar* alleles have occurred without the duplication. Moreover the normal genotype obtained from crossing over between two *Bar* chromosomes is not a reversal of position effect but merely the normal genes separated from the duplication and position effect by simply crossing over. Griffen (unpublished)

has shown that reversal to + can be obtained by irradiation of *Bar* without changing the duplication cytologically; Hansen (1928) also obtained reversals of *Bar* to + by irradiation, but these were not checked cytologically. The cases of *h* and *cu* were reversible by crossing over; hence they also were association effects. Grüneberg's (1937) reversal of *roughest* most probably was evidence for association effect, but the fact that in w^{m5} the *white* locus(3C2) may give normal eye color at some positions and mottled at others indicates that Grüneberg's reversal might not have been an exact return to the old normal association even though the cytology seemed to be normal. Grüneberg's case is not reversible by crossing over, and only the one reversal has been reported.

Since w^{m5} could not be returned to the normal association by crossing over, irradiation was employed to test the relation between position and effect on the *white* locus. In our experiments with w^{m5} and its derivatives, the phenotype was changed many times by moving the *white* locus (3C2) to various cytological positions. In no case did we return it to its original location. In some positions the eye was normally pigmented; in others it showed varying degrees of mottling (Table 3). In the majority of all the cases obtained each reversal of phenotype was accompanied by change in the position of *white* (3C2); a few partial reversals were obtained also by simple mutation at the *white* locus or elsewhere in 4 without detectable change in position. Since the changes in the amount of mottling were accompanied by breaks next to band 3C2, and on the right in each case, there are two alternative explanations for the phenotypes: either the effect is an association effect with changing associations responsible for the change in phenotype, or the breakage caused the effect independent of the association change. This last possibility is not tenable as it has been demonstrated that often a chromosome may be broken and reattached to the same point (Sax, 1940). Since it is necessary to have two breaks present at the same time for chromosome rearrangements to occur, breakage and reattachment at one point should occur more often than breakage at two points accompanied by rearrangement. Therefore we must conclude that this w^{m5} mottled effect is due to associations of genes and not to the lesions themselves. Obviously there are many associations that will cause the *white* locus to produce a mottled color. We can infer that other variegated stocks involving this locus are association effects, particularly as none have been found which are not chromosome rearrangements; furthermore we have produced a number of reverse or partial reverse mutations through X-ray induction of further rearrangements in several others of these *white* mottles, proving that they are of this nature.

In these experiments we have obtained mottled eyes with many different associations at numerous points in the chromosome complex (see the outline map forming the frontispiece to these papers). Schultz (1936), Schultz and Caspersson (1939) and others have claimed that mottling is dependent upon the association of eye color genes with the heterochromatin of the cell. This could be true only provided there is heterochromatin scattered through the chromosome complex and located at the points

where our *w* associations have produced mottling; this is obviously not the case. We may further state that there is little or no possibility that the partial reversals have carried a small amount of heterochromatin into the euchromatic portions of the chromosomes to produce mottling. In such a scheme, partial reversals would be accompanied by heterochromatin while completely normal reversals would be free of any such material; the complete reversals, however, revert to mottled without returning to the heterochromatin for a new supply of "mottling material." Finally we may state that chromosome 4 has been shown to contain no heterochromatin (Heitz, 1933; Griffen and Stone, 1940) so that the original w^{m5} stock itself is free from any heterochromatin association effect as we have defined the term.

It is true that most mottles have been caused by breaks involving the heterochromatic regions. There is however, a decided tendency for breakage to occur in these proximal portions of the chromosomes. There is also a decided tendency toward breakage in the w–fa region of the X-chromosome as evidenced not only by the frequent mottled cases but also, and even more convincingly, by the high frequency of crossingover or spontaneous breakage and reattachment in this cytologically short interval. These points must account for the fact that most original *w* mottles have been breaks at these two structurally weak (highly breakable) regions; also there are as yet few, if any, cases except those reported here in which any new association of the *white* locus has ever been formed without the production of variegation.

In the w^{m5} series there has been no evidence for cytologically detectable loss of part of the chromosome as claimed by Schultz (1936; Schultz and Caspersson, 1939) for some of his cases. It is difficult to conceive of a mechanism whereby, if mottling may be directly associated with somatic deletion, the presence of the Y-chromosome might decrease the degree of elimination and hence the degree of mottling. The fact that the Y-chromosome can have this effect and can be demonstrated as an example of maternal inheritance (Noujdin, 1936) seems most conclusive evidence that these are interaction effects through the cytoplasm. Mottling has also been claimed by Schultz and Caspersson (1939) to be related to nucleic acid metabolism; if this relation is true there is no evidence in w^{m5} that it operates through losses of chromosome parts. Schultz has pointed out that Patterson's case (1932) may not have been due to the loss of the translocated part of the X-chromosome despite the cytological evidence. However it is not in agreement with Schultz' (1938) argument that the genes nearest the break were lost most frequently by deletion and those farther away less frequently. The break in Patterson's case was to the right of *echinus;* but *echinus* did not show mottled although *notch* and *white,* genes lying farther from the break, did show variegation.

There is a remarkable similarity between the *ci* effect described by Dubinin and Sidorov (1934) and these effects of w^{m5} and its derivatives,

as well as other mottles. In each instance, some associations give observable effects on gene action, while others do not. All the $w^{mottled}$ breaks which we have checked have been at the same place, between 3C2 and 3C3; but it is not known how precise the breaks in chromosome 4 must be to produce the *ci* effect.

Plum mottles have been found to revert to normal with change in association, as well as *Cy* and *Gla* and some lethals associated with rearrangements (Suche, Parker, Bishop and Griffen, 1938). Several of these cases in addition to *Bar* are not mottles or mosaics.

Demerec (1931), while studying variegation in *Delphinium*, found that color varied with the developmental stage. He also found mottles in *D. virilis* in which variation was related to development. Rhoades (1938) has found a dominant gene *Dt* which causes mutation of a_1 to A_1 in maize. *Dt* and a_1 are not in the same chromosome, but merely have to be in the same cell to give the effect. Demerec (1937) found a recessive gene in a Florida strain of *D. melanogaster* which causes the mutation rate of numerous genes to be higher than normal.

When all of these cases are considered, there is no one explanation for all. However they do show one feature in common; the final effect is the result of gene association through the internal environment of the cell. Some of these cases have been proved to be either somatic or germinal mutations or both, as for example Rhoades' a_1 to A_1 case in corn and Demerec's mutations in *D. virilis* and *Delphinium*. We do not know whether our variegations are due to somatic mutation or not; it is certain that they seldom, if ever, give germinal mutations.

The effect of the presence or absence of the Y-chromosome and that of variations in temperature are consistent with the hypothesis that these mottles and perhaps other position effect mutations are effected by the amount, the rate of reaction, or the rate of diffusion of some substance present. The actual association effects would give varying phenotype due to the limited amount of reaction products of some genes. Some genes have certain effects in the internal environment of the cell, so limited that although they may affect the function of another gene, they must be very close together to produce their effect.

There is another group of mutations which are associated with breakage but are not variegated. Several of the different *scute* alleles, for instance, are associated with different rearrangements of the chromosomes. Muller and Prokofjeva (1934) found two *scute* alleles that had the same phenotype and the same rearrangement; they concluded that this was "a virtual proof that the nature of the phenotypic change was dependent upon the nature of the rearrangement." In view of the numerous associations that give mottling and numerous others that give normal red eye in our w^{m5}, it is by no means universally true that a certain rearrangement is necessary for the production of a particular phenotype.

When we consider these various cases of position, interaction and association effects we find that some associations must be very exact; others, such as the Y effect on mottling and the *Dt* gene in maize, need only to be in the same cell. All these cases are effects through the internal environment and are therefore genic interaction phenomena although some demand much more intimate relations for their effects. The so-called position effects are unique only in that the association effects are produced through very limited distances.

SUMMARY

(1) By means of irradiation, the *white* locus has been moved to many different positions in the chromosome complex. In some of these arrangements the eye color has been red, in others, variegated.

(2) Starting with +, a change in association by means of X-rays gave mottled which could be returned to red if some new associations were formed and which changed to a different variegated phenotype at others. It was possible to carry a series thus: + to mottled to + to mottled, etc., by changing the association of the *white* locus.

(3) Variegation was obtained with many associations in the euchromatic regions of the several chromosomes. Therefore it is not necessary for the *white* locus to be associated with heterochromatin to produce variegation. The claim that the *white* locus produced variegation only in association with heterochromatin has been advanced by many workers. Their evidence consisted only in the fact that the *white*^{mottles} studied by them were in association with heterochromatin. They never presented the complementary evidence that the *white* locus produced variegation only in association with heterochromatin. The evidence presented in this paper proves their contention to be incorrect.

(4) Variegation of w^{m5} is due to association and not breakage.

(5) The attachment of w^{m5} between the *white* locus and its fourth chromosome associate proved to be a weak spot as compared to the normal attachments.

(6) At this time it is impossible to give an explanation for all of the phenomena which have been described as position effects. All seem to fall within the general genic interaction phenomenon. True association effects seem to differ from other gene interactions only in that they depend on reactions of such a limited nature that they can be carried out only if the genes are in close juxtaposition along the chromosomes.

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IX. THE SECOND ARM OF CHROMOSOME 4 IN *DROSOPHILA MELANOGASTER*

A. B. GRIFFEN AND WILSON S. STONE

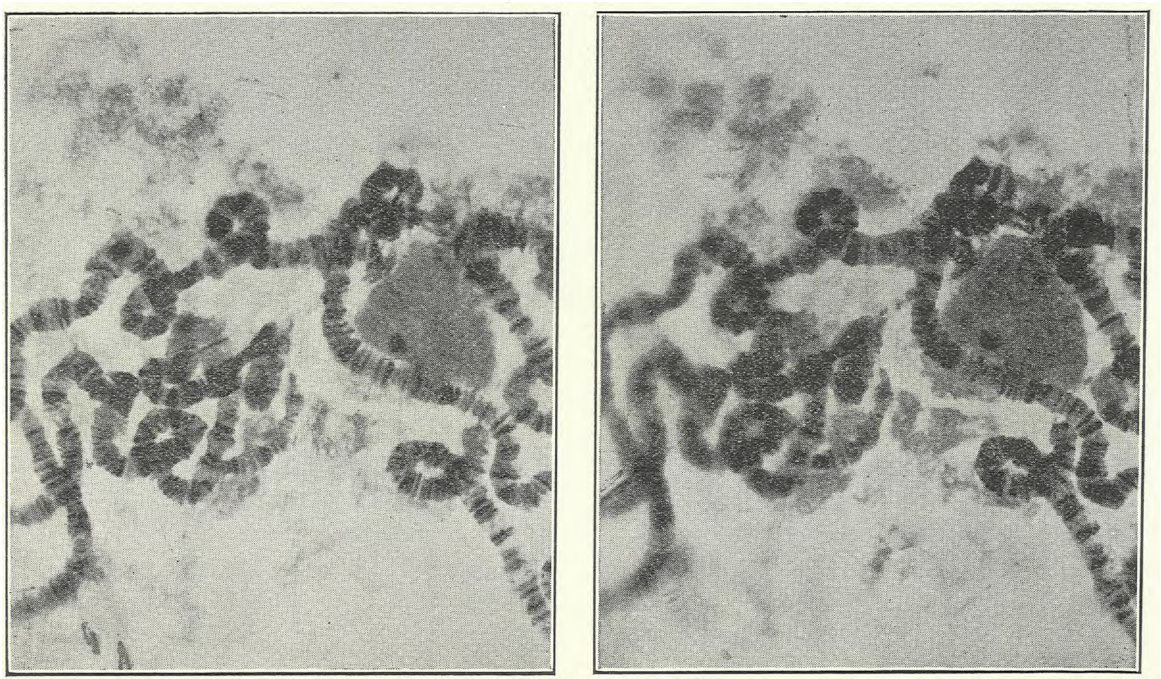
The chromosome complex of *D. melanogaster* has been supposed since a study by Metz (1916) to have a rod-shaped X, a J-shaped Y, a V-shaped second and third, and a dot-like fourth. More recently the position of the centromere in the rod and dot-like chromosomes has been questioned and it has been asserted that the centromere is not terminal. Kaufmann (1934) and Prokofyeva-Belgovskaya (1935, 1937) have presented cytological evidence that the so-called rod chromosomes are in fact J-shaped with one arm of the J very short. Muller (1938) referred to genetic and cytological evidence of Panshin and of Khvostova (later published, 1938) and of this case (published as note, 1938) that the small dot-like chromosome is in fact a small J, although the short arm is too small to be seen in an ordinary metaphase configuration. Even in the salivary gland nuclei the presence of this short arm is ordinarily difficult to demonstrate due to the facts that the two arms of a V- or J-shaped chromosome most often are separated and also to the variable nature of the so-called chromocenter region. This paper presents the details of the structure of the small second arm of chromosome 4 in *D. melanogaster.*

MATERIALS

The stock used is a translocation, T(1;4)A 18, w^{mA}, produced by irradiation of normal males. The males in this stock have very light eyes showing white or cream colored backgrounds with small red or brown spots unevenly distributed over them. When these males were crossed to *yellow white* females the F_1 female offspring were mottled, showing this case to be a mottled effect at the locus of *white.* If males were crossed to *yvf bw e ey* females* and the F_1 males backcrossed to such females, the F_2 males were all non-*eyeless* although they might or might not be *brown* or *ebony.* The *yvf* females were all eyeless. However *vf* females, hyperploid for XL, were all non-*eyeless* as well as non-*yellow.* This proved the presence of a translocation between chromosomes X and 4 of such a sort that XL was attached to the part of 4 which carries the normal allele of *eyeless,* therefore either distal to or across the centromere from this gene. The w^{mA} males crossed to *yw* attached-X females gave gray mottled females hyperploid for XL. Therefore both the *yellow* and *white* loci are in XL in the translocation.

*The *yvf bw e ey* stock carries *yellow, vermilion* and *forked* in the attached X chromosomes, *brown* in 2, *ebony* in 3, and *eyeless* in 4. This is a stock used to test for the presence of translocations between the chromosomes of *D. melanogaster.*

Plate I. Stereophotomicrograph of $w^{mA}/+$ showing the attachment of XL at *white* (3C2) to 4L near its distal end; reference to the drawings of Plate II will identify XL and 4 in the center of the photograph. The best stereoscopic effect is obtained when the page is made horizontally concave with the photograph in place before the stereoscope.



CYTOLOGICAL OBSERVATIONS

Acetocarmine smears prepared from the ganglia of female larvae heterozygous and homozygous for w^{mA} showed a striking condition of the fourth chromosome. In metaphase plates from wild-type flies chromosome 4 normally appears as a very small round or dot-like body, often spoken of as the micro-chromosome of the complex (Plate II, Fig. 1a). In metaphases from larvae heterozygous for the translocation, one chromosome 4 shows this characteristic form, while the other appears as a distinctly bilobed body with a more or less definite constriction between the lobes (Plate II, Fig. 2). This extra lobe or arm on the metaphase 4 represents in part the X fragment, including the region *yellow* through *white;* it is not possible in the metaphase condition to distinguish between the 4 and X arms of this small V, since the metaphase volume of the *y w* fragment is approximately equal to that of a normal 4. A further condition noted in the heterozygous female is that one X-chromosome is usually somewhat shorter than the other, although the difference in length is not pronounced. Finally, metaphase plates from female larvae homozygous for w^{mA} show, as would be expected, two of the small V-shaped bodies described above (Plate II, Fig. 3). These observations indicate that the X fragment is attached across the centromere from *eyeless,* as suggested in one of the two possibilities deduced from the genetic tests. The salivary gland chromosomes give full confirmation of this condition.

The chromosomes in the salivary glands of females heterozygous for w^{mA} show the details of the abnormality. As can be seen in the stereoscopic photomicrograph of Plate I and in the drawing of Fig. 4, Plate II, the *eyeless* region of chromosome 4 is intact from tip to centromere; the X fragment lies always close by, ordinarily appearing to have no relation to 4. When this translocated end of the X is studied in carefully prepared slides, the two sharp bands marking the *white* region of the X-chromosome can be seen distinctly. These bands are those beginning section 3C of Bridges' maps, of which the second, 3C2 (Bridges, 1935a; or 3C2–3 Bridges, 1938) is generally considered to represent the locus of *white;* but between this point and the clearly visible centromeres of chromosome 4 there is a peculiar expanse of material containing several distinct bands, with additional finely dotted bands often visible. Immediate checking of the long portion of the X to the right of the point of breakage revealed that none of the bands to the right of 3C2 were missing, and that in place of the translocated tip there were only two very faint new lines preceding band 3C3 (Bridges, 1935a; 3C4, Bridges, 1938), "capping" the broken end. Therefore it was evident that the left end of the X-chromosome, including the sector from *yellow* through *white* had been translocated to a second arm of chromosome 4 and that the break in this new arm was at the extreme tip, since only the two faint lines described above were visible on the proximal segment of the X.

The morphology of 4–L, as determined from the study of many w^{mA} nuclei, is not unusual. Although frequently obscured by the chromocenter,

4–L greatly resembles 4–R in its chromomeric appearance and lack of solid lines. There is no heterochromatin present in either arm (the completely euchromatic nature of 4 was first pointed out by Heitz in 1933), and the new arm can therefore be distinguished readily from any heterochromatin which may partially hide it. The presence of the X fragment on this arm tends to pull 4–L clear of the chromocenter and greatly facilitates its study. However the X is often broken away in the smearing process, leaving 4–L–R as shown in Figures 5 and 7 of Plate II, with the distinct centromere (cm) between the arms. In stretched chromosomes the total number of bands in the new arm appears to be about twelve, of which five are rather heavy and easily observed; the rest are of the finely dotted type, as indicated in the drawings.

Figure 4 is a drawing of heterozygous w^{mA}. The centromeres (cm) of 4, as well as the proximal bands of both arms, are partially covered by the chromocenter material; only the heavier bands of 4–L are visible but all the bands of the X fragment through *white* (3C2) are distinct. In Figure 7, likewise taken from heterozygous w^{mA}, chromosome 4 has been freed of the chromocenter material, but the X fragment has been torn away; here the entire chromosome can be seen in considerable detail. It will be noted that the four bundles of chromonemata representing the four original chromatids, two for each homologue, can be seen and that each chromatid bundle has its own distinct centromere; through a mechanical twist the upper chromatid pair (homologue) has been broken just to the left of the centromere, leaving the point of lesion visible on the lowermost half of 4–L; the other homologue is intact on both sides of the centromere. The heterochromatin shown in the drawing comes exclusively from chromosome 3. Similarly Figure 5 represents the fourth chromosome removed from the chromocenter with the X fragment again mechanically torn away; here, however, most of the translocated 4–L has gone with the X, leaving only the normal 4–L intact. The heterochromatin in this figure comes from chromosome 2. Finally, Figure 6 shows 4 as it appears in a normal cell; 4–R has its distal end hidden in the chromocenter material, which also partly obscures the left arm; yet the major bands of the latter arm are distinct, as are the centromeres. The fact that 4–L is clearly visible in many such normal cells precludes the possibility of the insertion of a small section from some other chromosome between 3C2 and the centromere, involving the assumption that a very complex rearrangement might have produced this new material.

Discussion

Some explanation as to why 4–L has not been seen in normal cells before the present time should be offered in this account. This body has not been definitely recognized in salivary nuclei because of its small size and its usual inclusion in the smeared-out, delicate, proximal regions of the other chromosomes to which are often added nucleolar material and considerable amounts of trapped cytoplasm; the resulting conglomeration is in most cases sufficient to obscure the proximal region of 4–R as

Plate 2

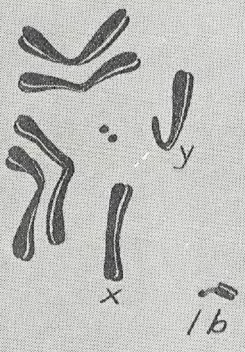


Fig. 1a +♂

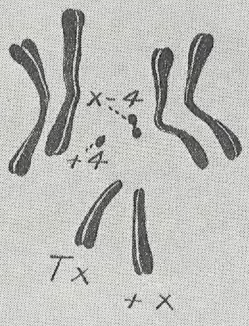


Fig. 2 A/+♀

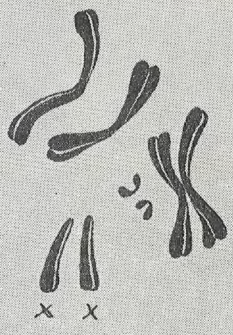


Fig. 3 A/A♀

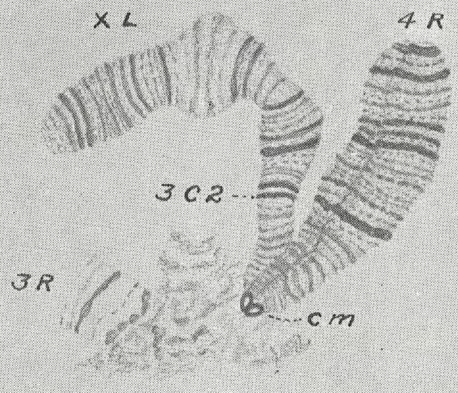


Fig. 4 mot A/+

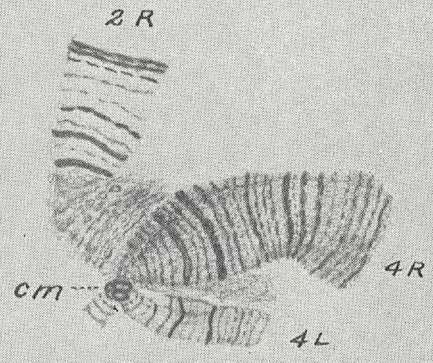


Fig. 5 mot A/+

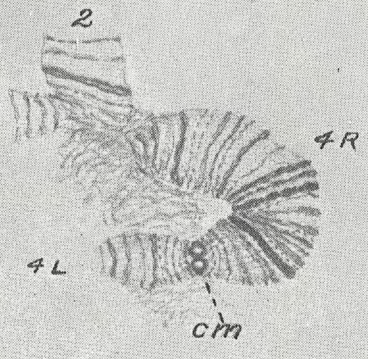
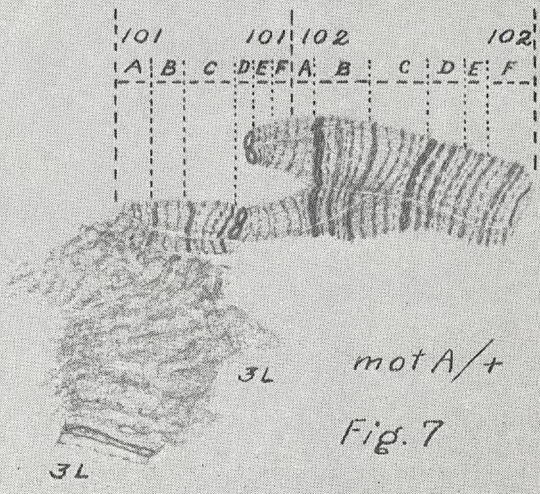


Fig. 6 +♂



mot A/+

Fig. 7

well as all of the short 4–L. After the left arm has been pulled out into view, as in w^{mA}, in a number of nuclei sufficient for convincing study, the observer can learn its morphology and then find and identify 4–L in a surprising number of normal cells which have been crushed out in the most favorable manner. The technique for such preparations merely involves the exercise of great care in the smearing process and the exclusion of fresh (temporary) mounts, in which never more than four of the most obvious bands of 4–L have been seen even in otherwise excellent preparations. It seems that the clearing which results in Bridges' technique for permanent slides is very important for this delicate region.

Although we are not presenting a detailed map for all of chromosome 4 at this time, we shall say that since Bridges has represented the known genetic portion of this body with its centromere on the left, our designation of the new arms as 4–L is in agreement with Bridges' map and allows for the use of region 101 of the salivary complex in the mapping of this body as indicated in Figure 7. With the new arm as 4–L, the arm bearing the genes of chromosome 4, becomes 4–R and its map divisions remain as in Bridges' figure (1935a).

The idea of a second arm for chromosome 4 is not a new one. Kaufman (1934) has shown this body in his Figure 4, which represents the chromosome complex as seen in somatic prophase; the fourth chromosome from Kaufman's Figure 4 has been redrawn in Figure 1-b of this paper. Also Prokofieva (1935) has suggested that 4 has a second arm, and she states that this structure tends to conjugate with the heterochromatin of chromosome 3; the diagrammatic nature of her single illustration and the lack of any other evidence supporting the observation leave little basis for any judgment of this claim. Finally Panshin and Khvostova (1938), have found a case similar to ours. These investigators, however, state that 4–L (4–R in their terminology) is microscopically invisible. It is probable that the 4–L break in their stock, as in ours, is at the distal end of this arm, since apparently no material of 4 replaces the *y–w* area on the X-chromosome; the proximal region of their 4–L is very likely obscured by the chromocenter material as we have already discussed, or it may have been deleted. The genetic evidence for a second arm as presented by Panshin and Khvostova is, nevertheless, conclusive. Since w^{mA} was analyzed, another translocation T(3;4)A, 27 has been determined to be a break in 4–L (Brown, 1940).

The proof of the existence of this short arm of chromosome 4 independently by Panshin and Khvostova and ourselves makes it probable that the cases of linkage between the X and 4 described by Painter and Stone (1935) as fusions of terminal centromeres are in fact translocations.

The clearest cases of change in chromosome number by fusion (or fragmentation) other than those experimentally produced are the cases in *D. virilis*. Here Chino and Kikkawa (1933) found a fusion of chromosomes 3 and 5 in material that had not been X-rayed. Later Hughes (1939) showed that *D. virilis virilis* differed from *D. virilis americana* in that the former had five pairs of rod-shaped chromosomes plus the dot-like

pair while the latter had only one pair of rods plus the dot-like pair. If we assume *D. virilis virilis* to be the parent form, then the other rods had become fused so that two pairs of autosomal rods had formed a pair of V's and an autosome had fused with the X. This gave a pair of X-autosome V's in the *D. virilis americana* female but there was only one V in the male for this pair as the Y and homologous autosome have not fused. Patterson, Stone and Griffen (1940) have shown which chromosomes were attached. There is a third strain, *D. virilis texana,* which has only one pair of V-shaped chromosomes.

The experimental production of change in chromosome number in *Drosophila* has been reviewed and discussed by Stone and Griffen (1940).

SUMMARY

1. The genetic and cytological study of translocation, T(1;4)A 18, $w^{\text{mottled A}}$, has shown that the distal end of the X chromosome has been translocated to the second (left) arm of chromosome 4 in *D. melanogaster.* This made possible the identification and study of this region.

2. There is no heterochromatin in chromosome 4, and therefore probably no "inert" material.

3. Since the genetically known arm of 4 has been mapped as a right arm by Bridges this arm is designated as 4–R while the new arm described here is designated as the left arm, 4–L.

4. For the purposes of mapping, region 101 (A through D) is reserved for 4–L; the known genetic or right arm then remains as mapped by Bridges.

5. 4–L can be seen and identified readily in many cells of wild type *D. melanogaster,* once its morphology is familiar to the cytologist.

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X. CHANGING THE STRUCTURE OF THE GENOME IN *D. MELANOGASTER*

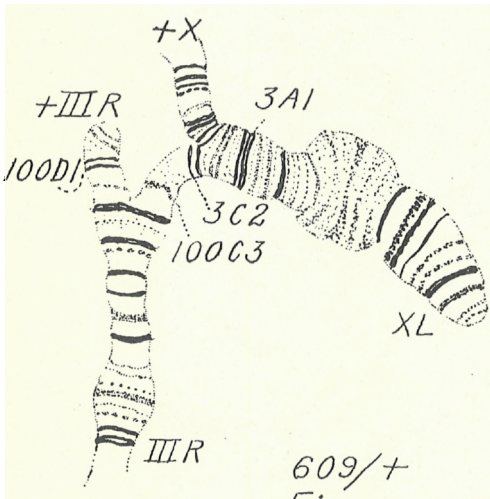
WILSON S. STONE AND A. B. GRIFFEN

Differences in chromosome number have often been associated with differences between species, doubtless sometimes correctly. The experiments discussed in this paper deal with alterations, increasing and decreasing the number of chromosomes in *D. melanogaster* as well as changing the genic balance systems. A preliminary report has been given by Stone and Griffen (1939).

MATERIAL AND METHODS

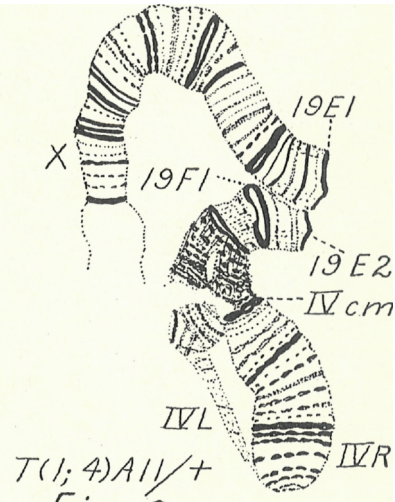
The material used consisted of several translocations. T(1;4)A w^{m5} is a mutual translocation with XL through 3C2* exchanged for 4R through 101F2. T(1;4)A 18 w^{mA} is a mutual translocation with the XL again

*The points of breakage on the cytological maps will be given from Bridges (1935) maps of the salivary gland chromosomes, see frontispiece.



609/+

Fig. 1



T(1;4)A11/+

Fig. 2

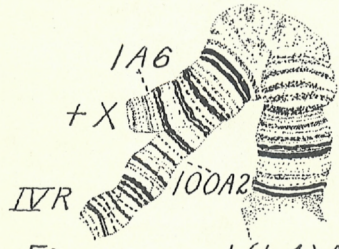
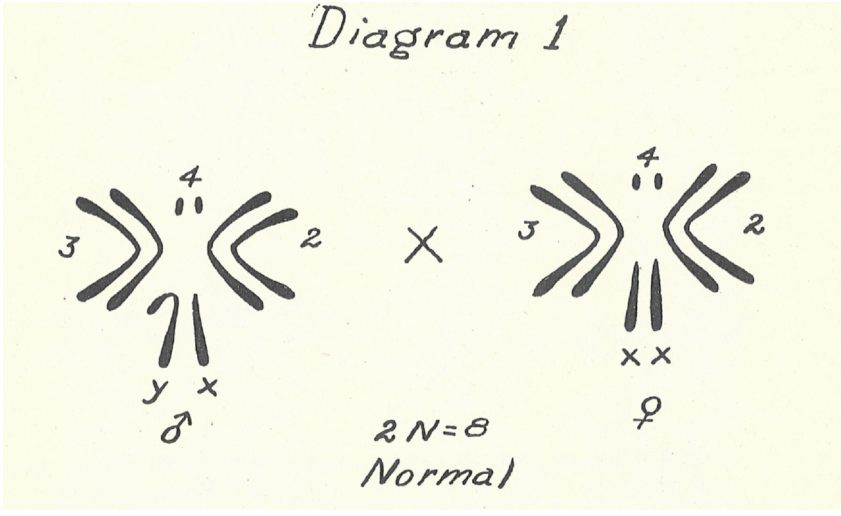


Fig. 3 T(1;4)A10/+

Fig. 4 T(1;4)A10/+

broken at 3C2 and exchanged for a very small portion of chromosome 4; here the break is in 4L near the end of this short arm of chromosome 4 beyond the fifth heavy band (Griffen and Stone, 1940). T(1;3)A w^{m609e} is a third *white* mottle with the break in the X at 3C2; the XL was exchanged for the tip of the right arm of chromosome 3 beyond the *claret* locus at 100C3 (Figure 1). These cases were selected because the breaks in the X-chromosome were at the same place (3C2/3C3) and the XL fragments could be exchanged without aneuploidy of the X. T(1;4)A 10 is a mutual translocation in which X is broken at 1A6 and exchanged for 4R through 102A1 as shown in Figures 3 and 4. T(1;4)A 11 and T(1;4)A 14 are "fusions" of the X and 4 chromosomes in the centromere region (Painter and Stone, 1935); in 11 the X apparently attached to the centromere of 4, replacing most or all of 4L by ordinary translocation (Figure 2). Two new "fusions" T(1;4)A 19 and T(1;4)A 20 were also checked. Another case, w^{m5}r123, consists of a secondary translocation from irradiated w^{m5}; here XL is transferred back onto 4R, which is attached to XR. The break in 4R is at 102F1; hence the order of the genes is O–3C2;102F1 through 101 F2; 3C3 to the centromere of the X. In effect the major portion of chromosome 4 is inserted into the X.

The normal chromosome number for *D. melanogaster* is 2n = 8 and the configuration is as shown in diagram 1. Two general methods were used in changing the chromosome number and in transforming sectors of the genome from X-chromosomal elements into autosomal elements and vice versa; these were segregation from the heterozygote and nondisjunction.



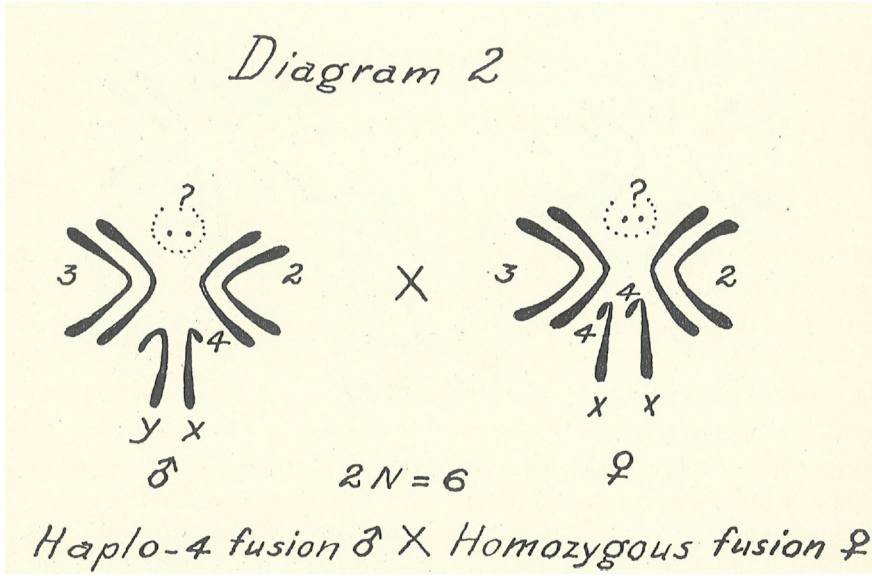
Reduction in Chromosome Number

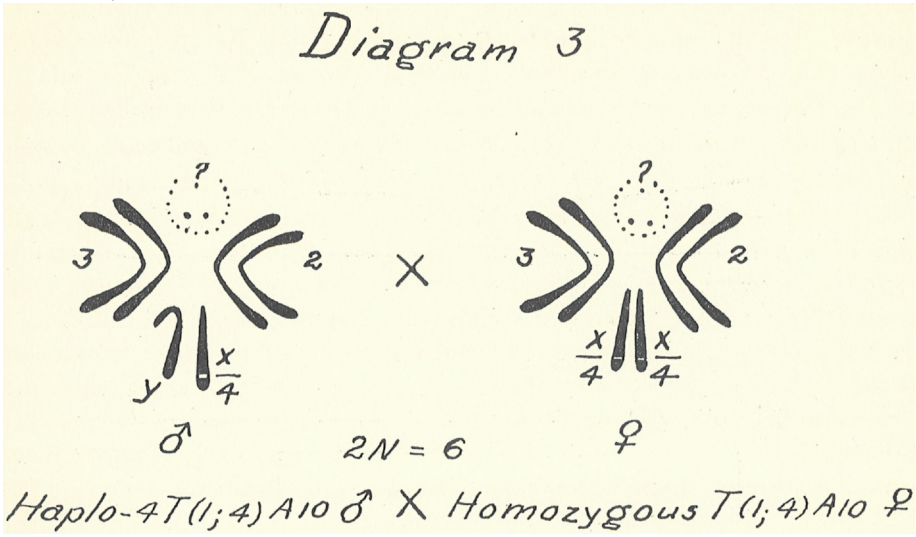
Reduction in chromosome number consisted in the elimination of the free chromosome 4 from a "fusion" stock in the male, while the females were homozygous. In this condition chromosome 4 is haploid in the male

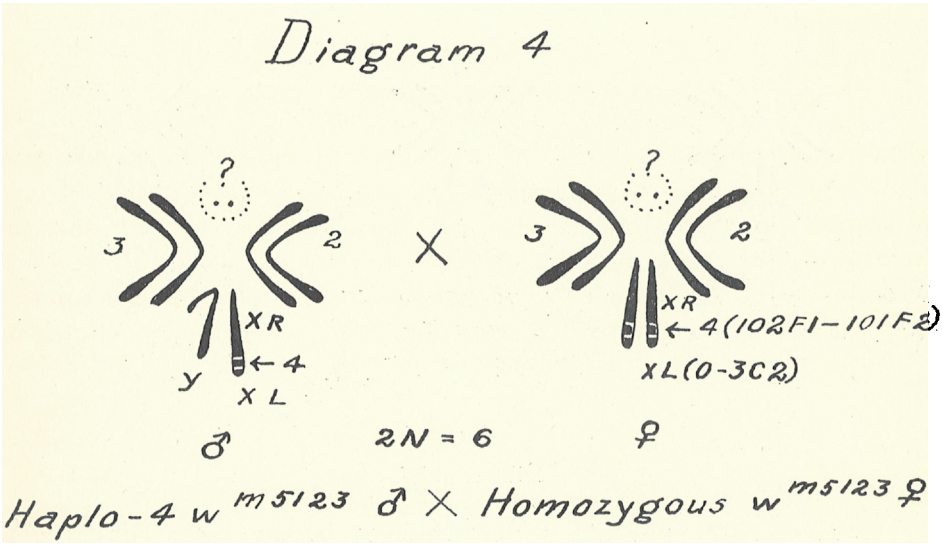
and diploid in the females; the chromosome complex then consists of a J-shaped X and V-shaped chromosome 2 and 3, making 2n = 6 as shown in Diagram 2. The other cases, T(1;4)A 10 and w^{m5123} are similar in that the free chromosome 4 is eliminated so that the male is hemizygous and the female is homozygous. In this case the chromosome complex consists of an elongated rod-like X and a V-shaped chromosome 2 and 3. In both of these cases the very small complementary part of the translocation may be present. In the case of T(1;4)A 10, the XL — 4L microchromosome may be seen in very exceptional cases (see Diagram 3 and the small body lying beside the + 4 in Figure 4); it is impossible to be sure that it is completely lost. These eliminations of the free chromosome 4 occur as the result of nondisjunction and stocks then established may be carried in this new condition. In w^{m5123} almost all the 4R part of chromosome 4 is inserted into the X between 3C2 and 3C3; when this condition is homozygous the chromosome number is reduced, provided no free 4 is present (Diagram 4). A comparison of Diagram 1, the normal condition, with Diagrams 2, 3 and 4 will illustrate how these changes are accomplished.

Increase in Chromosome Number

Diagrams 5 and 6 show how the chromosome number may be increased by utilizing the centromere of chromosome 4 in duplicate. In w^{mA} only the small tip of 4L is exchanged for XL through 3C2. In w^{m609e} only the small tip of 3R is exchanged for the 0–3C2 sector of the X. However in w^{m5} almost all of chromosome 4, 4R through 101F1 is exchanged for this same segment of the X. By segregation from the heterozygous or by

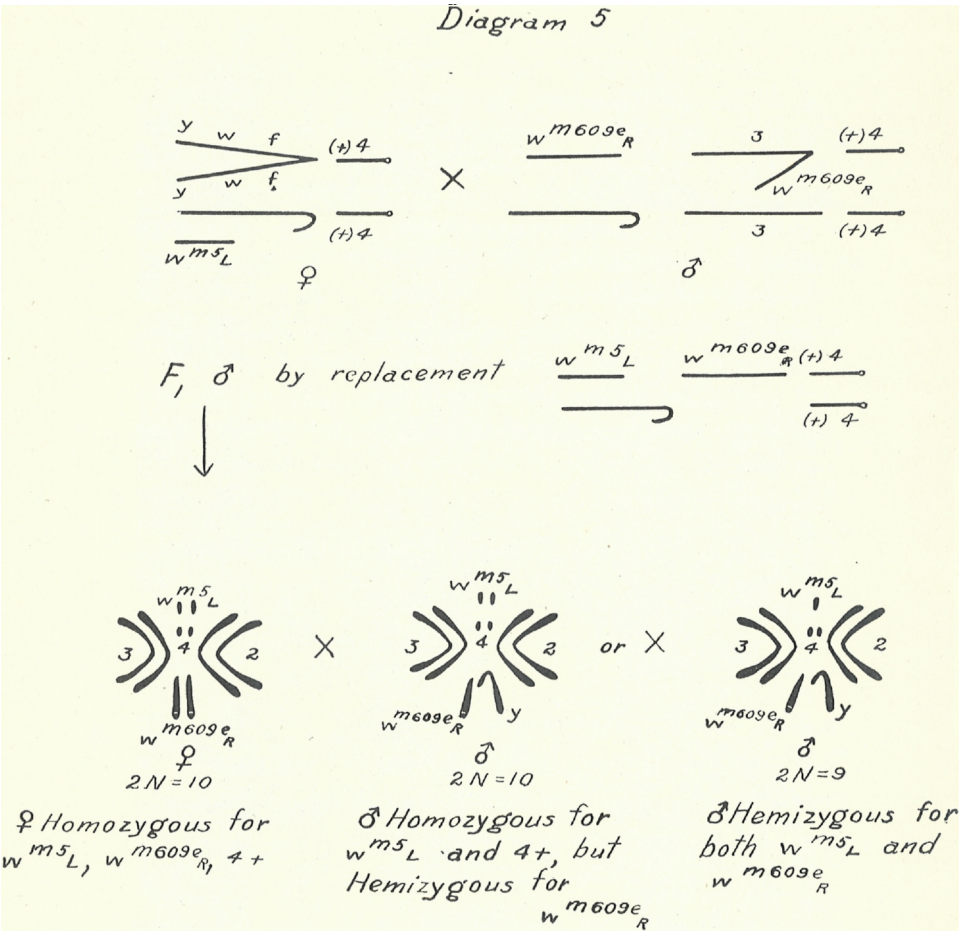






replacement from suitable crosses to females hyperploid for w^{m5}L the combinations w^{m5}L, w^{m609e}R and free (+) 4, or w^{m5}L, w^{mA}R and free (+) 4, may be obtained. A stock may be established having this complex homozygous in the female with only w^{m609e}R or w^{mA}R hemizygous in the male, but the stock must be selected or the hyperploidy for w^{m5}L will be lost by nondisjunction.

Another case of change in balance consists in choosing from a w^{mA} stock the males hyperploid for w^{mA}L without a free chromosome 4 and making a stock with these. Chromosome 4 is in normal balance; but XL is hyperploid in the male although normal in the female. Diagram 7 shows this condition which breeds true without selection although the males are reduced in viability.



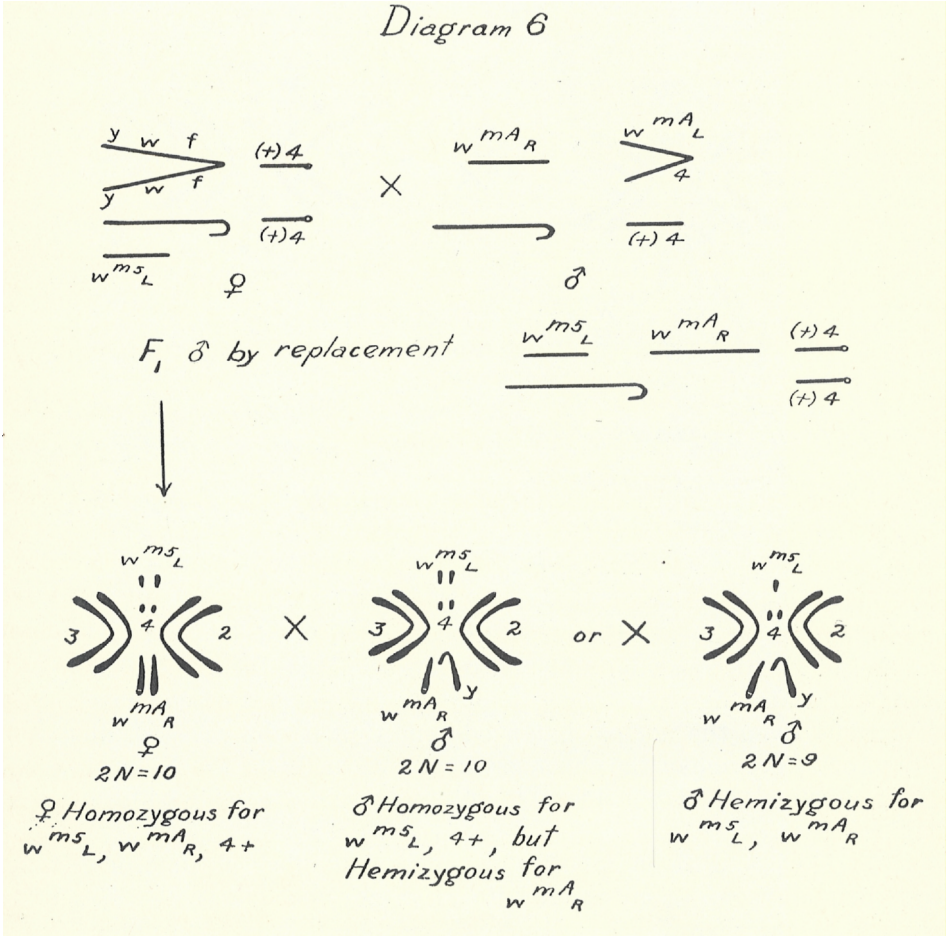
Discussion

Navashin (1932) postulated that changes in the basic chromosome number might be accomplished by translocation. Dubinin (1934, 1936) was able both to decrease and to increase the chromosome number in *D. melanogaster* through the use of techniques somewhat different from those employed in these experiments. However he was unable to change to any degree the genic balance system. We have been able to change the balance although the aneuploidy is detrimental to the viability of the hyperploid or hypoploid males in each case.

When stocks of "fusions" such as those described by Painter and Stone (1935) are established with the only chromosome 4 of the complex attached to the X, the X and small 4 form a J with one of the arms very short. Among the twenty-two X–4 translocations thus far produced in the Austin laboratory eleven were ordinary mutual translocations and eleven were

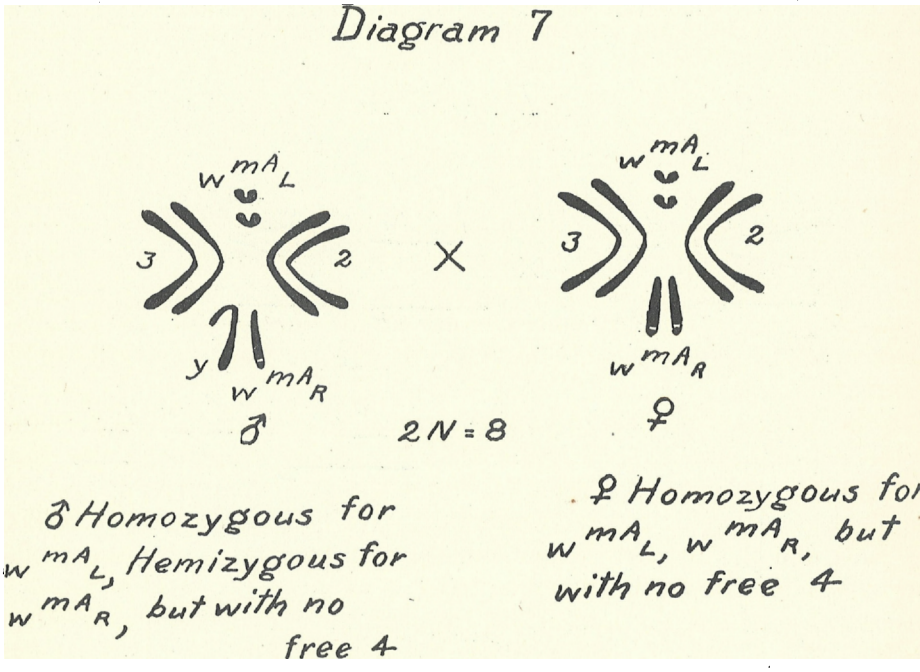
"fusions"; the latter group, like T(1;4)A 11, probably will be found to be ordinary mutual translocations between obscure portions of X and 4, such as 4L. Despite this relative frequency between breaks and "fusions" it is very interesting to note that most *Drosophila* species described have this small microchromosome free. In the forms reported without it the loss does not appear to be due to a "fusion" but most probably is simliar to the T(1;4)A 10 or w^{m5123} cases since the complex does not have a short-armed J. In *D. melanogaster* reduction in number by X–4 fusion must be accompanied by hemizygosity of 4; but many other species of *Drosophila* have rod-shaped chromosomes other than the X, so that fusion would not involve changes in the balance relations of the microchromosome.

This situation is made the more remarkable because there have been numerous changes in the chromosomes between the several *Drosophila* species and subspecies. In *D. virilis,* for example, there are four spontaneous changes from rods to V's: Chino's 3–5 "fusion" (Chino and Kikkawa, 1933) detected in the Japanese *virilis* and two in *D. virilis*



americana (Hughes, 1939), and one in *D. virilis texana* (Patterson, Stone and Griffen, 1940).

In our opinion this situation in the *Drosophila* where chromosome 4 is usually free is the result of selection for freedom of recombination. This hypothesis is made the more probable by the fact that crossing over is infrequent in the 4 chromosome. With this low crossing over, freedom for random segregation gives much more freedom for recombination than if the microchromosome were fused with a rod. If it were placed somewhere other than at the centromere, it probably would be more free to cross over, and this may explain why species lacking this dot-like body have no J-shaped chromosomes. The fact that the euchromatic chromosome arms of both rod and V-shaped chromosomes in all species of *Drosophila* are long enough ordinarily to form at least one chiasma, tends to present parallel evidence and substantiate this view.



In these experiments the other phase which is of interest is the conversion of X-chromosomal material to autosomal material or of autosomal to X material; this phenomenon is observed in several of our cases. Such deviations from the normal balanced condition in the cases studied were obligatory since one of the chromosomes concerned was the X-chromosome. Their survival is of considerable interest from the viewpoint of genic balance.

In each case of reduction in chromosome number chromosome 4 was transformed from an autosome into an X-chromosome, regardless of its position in the several cases. In this condition it was homozygous in the

female and hemizygous in the male where with the rest of the X it segregated from the Y-chromosome. In most of these cases there may or may not be present one, two or even more very small microchromosome remnants; in exceptionally good preparations of T(1;4)A 10 this microchromosome can be seen both in salivary gland cells and in ordinary oögonial or brain metaphases. These various michochromosomes consist of a centromere and two very small terminal fragments. In T(1;4)A 10 the minute body represents 4L, the centromere of 4, a fragment of 4R and the free tip of the X, making a total of approximately twenty small chromomeres carried by the centromere of 4; in w^{m5123} it is 4L, the centromere of 4, and the base and free tip of 4R, since most of 4R has been inserted into the X by the two translocations. It may be argued that as these microchromosomes can be seen only in exceptionally good cytological preparations, usually making it impossible to detect their presence, the cases presented are not, in fact, reductions in chromosome number. This is true, yet it should be pointed out that only the knowledge of their possible presence makes feasible the tedious search for these small bodies in the chromocenter regions and in the minute metaphases. We have no reason to believe they are always present twice or even once. These chromosomes would remain undetected in almost any animal or plant form as had 4L, for example, from 1932 to 1937 in the salivary glands. Such "free centromeres" can be present in addition to a normal complement of chromosomes without abnormality or detection; they could therefore play an important role in changing the chromosome number. It is our opinion that such microchromosomes are the explanation of the apparent *de novo* origin of centromeres. They might even have a positive selective value if hyperploidy for this small part were beneficial to the organism.

In the cases of increase in chromosome number there is also a change in the balance relations, provided that XL is homozygous in the male. In each of these cases XL from O through 3C2 is then present twice in the male, and 4L plus the centromere of 4 are present four times in both the male and the female. In the case of w^{mA} homozygous in the female and hyperploid for XL in the male, there is again a change in balance.

In all these cases the hyperploid male shows a reduction in viability; see for example the effect of hyperploidy of w^{m5}L as determined by Patterson, Stone, and Bedichek (1935). Despite this fact, stocks of all may be maintained with the proper care. In the stocks with the chromosome number increased, selection must be carried out each generation since nondisjunction will produce some males which are not hyperploid for w^{m5}L. These are much more viable than the hyperploid males and therefore will replace them in the stock except for selection.

In these cases we have an odd number of chromosomes in the male (see diagrams); these stocks breed true, and since the hemizygous w^{m5}L has no homolog, random segregation leads to the loss of approximately half the eggs. All other stocks will breed true despite the reduced viability of the males.

It will be most interesting to see whether and how soon these unbalanced forms can be modified by mutation to form a new "normal" balance.

These conversions of genes from X-chromosomal to autosomal genes and autosomal to X-chromosomal genes is a process which has a natural counterpart in the relations between *D. pseudo-obscura* and *D. miranda* described by Dobzhansky and Tan (1936) and MacKnight (1939). In our cases where most of 4R has become part of the X-chromosome system the genes have all been rearranged by the translocation so that they are hemizygous in the male. It is thought that in the conversion of the chromosome complex of *D. speudo-obscura* to that of *D. miranda* this transference to the hemizygous state has been gradual, by the loss of genes through mutation or otherwise from the original condition after the Y and 3 had become attached.

SUMMARY

1. By means of certain translocations, the chromosome number in *Drosophila melanogaster* was both increased and decreased.

2. Certain of these changes, in effect, transform part of the X-chromosome into an autosome, while others transform part of an autosome into X-chromatin.

3. After the change has been accomplished the X-chromatin made from the autosomal material acts as the differential segment of the X in sex determination; that is, it is present twice in the female and once in the male.

4. The autosome made from X-chromatin now is no longer part of the differential segment of the X for it is present twice in each sex. It should be pointed out that each region of the X except one can be converted to autosomal material in that it can be present twice in both sexes.

5. Each conversion studied was at some disadvantage in viability, in fertility, or in both when compared to the normal strain of *D. melanogaster.*

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XI. EVOLUTION OF THE VIRILIS GROUP IN DROSOPHILA

J. T. PATTERSON, WILSON STONE, AND A. B. GRIFFEN

It is becoming increasingly clear that the genus *Drosophila* offers unusual opportunities for studies on the origin of variations which, in some cases at least, have evolutionary significance. The clearest and simplest case thus far reported belongs to the species known as *Drosophila virilis* Sturtevant. For a number of years this species has been utilized as material for the study of genetic phenomena, but in 1936 Spencer discovered a new strain which showed that the group was not homogeneous. In that year he captured a fertilized female in Wayne County, Ohio, which showed a number of phenotypic and physiological characters that differ from those of *D. virilis*. He gave sub-species rank to this new form and named it *D. virilis americana*. In a recent article Spencer (1940) gives an account of the original discoveries of *D. virilis virilis* and *D. virilis americana* and shows that the two sub-species differ from each other in a number of important characters. He also gives the results of his genetic tests.

Since the early summer of 1938 we have been collecting the wild strains of *Drosophila* in Texas, and among the specimens captured is a number of *virilis*-like forms. Genetic and cytological studies have been made on three of these new stocks, and tests with several stocks from other places have been carried out. It is the purpose of this article to present the results obtained in these investigations. We will have occasion to refer to several other Texas strains, but these will be reported on more fully at some later time.

MATERIAL AND METHODS

The sources of the stocks referred to in this article are as follows:

(1) *D. virilis virilis* Sturtevant. This represents the Pasadena stock of *D. virilis* which we obtained from Dr. W. P. Spencer of Wooster, Ohio. This stock originally came from a single pair bred from a pineapple exposed at Columbia University in November, 1913, but Professor Sturtevant informs us that it may have been crossed at one time or another with other strains and thus may be a mixed stock. It is referred to in this article simply as *virilis*, or *V* for convenience (*gray*).

(2) *D. virilis*, Japan Stock. This is the ix[1] line obtained from Dr. G. A. Lebedeff and originally came from Japan. It is referred to as *Japan*, or ix[1], or simply *J* (*gray*, asiatic).

(3) *D. virilis*, China–a Stock. This was obtained from the Carnegie Institute, Cold Spring Harbor. This stock originated in China and in this paper is called *China*, or C (*gray*, asiatic).

(4) *D. virilis*, New Orleans Stock. This also was obtained from Cold Spring Harbor Laboratory, and was derived from an individual or individuals collected in New Orleans. It is called *New Orleans*, or *N* (*gray*, southwest).

(5) *D. virilis,* Henly Stock. This stock came from several flies which emerged in a trap bottle that had been exposed in a store at Henly, Texas, on September 15, 1938. Since *D. virilis* is a rare species, it is probable that the eggs from which this stock arose were all laid by a single female. In this paper it is called *Henly,* or *H* (*gray,* southwest).

(6) *D. virilis,* Victoria Stock. This stock came from a single female captured in Victoria, Texas, on November 19, 1938. It failed to lay eggs within a week after it was captured, and was then mated to males of the *Henly* stock. The stock is therefore probably hybrid. It is called *Victoria,* or *O* (*gray,* southwest).

(7) *D. virilis americana* Spencer. This is the first member of the second group to be discovered. Professor Spencer has very generously allowed us to use this sub-species in our tests. It is called *americana,* or *A* (*red*).

(8) *D. virilis texana* Patterson (unpublished manuscript). This stock arose from a single fertilized female captured at San Gabriel Park, Georgetown, Texas, on September 13, 1938. It is called *texana,* or *T* (*red*).

There are several physical and physiological differences between the several stocks. Examination and tests of all the stocks show that they may be placed in two main groups. The first of these will be referred to as the *red* group, and includes *americana* and *texana.* The members of this group have broadly clouded crossveins, etherize almost immediately, produce red pupae and the larvae pupate at the edge of the food.

In contrast to this, members of the second or *gray* group have narrowly clouded crossveins, etherize slowly, produce gray or black pupae and pupate up on the sides of the container away from the food. While there is some variation in the color of the pupae among members of this group, yet they fall into two classes with respect to this character. In general *virilis, China* and *Japan* have tannish gray pupae, while those of *New Orleans, Victoria,* and *Henley* are almost black, provided the population is small and the food conditions are suitable. The pupa color is variable for all. Although all forms will have tannish-gray pupae when the food is poor, the southwest stocks will form much darker pupae on optimum food. For several reasons, including a geographic one, the gray group is subdivided into the asiatic and southwest groups, and *virilis* is set off by itself.

Relationship may be tested in a number of ways by cross matings. In the first place there are tests of ability to cross between the several strains. This is better described as the effectiveness of opportunity to mate when one or more members of the opposite sex from two stocks are placed in the mating vial or bottle. In all of the matings reported in this paper the banana-karo-yeast-agar medium was used.

In the experiments, the crosses which gave the data recorded in Tables 1, 2, 3, and 4, were pair matings between flies that were at least three days old. To obtain the data listed in Table 1, enough matings were made so that when the vials were examined eight days later there would be at least one hundred with both flies living for the test. All vials in which

one or both members of the pair had died during the eight day period were not considered. In each test the number of flies was counted on the twentieth day after mating. This gave comparable data for the whole series, although it is to the disadvantage of members of the *red* group which develop somewhat more slowly than members of the *gray* group. This table gives both the total number of pairs fertile and the average number of flies per culture.

The fertility of F_1 flies was tested by inbreeding. The results are given in Table 2. Here only fifty pairs were tested. In several cases fifty pairs were not obtained, either from repeated pair or mass cultures. In all such cases the number of pairs tested is indicated in the table in parentheses. In the F_1 backcross data, and other crosses shown in Tables 3, 4, and 5, the number of pairs tested can be ascertained by adding the number of those fertile to the number sterile.

Other types of tests were carried out both for the initial and subsequent crosses. In several cases egg counts were made in order to determine how many eggs from a pair developed under various conditions. Another test employed was the dissection of both members of pairs of flies to determine whether normal sperm (motile) were present in the male, and also whether such sperm were present in the spermathecae and ventral receptacle of the female.

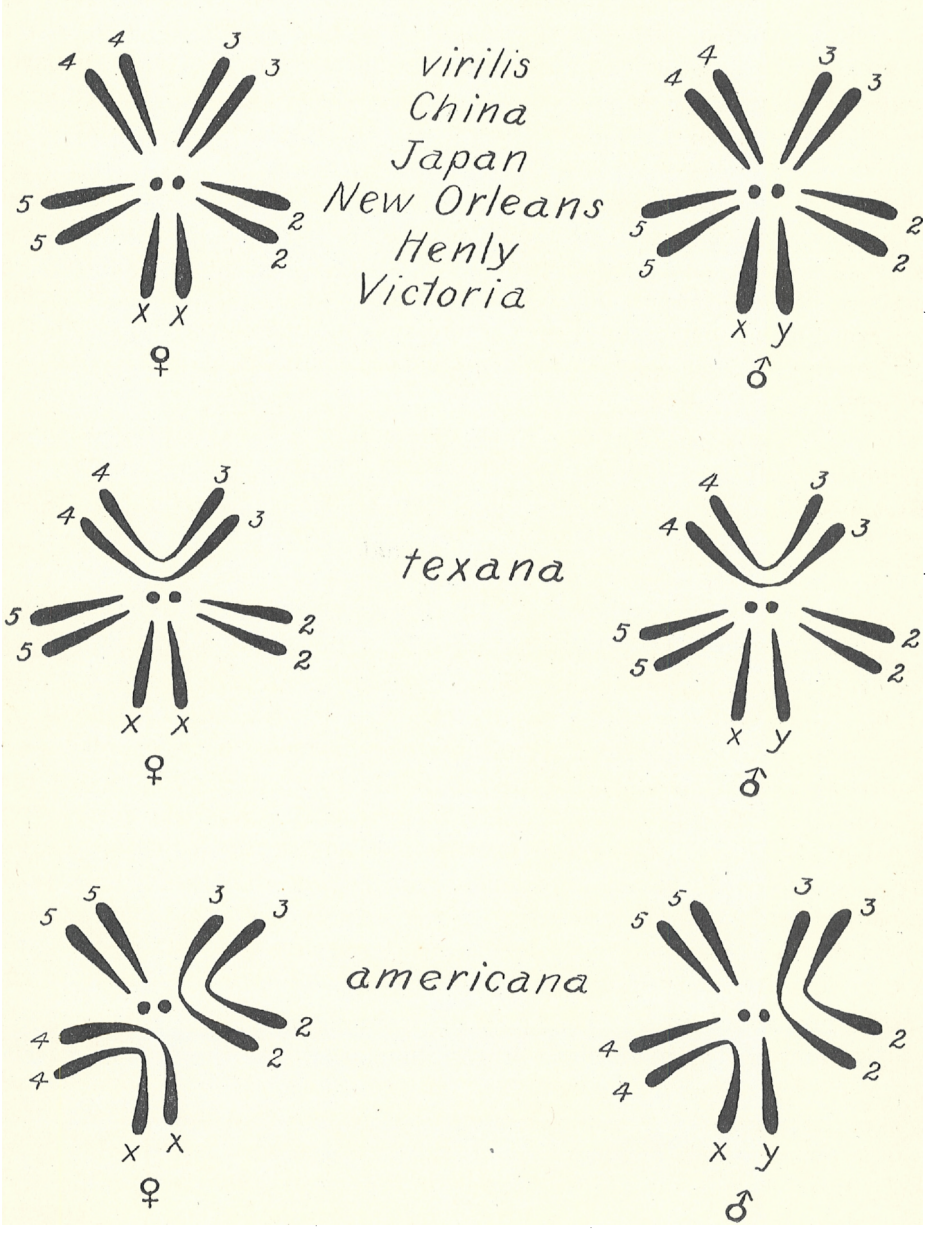
The cytological differences were determined by examining both the ordinary metaphase and the salivary gland chromosomes. This was done from aceto-carmine smears of the larval ganglion and salivary glands, respectively.

Cytological Results

The first salivary chromosome maps of *virilis* were made by Fujii (1936) and Hughes (1936), both of whom correlated linkage maps with the salivary chromosomes. The results reported by Hughes (1939) on the differences in the gene order within the chromosomes between *virilis* and *americana* may be compared to the following. Figure 1 shows the ordinary metaphase configurations of the several strains. All of the *gray* group included here have rod-shaped chromosomes and a pair of dots. *Texana* has one pair of V-shaped autosomes, while *americana* has a pair of V-shaped autosomes, and in addition the X and an autosome are attached. However, the Y and the homologous autosome are not attached so that in the male there are three V's, four rods and two dots, and in the female there are four V's, two rods and a pair of dots.

The metaphase chromosomes do not allow us to determine if the same changes from rods to V's are present in the autosomes of *americana* and *texana*. In the salivary glands Hughes has shown that *americana* differs from *virilis* in the arrangement of the genes along the chromosomes. These differ in that the order of genes in *america* is inverted simply or in a complex manner in four of the five long chromosomes, the X, 2, 4 and 5.

The texana stock differs from *virilis* in three chromosomes, the X, 2 and 5. Those in 2 and 5 are simple inversions, which are equivalent and apparently identical with those of 2 and 5 in *americana*. The X of texana has two of the three inversions which cause the difference between *virilis* and *americana* but lacks the third. The following statement of the attachments which are responsible for the changes from rods to V's may be made.



The fusion of rods to form V-shaped chromosomes involves chromosomes 3 and 4 of *texana*, both of which have the same order of genes as *virilis*. The fusion of autosomes in *americana* involves 2 and 3, so that 3 is attached both in *texana* and *americana*, although to different chromosomes. The X has fused with chromosome 4 in *americana* so that the free 4 has become a male sex limited chromosome such as the Y-chromosome. It is not, however, genetically nearly blank as is the Y, as inactivation of many genes would lead to abnormal sex ratios in crosses to other members of the group. The chromosome maps and diagrams show the difference between the several stocks. We shall consider later the evidence for the direction of the change found.

The differences due to rearrangement within the chromosomes may be seen in the heterozygous larva and thus these may be located on the salivary gland chromosome. Smears of larval brains of both males and females in the pure stocks and the crosses show ordinary metaphase configuration. The difference between the male and female brain in *americana* is apparent in the pure stocks. Also if *americana* males are crossed to *virilis* females, the female larval offspring have two V-shaped chromosomes, six rods and two dots, while the male offspring have only one V plus eight rods and two dots. In these checks both brain and salivary glands were prepared from the same larva.

As the attachment or fusion of two rods to form a V could be checked quickly cytologically, it was done that way. Theoretically the changes could have been in either direction. That makes no difference as the rods are the equivalent of the two arms of a V. Both *texana* males and females have one pair of V-shaped chromosomes. The hybrid with *D. virilis* has only one V whichever way the cross is made. The following crosses prove that it is autosomes 3 and 4 of *virilis* which are joined together in *texana*.

T ♀ X V ♂
V ♀ X F_1 ♂ (T V) individually
V ♀ X F_2 ♂ V (T V) individually

This F_2 cross gave some cultures with only gray pupae and some with both gray and red pupae. It is to be noted that the history of a test may be determined from the symbols. For example, V (T V) indicates that this is an individual derived from a backcross to a *virilis* female of a male F_1 from a cross of *texana* female by *virilis* male.

Some of the cultures having only gray pupae had a V plus eight rods and two dots in the metaphase of ganglion cells. As the X and Y from the male parent came from *virilis* the V must be autosomal. Also the *texana* inversions of chromosomes 2 and 5 were not present, i.e., all chromosomes in the salivary glands were synapsed perfectly. The V. must therefore represent chromosomes 3 and 4 of *texana*. These have the same gene order as *virilis*.

In *americana* the following tests show that chromosome 4 is attached to the X.

P_1 *A* ♀ X *V* ♂
F_1 *V* ♀ X *AV* ♂

F_2 larvae checked, brain and salivary chromosomes from each. The F_2 female larvae showed the inversion of chromosome 4 of *americana,* and in no sure case did F_2 male larvae have this inversion. The inversions of 2 and 5 of *americana* might or might not be present in either sex. If the F_2 female larvae had had two V chromosomes in the brain cells, i.e., if both the sex-linked and the autosomal V are present, then chromosome 2 having the *americana* inversion was always present. If the F_2 male larvae had a V, the autosomal one, this chromosome 2 was present. However, chromosome 5 of *americana* with its inversion might or might not be present. Consequently the autosomal V of *americana* must be chromosomes 2 and 3.

Results of Crosses

The results listed in Table 1 show that crosses between members of the *gray* group are quite fertile. Crosses between *americana* and *texana* are less fertile, while crosses between members of the two groups are least fertile of all. Three crosses were sterile, *A* X *V*, *A* X *J*, and *N* X *T*, within the limits of the experiment, but repeated crosses have proven that these are sometimes also fertile, so that none of the combinations is completely sterile. Crosses between the two groups went poorly and produced fewer offspring when they did go than crosses within the groups. The data suggest also that *americana* and *texana* cross less freely than either inbreed.

In Table 2 are presented the data on inbreeding the F_1 progeny of the several crosses. All but the cross between *China* females and *americana* males were at least slightly fertile. Certain of the data suggest differences between the reciprocal hybrids. Thus the F_1 flies from *C* X *A* were sterile but those from *A* X *C* were fertile. In general all heterozygotes of the *gray* group were quite fertile and produced a large number of offspring per vial. Usually the hybrids between the *gray* and *red* groups were more fertile than the initial crosses from which they came. Also these hybrids produced as many offspring as are produced within the *red* group in the same time period.

D. virilis virilis is more fertile to the *red* group than the other members of the *gray* group and the F_1 hybrids show higher fertility. These hybrids are more fertile both in numbers of pairs fertile and numbers of F_2 offspring than the *americana* and *texana* stocks or their hybrids, which in turn are more fertile in the numbers of pairs fertile but not in numbers of offspring produced than the hybrids between the other members of the *gray* group and the *red* group.

A count of the relative frequency of red to gray (or black) pupae in F_2 on inbreeding heterozygotes for the *gray* and *red* groups gave 4372 red to 1465 gray for twenty-three different combinations. All showed the general 3:1 ratio. On backcrossing the hybrids to the *gray* group the ratio of red to gray pupae on a small test was 234 red to 213 gray, which is the 1:1 ratio expected if red is dominant. These ratios show that red is dominant and due to one or at most several completely linked mutations.

The red pupa character of *americana* and *texana* are alleles or at least they give no recombination in the heterozygotes. This factor is located in chromosome 2 which has an identical gene order in *texana* and *americana,* so it seems most probable that the factors are alleles.

The F_1 backcross data are given in Table 3. In general these tests were quite successful, for when heterozygous females were tested only *T N* were sterile. The number tested here was small, and not all of the possible tests have been made and more were crossed back to the *gray* group.

The results suggest that the hybrids are more often fertile. Usually they produce a good number of offspring per vial. The pupa color is indicated in Table 3, and also in Table 5, where the F_2 crosses are recorded. In several cases only an approximation of the number of offspring was made. Such cases are indicated as *ca.* 25 etc. A number of crosses involved too few tests to give data on their relative fertility. Obviously F_2 flies tested together varied considerably in their genotype. Usually the female F_2 hybrids proved quite fertile. There are certain factors which influence the fertility of F_2 males. A most interesting relation is that between the fertility and a peculiar Y-autosome interrelation of both the *texana* and *americana* Y.

In this series of crosses,

P_1 *V* X *T*
F_1 *V* X *VT*
F_2 *V* X *V*(*V T*)

we find only about 25 per cent of the F_2 males are fertile and all of these give both red and gray pupa in this cross. When the cross had been made in the following manner,

P_1 *T* X *V*
F_1 *V* X *TV*
F_2 *V* X *V*(*TV*),

some 82.5 per cent of the males are fertile and give both red and gray pupae or only gray pupae. The females are alike from either cross.

These males have the same autosomes, both have the *virilis* X, but *V*(*TV*) has the *virilis* Y while *V*(*VT*) has the *texana* Y. The larval progeny from a number of the *V* (*VT*) males were checked cytologically. The *texana* V, which equals chromosomes 3 and 4, might or might not be present. The *texana* inversions of 2 and 5 always showed in some

progeny from each male, as did the red pupal color which is in chromosome 2. There is therefore some genic balance relation between the *texana* Y and certain of the autosomes, here 2 and 5, so that both these last must be present at least once before the male with a *texana* Y will be fertile. The small 6 chromosome could not be followed. The 25 per cent fertility, cited above, is of the order expected if both these *texana* autosomes must be present for fertility of the F_2 males although they can segregate at random in the F_1 (*VT*) males.

The situation is similar in *americana*. The *V* X *V(VA)* cross was only 10 per cent fertile which may mean a more complex effect. However, the Y-autosome relation is evident from the same sort of evidence. The females with the same genotype as the males were quite fertile in each case.

Table 5 shows a number of tests to determine if possible the relations of sterility factors, heterosis, etc. In these tests F_1 individuals from crosses between two members of one group were crossed to the members of the other group or similar heterozygotes between members of the other group. Sometimes the heterozygous males were somewhat more fertile than the homozygous strains, when numbers fertile are considered. There seemed to be little difference in the number of offspring per vial in these tests, so that heterozygosity of the parents, either male or female, did not materially increase the number of eggs to hatch. The heterozygous females showed a decided increase in fertility in most cases over either homozygous base stock.

Table 6 shows the results of a few tests which were made to determine if one reason for sterility was lack of mating. This proved to be the case. If motile sperm are present in the female, lack of mating or sexual isolation cannot be the reason for sterility. The data show that the low fertility in the crosses listed in Table 1 must be due in part to lack of mating. Those cases of low fertility correspond, as far as this test will show, with the cases where sperm were not often present in the females. Some of the males were checked to see if normal appearing motile sperm were present, and it was found that in all except one case the sperm were normal.

Tables 7 and 8 show egg counts and offspring from certain crosses. All cases have approximately equal numbers of males and females. When *virilis* females are mated to *virilis* males, the hatch is 92 per cent. However, when *texana* females are crossed to *texana* males or *americana* males, only about 50 per cent of the eggs hatch. Tests with *americana* inbred give no better results. In all of these cases only those eggs were counted where the female was already proven fertile by the presence of larvae in previous tests. By using this method, the eggs laid by unfertilized females did not confuse the issue and reduce the apparent hatch from the cross-fertilized females. These tests do not represent the total output of these pairs but only a short count.

Table 8 shows the effect of replacing the male of one species by that of another. In almost all cases the sperm from the second male replaced that of the first in fertilizing eggs, although in a few cases mixed cultures were obtained. Pupa color allows one to determine this in each of these cases.

Discussion

The experiments with egg counts and with dissections of females and males make it obvious that in a mixed population the red and gray groups have certain barriers restricting crossing, which in this case is due to sexual preference. Such sexual isolation was found to exist between *D. pseudoobscura* and *D. miranda* by Dobzhansky and Koller (1938). Several strains of these forms showed differing reluctance to mate. These authors also found that sexual isolation was operative but to a lesser degree between *D. azteca* and *D. althabasca*. Spencer (1940) has reported sexual isolation between some of these strains of *D. virilis*.

Moreover, if a female mates with several different males, she can under the proper circumstances, produce offspring by each of these males. In each case the sperm from the last male to mate with her would most usually be effective in fertilization, although some mixing does occur. This type of result was already known to occur in *Drosophila*. It is not restricted to *Drosophila* for Nabours (1927) reports that a similar situation exists in the grouse locust *Paratettix texanus*.

In addition to the barrier due to inhibitions which prevent cross mating, the eggs do not hatch well even from fertile females. The egg counts shown in Tables 7 and 8 are all from females that had already produced larvae and therefore were fertilized. As there is no information on the number of eggs laid by unfertilized females in wild populations, we can only relate the fertility to the egg hatch after fertilization with males from the several different stocks.

Under the food and temperature conditions of these experiments the several strains crossed with different frequencies. The egg counts showed that when a cross was affected, different percentages of the eggs hatched. This is true even for the reciprocal crosses between *texana* and *virilis*. These differences between the strains may be taken as one measure of their relationships.

The cytological differences between the strains can contribute nothing *per se* to the sterility of the initial cross between them, although they may affect the fertility of hybrid offspring. The number of aneuploid gametes of the hybrids might be increased by the differences present, but these are not sufficient to produce sterility due to aneuploidy of all gametes. The hybrids between *texana* and any one of the gray group are heterozygous for one double inversion in the X and the relatively short inversions in chromosome 2 and in one in 5. Beadle and Sturtevant (1935), Sturtevant and Beadle (1936), and Stone and Thomas (1935) have shown simple inversions do not give many abnormal gametes in *D. melanogaster*. Even

though crossing over is more frequent in *D. virilis* (as one would suppose from the maps), these inversions should not lead to a serious loss of zygotes from abnormal sperm or egg nuclei. Single crossing over within the bounds of heterozygous simple inversion segregates only non-crossover strands to the gametes after the maturation divisions. Only four-strand double exchange (i.e., only about one-fourth of the double exchange) leads to chromosome loss. Neither causes an appreciable amount of nondisjunction. These simple inversions are autosomal and would produce inviable zygotes as frequently as four strand double exchange occurred. We presume that crossing over occurs only in the female. This, however, cannot explain a serious reduction in egg hatch from a hybrid female and should not give rise to any abnormality in a hybrid male. *Americana* has the same simple inversions in chromosomes 2 and 5 as *texana*. In addition, there are complex inversions in the X and in 4, and the complex related inversion in the *texana* X. These would form two chiasmata within the inversion even less frequently than the simple inversions. These inversions can explain only part of the reduction in fertility of the hybrid females, and should produce little effect in the hybrid males. Although *americana* and *texana* have V-shaped chromosomes, there are no rearrangements involving two arms across a centromere. All rearrangements are within one chromosome arm. Kikkawa (1936) did report a change which resulted in two stocks of *D. montium* which differed in that one had a rod and another had a V, seemingly by inversion (i.e., rearrangement within the chromosome). However this case seems to involve heterochromatin for the most part and we do not know how much effect that it had on disjunction.

Inversions are quite widespread within species. Sturtevant (1931) and Dubinin et al. (1936, 1937) have shown inversions to be frequent in the wild populations of *D. melanogaster*. Tan (1935), Sturtevant and Dobzhansky (1936a), Dobzhansky and Sturtevant (1938), Dobzhansky and Queal (1937), and Koller (1939) have shown the extreme frequency of inversions in both races A and B of *D. pseudoobscura* (see also review by Dobzhansky, 1939). In fact it cannot be said that any one arrangement is the normal one for the third chromosome where at least 18 different arrangements are known, which occur with different frequencies in the several localities occupied by this species. Kaufmann (1936) and Kikkawa (1938) have described some six inversions in *D. ananassae* which were remarkable in that four described by Kaufmann for the eastern United States were present in the Japanese strains.

Numerous inversions have been described in *D. azteca* by Dobzhansky and Socolov (1939) and in numerous other species of *Drosophila*. This widespread and general occurrence of inversions in *Drosophila* proves that simple inversions do not suffer in selection or cause abnormality by their effect on disjunction or elimination of chromosomes.

There remains another possibility. Due to the complex nature of the inversions in chromosomes X and 4 in *americana*, and the X of *texana*

single crossing over might give rise to aneuploid gametes. This in effect is equal to exchange between two similar but not identical inversions. However, Dobzhansky and Sturtevant (1938) showed that just such similar inversions were very common together. It is probable that even less crossing over would occur between the *virilis* or *texana* and *americana* X-chromosomes and 4 chromosomes than would occur in such heterozygous populations of *D. pseudoobscura* so that this factor must be negligible.

In addition to the differences due to inversions, there are one or two fusions which can effect disjunction in a hybrid. These are the replacement (effectively at least) of the two centromeres of two rods by one centromere, presumably one or the other of the two present on the rods. These change two rods into a V-shaped chromosome, which is its genotypic equivalent. In these cases no gene order change was effected. These fusions, the V-shaped chromosome, have certain effects on disjunction when heterozygous with their homologues, the two rods. As far as analogy is concerned, they are similar to the 3–4 translocations 36, 31, and 27 studied by Brown (1940) in *D. melanogaster.* In these latter cases the males gave slightly over 50 per cent disjunctional gametes and nearly 50 per cent nondisjunctional gametes. The females gave over 60 per cent disjunction. In fact one of them, 31, gave between 70 per cent and 80 per cent normal disjunction. If *virilis-texana* hybrids crossover normally when no gene order change is present, the V-shaped chromosome in *texana* should effect disjunction in the hybrid females even less than those heterozygous translocations in *D. melanogaster* effected disjunction, as chiasma frequency is higher in *D. virilis.*

However, it would affect hybrid males if the condition acts at meiosis as in *D. melanogaster,* so that aneuploid gametes would be formed. The X-autosome fusion of *americana* would not crossover normally, due to the presence of the rearrangements, so it might act more like the condition in the male. This would lead to more nondisjunction in *americana* hybrids and the fusion of the 2 and 3 autosomes would contribute to some further nondisjunction. These would lead to a reduction in egg hatch from some of both male and female *texana-americana* hybrids as well as all *red-gray* hybrids, but not to complete sterility.

There is another factor. A considerable number of tests within *texana* and within *americana* (Table 1) are sterile. This sterility, which so far as we know has nothing to do with hybridity in these stocks, may contribute markedly to the sterility of the hybrids. In this case sterility would be due to factors other than hybridity. It does not seem probable that this is more than a contributing factor.

There is another effect apparent from Table 2. When *red-gray* hybrids are inbred, on an average they are only 60 per cent as fertile (in numbers fertile) when the *gray* group is used as the female parent as when it is the male parent. If *virilis* is omitted the other five *gray* stocks as female parents give hybrids only about 46 per cent as fertile as those when they are used as male parents. The reason for this difference is not known. It might be due to the genotype of either parent.

Another factor affecting sterility of F_2, F_3, etc., males is the Y-chromosome-autosome relation of *texana* and *americana* stocks. This is a genetic and not a chromosomal relation. The effect is through some activities controlled by the Y and necessary for fertility in the male. Heterozygous *americana* or *texana* autosomes may be fertile without their usual Y (i.e., with a *virilis* Y), but for a male to be fertile with a *texana* Y, autosomes 2 and 5 must be present. The autosomal factors necessarily must be dominant as they have to be present only once. It is not known how many genes are involved. The situation with *americana* is similar although it may be more complex. This peculiar interaction relation causes a high percentage of hybrid sterility in the F_2 and F_3 males where the correct chromosomal combinations may be missing. This interrelation may be due to a previous translocation between the Y and autosome(s), but no proof has been found.

Whether this Y-autosome relation has any additional functions in the homozygous stock is unknown. These autosomal factors do not seem to affect female fertility. The one test of the *virilis* Y, *T(TV)* X *T(TV)* was 86 per cent fertile. So there is no indication that the *virilis* Y is similarly dependent on an autosomal factor. In many of the possible cases the Y-autosome interrelation reduces the effective recombinations possible, as it makes many of the recombination males sterile, instead of merely reducing their fertility as do the chromosomal rearrangements. It thus provides a very efficient, if not complete, barrier between the *red* and *gray* groups.

The role of the Y-chromosome is not like that reported for *D. pseudoobscura* by Sturtevant (1937), although it is similar in reducing the number of recombinations and affecting the females to a much less extent, or not at all. In *pseudoobscura* the interaction leads to the death of part of the males, here it leads to sterility.

There are numerous differences within and between the groups in both morphological and physiological characteristics. Some of these have been mentioned and were utilized in the experimental work, and some of them have been described by Spencer (1940). Differences exist also in different stocks elsewhere as mentioned by Kikkawa and Peng (1938) and Fujii (1940). A discussion of these relations will be reserved until a more thorough and complete study can be made. A number of morphological differences have been demonstrated between the races of *D. pseudoobscura* by Mather and Dobzhansky (1939) as well as many variations in the shape of the Y-chromosome, Dobzhansky (1937b).

There is evidence in size and other characteristics that the F_1 hybrids between the *gray* and *red* groups have the vigor often associated with heterosis. This is true also when crosses are made within the groups. When hybrid males are backcrossed the viable offspring are usually normal in phenotype, despite the sterility of some individuals, and the sex ratios obtained in numerous experiments have all been approximately normal. Therefore the general genic balance is sufficiently similar so that when a chromosome of one group is replaced by one of another group—and this

is of course limited by the fusions—the resulting individual is phenotypically normal.

The strength of linkage and ability to recombine is necessarily different in the several strains. The effect on the genotype and balance within the chromosomes is yet to be determined, as well as more critical evidence, that all types of chromosome recombination are normally viable. This is not so for fertility, due to the Y-autosome relations of *texana* and *americana* in certain cases in the male.

Relationships in D. Virilis

The members of this species here reported may be divided into groups by means of the several tests employed. First there is the asiatic group consisting of *Japan* and *China*. Next is the southwest group of *Henly, Victoria,* and *New Orleans*. *Virilis* must be separated off by itself. Then there is *texana* and finally *americana*. Spencer (1940, and previous notes) has given *americana* subspecies rank. Patterson has also given *texana* subspecies rank. Although the other three classes are different, yet they cannot at present be separated; therefore we place them all in the subspecies with *virilis*. We have temporarily divided them into five subgroups, but this does not imply that either the asiatic or the southwest group is homogeneous.

There is evidence from geographical distribution, phenotype, genetic, and cytological relationships which should be considered.

The asiatic stocks are from China and Japan. Stocks from different parts of Japan differ in phenotype (Fujii, 1940), and Spencer (1940) states that some China and Japan stocks examined by him are also dissimilar.

According to Kikkawa and Peng (1938), the species *D. virilis* is common in these asiatic countries and has been collected at some forty stations in Japan and Korea. The species *D. virilis* is rare in the United States. Spencer (1940) reports that members of the *gray* group have been collected three times, once each at New York (the *D. virilis virilis*), Terre Haute, Indiana, and Los Angeles, California. In addition, there is the New Orleans strain. Spencer reports four collections of members of the *red* group, two from Ohio and two from Tennessee. None of the data gives any idea of the comparative frequency of these in the population, except that *D. virilis* is fairly common in Asia but rare in the United States. Patterson has the following data. Members of the *red* group have been collected at four places in Texas, one on each occasion. Members of the *gray* group have been collected at one place in Louisiana and six places in Texas. In all, nineteen adults have been captured (exclusive of twenty-three raised from one trap bottle) in these places. In a total collection of approximately 400,000 adult flies, this gives an average of about one in 20,000 adults for the *gray* group and one in 100,000 for the *red* group. This represents samples from over seventy localities in the southwest. At only one locality have both groups been caught, and here over 120,000 specimens have been taken.

Although not the rarest species, *D. virilis* is one of the very rare forms in the southwest. We are not able to determine from the data at hand its comparative density elsewhere in the United States, except that it seems to constitute a comparatively sparce population or a residual one.

We could take as our hypothesis that the asiatic is the parent stock because of the extent of the asiatic population. That might also mean that it was better able to compete and survive there, not that it originated there. Of the stocks studied, the divergence is such that the *Japan* and *China* stocks fall at one end of the series with *americana* at the other. The southwestern stocks behave as though as far removed from *americana* as do the asiatic stocks. This relation depends on gene differences as shown by cross tests. *Texana* lies next to *americana* linking it to *virilis*.

There are two probable arrangements in the *virilis* complex. These are:

			gray	red	
1.	Asiatic	Southwest	*virilis*	*texana*	*americana*
	(*Japan*, *China*)	(*Henly*, *Victoria*, *New Orleans*)	(New York)	(Texas)	(Ohio)

			gray	red	
2.	Asiatic		*virilis*	*texana*	*americana*
		Southwest			

Any one of the several stocks may have been the original one. It seems most probable that *virilis* is the nearest one of its group to the *red* group, as it crosses more readily and the F_1 hybrids are more fertile. It may be argued that *virilis* is a hybrid stock and that cross-sterility factors, of necessity recessive, had been lost from the heterozygote. This lacks proof. There is no evidence that *virilis* is more closely related to the Southwest stocks than to the asiatic stocks except the geographical factor of distance. All the members of the *gray* group are alike cytologically so their relations cannot be checked in that way. The only exception, and it throws no light on the present question, is that reported by Fujii (1940) that the small 6-chromosome of *New Orleans* stains less intensely. *Texana* is more closely related cytologically to the *gray* group than is *americana*. In gene sequence *texana* has a simple inversion in chromosome 2 and one in 5, although chromosome 5 may have an additional change that is incompletely analyzed. Also the X has two overlapping inversions. *Americana* has an additional overlapping inversion in the X, those in 2 and 5, and a double inversion in 4. As to gene grouping, *texana* has one fusion with respect to the *gray* group while *americana* has two different fusions. The ease of etherization, length of period of development, red pupa color, clouded crossveins, pupation pattern, and Y-chromosome interrelated genic balance are common to both *texana* and *americana*.

This designation of relationships is tentative. It fits the data at hand but there are as yet too few stocks available to test it thoroughly. Furthermore the stocks reported here and several others from the Southwest are

appreciably different in genotype. From the reports this is also true for the asiatic stocks.

The populations of the *virilis* group in the United States are very sparse. They therefore are subject to the effects inherent in the evolution of small populations (Wright, 1931). They are expected, and do, show the effect of considerable variation. It is perhaps more remarkable that members of the *gray* group are so alike cytologically, and that they are so cross fertile. None of our tests has demonstrated marked differences between the asiatic and southwestern stocks except that hybrids between them cross slightly more readily with the *red* group. *Virilis* is similar to both except that it crosses more readily as females to the *red* group. In addition, all female hybrids between *virilis* and either the southwestern or asiatic groups cross to the *red* at least as readily as *virilis*.

This suppression of some isolating factors of both asiatic and southwest groups, even though hybrids between the asiatic and southwest groups still have isolation, argues against the hypothesis that *virilis* is a hybrid stock unless it is an introgressive hybrid (Anderson and Hubricht, 1938), between the *reds* and *grays* back to the *grays*. Other evidence that *virilis* is more closely related to the *red* group than other members of the *gray* group consists in two types of evidence from Table 2. The *virilis-red* hybrids are more fertile in numbers fertile than other *gray-red* hybrids. Also reciprocal crosses give hybrids which are equally fertile on inbreeding. Other *red-gray* hybrids are at least twice as often fertile on inbreeding if they come from *red* females by *gray* males. The number of progeny for a cross is not different, so it seems most probable that it is the sexual isolation factor which has been changed. There is some indication of heterosis here also. The factor or factors which cause the asiatic and southwestern stocks to cross less readily with *americana* and *texana* are therefore recessive to genes present in *virilis* that allow the cross. It is at present impossible to say whether the isolation genes are alleles in the asiatic and southwestern stocks, or multiple factors which sum to about the same effectiveness in both parent stocks and in the heterozygotes.

There is a number of differences between the nearest relatives of the *grays* and *reds, virilis* and *texana*. First, there are the inversions, but these are so common in *Drosophila* populations that they cannot *per se* be of primary importance.

They cannot be the initial isolating mechanism. Their wide occurrence within other species has been pointed out. We do not know whether any of the inversions were associated with position effect mutations.

Second, there are the gene differences which are of several categories. Among these are Y-autosome interrelation and those causing morphological and physiological differences, such as pupa color, clouded crossveins, length of developmental period, and etherization reaction. Finally, there are those directly concerned with crossing, some causing inhibitions to mating while others are responsible for poor egg hatch from crossfertilized females. We do not know if this poor egg hatch is due to developmental abnormalities or to lack of fertilization of the egg by sperm even

in a fertilized female. However there is no such drastic effect on development in the *red-gray* hybrids as that described by Kaufmann (1940) in certain *D. pseudoobscura–D. miranda* hybrids. In addition to the gene differences and inversions there are also the fusions to form V-chromosomes from rods.

There are several differences in the genotype between *texana* and *americana*. There is the additional or stronger Y-autosome relation of *americana* as shown by hybrid fertility tests. Also there are some further modifications of the tendency to cross. There seem to be some barriers to mating between *texana* and *americana*. In addition, there are the inversions, one in the X and two in the 4. Finally, there are the two fusions in *americana;* certainly the one between X and 4 is of independent origin. The one between 2 and 3 of *americana* may have been derived from that between 3 and 4 of *texana* by translocation. We have here a series of changes culminating in the many differences between the asiatic and southwestern forms at one extreme and *americana* at the other.

The question of "fusion" versus "fragmentation" to explain the changes in chromosome number cannot be settled. Fusion of two rods to form a V has been found in *D. virilis* by Chino (Chino and Kikkawa, 1933) in an untreated stock. It has been accomplished by radiation, indirectly by Dubinin (1934, 1936) and directly by Painter and Stone (1935) in *D. melanogaster*. In the direct cases one centromere of the two (in this case the X and 4) could be left free with very few and perhaps no necessary genes with it. Such "free centromeres" might be lost or they might be carried in excess. This would be particularly likely if the genic balance was such that hyperploidy had an advantage. Such free centromeres can be produced by mutual translocations if the parts exchanged are so unequal that a microchromosome is formed (see the discussion in Stone and Griffen, 1940).

The supposition that it is more probable that the "fused" V was derived from two rods depends on the fact that such a fusion is one event. Fragmentation depends on two events at least; either a microchromosome (free centromere) was present due to a previous translocation or deletion, or a complex segregation from two heterozygous translocations has occurred. Fragmentation could depend on one event only if *D. virilis* differed from *D. melanogaster* in that a broken (raw) end, in this case a centromere, could survive as such. If that were so and the centromeres of *D. virilis* were compound so that it could be broken into two functional parts, then fragmentation could occur in one step. The centromere in maize was so divided by McClintock (1932). This, however, was a deletion which formed a ring so there was no broken (raw) end and therefore is different from that necessary here.

In such a sparse population as *D. virilis* it is not difficult to see how chromosome rearrangements, either fusions or inversions, would become homozygous by inbreeding in semi-isolation. These homozygous strains would lose relatively few of their gametes through cross-breeding, so their reproductive efficiency would not be impaired. This situation would

still be such as to utilize some isolating factors, but certainly the need for genic isolation is much less here than in a dense population.

The situation with regard to the X–4 fusion in *americana* could become established as well as any other fusion. In fact it would be easier because the Y is unique and necessary. Males must have it to be fertile; they must also have a pair of chromosome 4. Therefore, once the present condition had occurred, the descendants of such a stock would breed true, even if the lack of Y-4 fusion caused the production of some aneuploid sperm. This would favor a mechanism that would ensure directed disjunction. Thus far it has been impossible to obtain a satisfactory egg count in *americana*. This may be due in part to this unusual sex chromosome mechanism. The situation is not comparable to *D. miranda* (Dobzhansky, 1935, 1937a; Dobzhansky and Tan, 1936; McKnight, 1939), as there is a cytologically normal male-limited chromosome 4 in *americana*. The situation is similar to that in *D. miranda,* in that a fusion or some similar mechanism must have given rise to it (McKnight, 1939). It most probably survived due to the fact that once the Y had become attached and the strain had become isolated, it could not be lost even if it did produce aneuploid gametes. *D. miranda* has progressed to the point that normal disjunction occurs by some regulatory mechanism that has not been determined.

Several types of both genic and chromosomal isolating mechanisms are present in these few stocks of *D. virilis*. Not only are these several mechanisms present but also the degree of their effect differs in the various strains.

Under the term genic isolation there are: (1) sexual isolation, (2) failure of eggs to hatch from cross-fertilized female, (3) sterility of some of the hybrids, and (4) sterility of some recombination hybrids in F_2 and after with the *americana* or *texana* Y chromosome.

Under the head of cytological isolation mechanisms there are: (1) inversions and (2) fusions (or fragmentation).

Let us consider the role and degree of effect which has resulted or could result from these several mechanisms.

Sexual isolation differs in effectiveness between the several possible crosses. There seems to be no sexual isolation within the *gray* group but it may operate to some extent between *texana* and *americana*. It differs in effectiveness between *virilis* and the *red* group as compared to other members of the *gray* group. Also it differs in reciprocal crosses (i.e., *V* X *A* as contrasted to *A* X *V*, Table 1). The genes which cause this isolation to be effective in the asiatic and southwest strain are recessive to those which allow crossing to occur with *virilis*. Thus this isolating mechanism depends on a recessive gene (or genes) which could spread in the heterozygote and cause isolation as they become homozygous. As the isolation is not quite complete, the mutation from the recessive to the dominant might easily have become established in such populations as those of *D. virilis*.

The percentage of eggs to hatch from a cross between the *red* and *gray* groups is small even from known fertilized females. Also it differs for

the several stocks and even reciprocal crosses. Therefore this is an extensive but incomplete barrier between the groups. The mechanism here is as yet unknown.

The sterility of part of the F_1 (and F_2, etc.) is also a factor in divergence. It varies with the direction of the cross in several ways and for different reasons. For example, part of the F_2, etc., recombinations are sterile due to the *texana* and *americana* Y-autosome interrelation. Also there is a difference in the fertility of the F_1 hybrids on inbreeding from reciprocal crosses. Part of this in the males may be due to the Y-autosome relation with nondisjunction. Table 3 shows that often hybrid males were less fertile in backcrossing than hybrid females.

This hybrid sterility is incomplete. It does not compare in effectiveness to that of *D. melanogaster–D. simulans* hybrids, *D. aldrichi–D. mulleri* hybrids (Patterson and Crow, 1940), or even *D. pseudoobscura A* and *B* hybrids which are sterile only in the male. The last mechanism, the Y-autosome interrelation, has already been described.

The cytological differences of gene order, inversions, are not present in the members of the *gray* group so far tested. *Texana* differs from the *gray* group in having two simple short inversions and two overlapping inversions in the X, while *americana* differs from both in that it has in addition two complex inversions, one of them in the X. These inversions would give, when heterozygous, an insignificant number of aneuploid gametes. They were present homozygous in the strains and therefore would be of no benefit through heterosis in the pure stock. There are no known cases where inversions, by the production of aneuploid gametes, caused isolation between species. Inversions can only affect disjunction in the heterozygote, and the fusions are limited in the same way. The fusions, one in *texana* and one or two in *americana* may reduce the number of normal gametes produced in the hybrids. This would be true particularly in the male as (and if) there are no chiasmata present in the hybrid males. They should be less effective in the hybrid females in relation to the amount of directive action imposed on disjunction by chiasmata (see Brown, 1940, for the equivalent case in *D. melanogaster*). The X–4 fusion must seldom form chiasmata in hybrids.

Both inversions and fusions have a role in promoting divergent evolution in the homozygous condition which should be considered. Recombination has a very important role in evolution, so important that sexual reproduction which utilizes its advantages has given rise to most of the more complex forms (Wright, 1931). Anderson (1939) has pointed out the hindrance to free recombination imposed by linkage. Both inversion and fusion effect this system, inversion by changing the amount of recombination between the several genes within the chromosome, and fusion by joining two formerly independent systems into one linkage group.

The pressure to change due to the "drift" inherent in small populations might place marked emphasis on such changes in linkage. This is not the same factor as that considered by Sturtevant and Mather (1938) in which

they discussed the differences that would follow from lack of free exchange in the heterozygous although this also would apply in *D. virilis*.

The inversions common to *americana* and *texana* must have a common origin. Dobzhansky and Sturtevant (1938) conclude that it is highly improbable that the same inversion would arise at two places independently. Kikkawa (1938) reported that there were four inversions in the Japanese strains of *D. ananassae*, the same as four described by Kaufmann (1936) for the United States. This would lend credence to the opinion that the asiatic and southwest strains of *D. virilis* were closely related. This could be inferred also from the fact that they are so similar in the tests reported although the southwest form must be subject to the rapid change due to the mutation drift inherent in the nature of its sparse population.

The many differences between the *gray* and *red* groups in phenotype and physiological characteristics must be due in part to the mutation drift of these sparse populations. We cannot differentiate between changes brought about by selection and changes through mutation and chance fixation for these differences. However the sterility encountered within *americana* and *texana* can best be explained by chance fixation against selection pressure.

Sturtevant and Dobzhansky (1936b) report that the male hybrids between *D. athabasca* and *D. azteca* are quite abnormal in phenotype from both reciprocal crosses although the females are more nearly normal, but also sterile. No abnormal F_1 hybrids have been detected in these crosses. Some of almost all crosses have been fertile, some especially so in certain crosses. Most of them in fact seem to have hybrid vigor. Even when we consider the several other detectable differences between the *red* and *gray* groups, we are still of the opinion that the time of their divergence was relatively recent.

This group might be classified in its evolution as a continental type using Kinsey's (1937) division into insular and continental. It is a special type of continental distribution, one with sparse population. These things lead to the remarkable diversity found here in this small group of stocks.

Sturtevant and Tan (1937) have compared the gene maps of *D. melanogaster, D. simulans* and *D. pseudoobscura*. They point out that most of the changes in gene grouping may be explained by inversion and fusion or fragmentation. The situation is the same so far in *D. virilis*. The changes found here are all of those types. The one possible exception is the Y-autosome relation of *americana* and *texana* which might have arisen by translocation. However it could also be due to mutation. This may be an example of the assumption of the Y function by substitute mutation in the autosomes as a step in the elimination of the Y-chromosome. Such fertile X–O males would be a distinct advantage in *americana* for they would eliminate any abnormal disjunction arising from competition there due to the complex sex chromosome mechanism. This last example is the only one so far found as the tests have been inadequate

to show the shifting of the function of one gene to another. Gordon, Spurway and Street (1939) have found tentative proof for this in *D. subobscura*. Harland (1936) has demonstrated it extensively in cotton by comparing several strains of two species. Serebrowsky (1938) has presented good evidence of a complementary nature on the subdivision of the function of a gene between the gene itself and what must have been an adjacent repeat of the same gene.

The high fertility of these hybrids argues that they have not diverged far in their primary gene functions. In contrast, Sturtevant and Dobzhansky (1936b) have shown that the reciprocal hybrid males from the cross of *D. azteca* and *D. athabasca* show complementary types of phenotypic abnormalities, one a dwarf and the other a giant.

Muller (1939) has expressed the opinion that substitution of the functioning of one gene by another with subsequent development of new reactions on this system will lead to divergence. Evidence for this has been presented and discussed by Harland (1936). This may culminate in a situation in which the hybrid will have gene interactions of a detrimental nature not present in either parent. The hybrid substance unknown to either parent discovered by Irwin (1939) in the dove-pigeon crosses is an excellent example of such a new interaction.

If a mutation occurs which carries out the same function as another gene present in the system, then whether the new or the old gene will remain after a length of time, will depend on many factors besides chance such as mutation pressure, drift, etc. But if the new mutation carries out the old function and some other beneficial function as well, then it will have the advantage and may replace the old gene or leave it free to mutate. If there are several functions, for each gene and they temporarily have one or more in common, mutation pressure would soon lead to separation of function and so to differences between related groups such as are encountered in cotton (Harland, 1936).

There is another type of balanced gene interaction that follows here also. There will be many reactions that genes will not carry to completion in the hybrid through incomplete dominance, i.e., those reactions in which two doses of a particular gene gives more effect than one. This inadequacy of reaction must account for a certain part of hybrid sterility. This would be particularly apt to explain the fact that a diploid hybrid can be sterile and the tetraploid derived from doubling this sterile diploid will be fertile. This, together with the possibility of abnormal (effectively aneploid) recombinations seems to us a much more probable explanation of the fertility of *Primula kewensis*, Newton and Pellew (1929), and Upcott (1939), as pairing and chiasma formation were too normal to account for the sterility of the diploid hybrid.

Sturtevant (1938) seems of the opinion that there is no explanation for the sterility of hybrids to develop if two groups are separated for a period of time. It seems to us, however, that these two mechanisms just discussed, namely, the transfer of function from one gene to another and non-dominance of either a transferred function or a new one, would

easily explain this divergence and consequent sterility. There has also been the question of why the sex system was the first to suffer in the hybrids. This, it seems, should be expected. In the first place this is the one most liable to be subject to shifts and modification by selection on mutants as they appear in the X or interact with those on the X (and Y). The shift from one to two X-chromosomes with the change in sex allows for most rapid selection in the X and consequently for the most rapid transfer of function. Also the systems other than sex are selected for stability whereas sex is selected for instability of a particular balanced sort. Consequently sex should be most liable to upset in hybrids and especially in the hemizygous sex. In the homozygous sex there is the 1X : 1A balance of each stock present in the hybrid; in the hemizygous sex, the one X is selected to balance in a particular way with its own autosome set, but has not been selected to balance with the other autosome set. Consequently it is the most liable to be abnormal, and the sex system in general is most liable to abnormal development.

Whereas the asiatic or southwest group is quite effectively isolated from the *red* group, *virilis* is not. The readiness with which *virilis* females cross either to *texana* or *americana* males implies that genes could be transferred from one group to the other. It is noteworthy that in a mixed population of *virilis* with other members of the *gray* group, transfer could be accomplished between all the several possible strains. This could occur without *virilis* being present but much more slowly.

That raises the question as to whether complete isolation is the most advantageous possible relation between two related species. If such a mixed population exists where isolation is nearly but not quite complete, a certain amount of transfer of genes could be accomplished between two related species. This could be done even if there was considerable sterility. Wright (1931, 1935) has stressed the importance of recombination in evolution and pointed out that the most efficient evolution would be the condition where a population was broken up into smaller breeding populations with occasional migration between the groups. Such a cross-sterility mechanism as postulated here would then function between species in the same way. This must be one of the factors in evolution following species crosses.

SUMMARY

1. The strains of *D. virilis* which we have studied may be divided into two groups, called the *red* and *gray*. The *gray* group is subdivided into *D. virilis virilis* Sturtevant, the southwest group, and the asiatic group. These are placed in the subspecies of *D. virilis virilis* despite some differences. The *red* group consists of two subspecies, *D. virilis americana* Spencer and *D. virilis texana* Patterson.

2. Except for a fusion found by Chino all of the *gray* group so far studied have had five pairs of rods plus one pair of dot-shaped chromosomes at metaphase. *D. virilis texana* has one pair of autosomal V-chromosomes which are the equivalent of two pair of rods, chromosomes 3 and 4

in the *gray* group, "fused" at the centromere. *D. virilis americana* has a pair of autosomal V's which are the equivalent of the pairs of rods 2 and 3 of the *gray* group. Also the X in *americana* is fused with chromosome 4 so that the female has two pairs of V-chromosomes and one pair of rods. The male has no Y-4 fusion so it has only three V-chromosomes and four rods.

3. The gene sequence has undergone several changes. Both *americana* and *texana* have simple inversions in chromosomes 2 and 5. *Americana* has a double inversion in 4, an inversion within an inversion. *Texana* has two overlapping inversions in the X and *americana* has a third. The points of breakage of *americana* agree well with Hughes. However, the order of the genes as we have analyzed it differs somewhat. The intermediate condition of *texana* makes the analysis somewhat simpler.

4. There are several other factors which contribute to the isolation of *D. virilis* into the several semi-isolated groups. These are sexual isolation, poor egg hatch from crosses, and complex interrelated fertility mechanisms. Sexual isolation is very strong between members of the *red* and *gray* groups unless *virilis* females are used. Also females heterozygous for *virilis* and any other member of the *gray* group are often fertile with *texana* or *americana* males. *Virilis* males, however, cross very poorly with *americana* or *texana*. The egg hatch is very low from all crosses tested.

5. Some hybrids between the several combinations are fertile.

6. There is a complex Y-autosome relation necessary for fertility in *texana* and *americana*. If the *texana* Y is present in hybrids, chromosomes 2 and 5 must be present for a male to be fertile. *Americana* has the same limitations. All recombinations are fertile in the female hybrids.

7. This Y-autosome relation causes high sterility in the hybrids where the initial cross goes with ease but is not present in the result of the reciprocal which is made with difficulty.

8. The nature and degree of relationship in *D. virilis* is such that the asiatic and southwest groups are at one end of a series connected by *virilis* through *texana* to *americana*, which represents the other end of the series.

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SALIVARY GLAND CHROMOSOMES

The salivary gland chromosome map of *D. virilis* was made from the *Henly* strain, which has been used as the cytological standard. This strain was chosen because its chromosomes seem to stain somewhat more sharply than those of the Pasadena standard *virilis;* structurally the chromosomes of these two strains are identical. In this map emphasis has been placed upon the structural details and upon the use of fully extended, but not stretched, mature chromosomes. The free tip of each chromosome is shown at the left, while at the right is shown the entire proximal region of each element extending through the centromeres. At the proximal ends of chromosomes 4 and 6 are shown the four centromeres which belong to these particular chromosomes, one centromere for each chromatid of the synapsed homologues. This four-centromere appearance is not typical for *D. virilis*, since all the centromeres characteristically fuse into a cluster such as is shown on all the other chromosomes of the map; it must be pointed out that these centromere clusters are the collected centromeres of the cell and that only four centromeres of the group belong to any one chromosome.

On chromosome 4 can be seen several of the bands of the small right arm of this body; it is quite possible that the X–4 attachment in *americana* may have come about through a translocation involving this small arm or a similar one on the X.

A new system for the designation of bands is also given. Each chromosome is divided into eight sections, A through H; section H always includes only the heterochromatin of the chromosome or in the absence of heterochromatin, as in chromosomes 3 and 6, only the centromere. Within the sections are divisions which are numbered *1, 2, 3,* etc., but not going beyond *9* in any case; thus it is possible to avoid the use of designations involving more than one digit. The bands composing the divisions are indicated by letters of the alphabet beginning with *a* and continuing as far as is necessary in any division; new bands, when found, will be called *a2, a3,* etc., keeping the original band *a* of the division as *a1*. In this system any designation includes the number of the chromosome, the section, the division and the line; thus 2D4b means chromosome *2*, section *D*, division *4*, line *b*.

In the naming of chromosome abnormalities the map designation will be used since it is possible both to name and to identify specifically the order of bands in the rearranged elements; thus the *texana* inversion in chromosome 2 is fully described by the formula In 2D5g–2F8b. Similarly the fusion (translocation) between chromosomes 3 and 4 in *texana* is designated T 3H;4H4 (here the identity of the centromere is doubtful, although it probably is that of 4); a more easily analyzed translocation may be designated T 3D2c;4F3f + 4F3e;3D2d, the semicolon indicating the points of breakage. In such a case either half of the name may be used, but in describing nondisjunction gametes it is most convenient to have both parts named.

The practice of giving a double-walled band a double designation as initiated by Bridges in his 1938 *D. melanogaster* map has been strictly avoided; there is no practical reason for numbering the two halves of a single chromomere, which at the present time remains the structural unit of the chromosome, since there has been no reliable case of chromomere breakage. Separation of the chromomeres in a compound band as in the case of *vermilion* (Mackensen 1935) in *D. melanogaster* cannot be considered as chromomere breakage.

Many structurally "weak" points appear in the chromosomes of *D. virilis*. Such regions are shown in 1D9, 1H4, 2G6, 2H4, 3E2, 3F1, and elsewhere. The weak point at A7 in chromosome 4 is the most striking of these; the left tip of this body is often completely severed from the right portion in ordinary preparations. In all cases the typical appearance of these regions has been given, although variations occur.

The cytological comparisons, based on *Henly*, are as follows:

V (or H) x all other members of the gray group:
Complete, perfect synapsis; no inversions or other abnormalities

V x *T (red group)*
Poor synapsis
Apparently two overlapping inversions in X, with outer limits at 1D5e and 1G3c
A simple inversion in 2, from 2D5g through 2F8b
No abnormalities in 3, 4 and 6
A single inversion in 5, from 5C5g through 5F8e

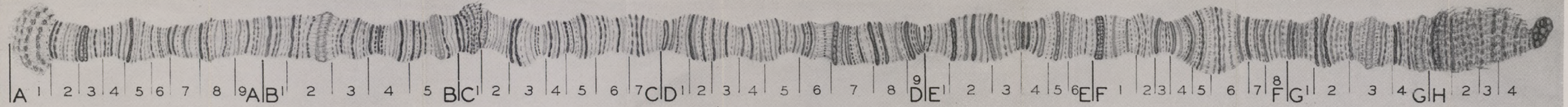
V x *A (red group)*
Very poor synapsis
X has the *texana* inversions plus a third simple inversion from 1C7d and extending far into the overlapping *T* inversions
Single inversions in 2 and 5, identical with *texana*
No abnormalities in 3 and 6
A large inversion in 4 from 4D1c through 4F7f; a second inversion within the first, from 4D5b through 4D6d

A x *T*
Perfect synapsis
A single inversion in X, and two in 4 described above
No abnormalities in 2, 3, 5 and 6
These cytological facts prove *T* to be intermediate between *A* and *V*

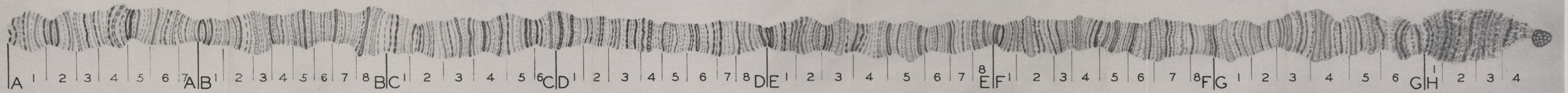
SALIVARY GLAND CHROMOSOMES

D. VIRILIS – HENLY

X = 1



2



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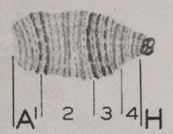
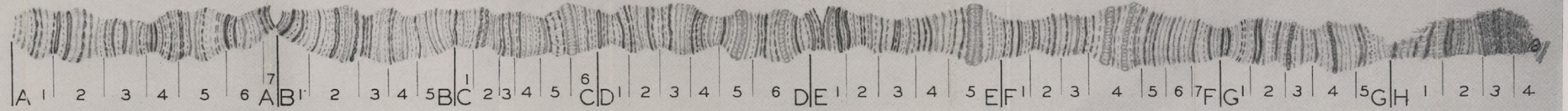


3

4



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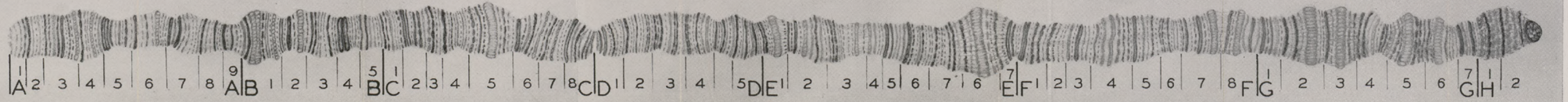

TABLE 1

P_1 ♀ ↓ / P_1 ♂ →	V	H	J	O	C	N	A	T
Per cent fertile	100%	96%	92%	97%	90%	90%	79%	44%
*V*irilis Average per tube	67.2	90.4	57.3	72.3	53.1	75.3	2.7	4.8
*H*enley	98% 80.2	98% 82.9	91% 57.7	89% 53.2	99% 56.4	82% 59.1	1% 1.0	8% 1.4
*J*apan	87% 36.6	92% 31.6	72% 36.4	95% 29.3	76% 37.4	90% 42.8	2% 1.0	2% 1.0
Vict*O*ria	97% 65.5	98% 65.6	87% 56.5	97% 45.1	99% 59.6	84% 65.3	3% 1.3	6% 1.2
*C*hina	99% 67.6	92% 55.5	93% 67.6	95% 62.5	93% 49.7	78% 49.2	1% 1.0	4% 1.2
*N*ew Orleans	76% 29.6	81% 10.4	70% 18.6	92% 12.2	70% 16.4	63% 19.2	1% 1.0	Sterile
*A*mericana	Sterile	3% 1.7	Sterile	1% 2.0	9% 2.3	6% 5.2	74% 18.7	44% 22.0
*T*exana	11% 7.4	1% 6.0	1% 2.0	18% 9.3	3% 7.7	4% 11.2	28% 18.8	54% 25.6

TABLE 2

F_1 X F_1

P_1 ♀ ↓ / P_1 ♂ →	V	H	J	O	C	N	A	T
Per cent fertile	100%	100%	100%	100%	100%	100%	64%	82%
*V*irilis Average per tube	67.2	90.9	82.7	79.1	69.9	64.6	19.5	41.3
*H*enley	98% 81.2	98% 82.9	100% 92.9	98% 71.8	98% 74.0	96% 74.2	34.1% (44 tubes) 16.1	20% (45 tubes) 20.2
*J*apan	100% 66.1	100% 85.8	72% 36.4	100% 71.1	94% 85.7	100% 67.8	28.9% (38 tubes) 11.9	22.2% (45 tubes) 14.4
Vict*O*ria	100% 88.4	92% 65.2	98% 82.9	97% 45.1	98% 52.2	62% 47.1	26% 16.5	26% 21.8
*C*hina	100% 74.5	98% 40.9	98% 92.9	98% 48.2	93% 49.7	98% 54.4	Sterile (24 tubes)	4.3% (46 tubes) 14.5
*N*ew Orleans	98% 79.5	86% 59.5	96% 64.2	92% 64.8	98% 57.6	63% 19.2	11.1% (9 tubes) 24.0	47% (17 tubes) 20.5
*A*mericana	72% 34.6	34% 26.9	23.5% (34 tubes) 7.5	46% 28.8	31.2% (32 tubes) 27.7	64% 31.9	74% 18.7	60% 14.2
*T*exana	66% 31.5	34% 29.2	44% 24.9	62% 35.2	78% 30.3	58% 31.1	58% 21.5	54% 25.6

TABLE 3

Fertility

F_1 Backcross ♀ X ♂	Number Sterile	Number Fertile	Per Cent Fertile	Average Per Tube	Pupa Color
OT X O	0	11	100.0%	4.0	Red and black
O X OT	6	19	76.0%	4.4	Red and black
H X AH	4	8	66.7%	35.0	Red and black
TH X H	10	64	86.5%	37.2	Red and black
H X TH	24	12	33.3%	42.0	Red and black
TH X T	13	4	23.5%	9.8	Red
T X TH	4	0	0.0%	----	----
TN X N	23	0	0.0%	----	----
N X TN	5	23	82.1%	(ca) 40.0	Red and black
TN X T	6	0	0.0%	----	----
T X TN	5	18	78.3%	(ca) 40.0	Red
VT X V	6	115	95.4%	41.5	Red and black
V X VT	49	143	74.5%	43.5	Red and black
TV X V	4	39	90.7%	47.5	Red and black
V X TV	12	28	70.0%	59.6	Red and black
VT X T	23	42	64.6%	28.6	Red
T X VT	33	38	53.5%	35.6	Red
TV X T	2	16	88.9%	29.2	Red
T X TV	14	10	41.7%	31.8	Red
VA X V	8	75	90.4%	43.5	Red and black
V X VA	24	29	54.7%	25.1	Red and black
VA X A	8	56	87.5%	35.4	Red
A X VA	55	19	25.7%	13.6	Red
AT X A	19	74	79.6%	25.2	Red
A X AT	25	52	67.5%	11.3	Red
TA X A	21	57	73.1%	27.5	Red
A X TA	19	29	60.4%	54.7	Red
AT X T	32	51	61.5%	29.7	Red
T X AT	32	54	62.8%	20.4	Red
TA X T	47	30	39.0%	16.3	Red
T X TA	28	16	36.4%	21.9	Red

TABLE 4

Fertility

Cross ♀ x ♂	Number Sterile	Number Fertile	Per Cent Fertile	Average Per Tube
TA x V	42	4	8.7	18.2
V x AT	10	32	76.2	9.3
V x TA	7	71	91.0	9.8
AT x H	44	1	2.2	2.0
TA x H	47	2	4.1	10.0
H x AT	34	7	17.1	2.6
H x TA	67	10	13.0	2.2
AT x J	39	0	0.0	---
TA x J	55	0	0.0	---
J x AT	43	2	4.4	2.5
J x TA	38	1	2.6	3.0
AT x O	51	0	0.0	---
TA x O	50	1	2.0	3.0
O x AT	36	0	0.0	---
O x TA	40	1	2.4	4.0
AT x C	44	2	4.4	6.0
TA x C	35	0	0.0	---
C x AT	52	0	0.0	---
C x TA	40	0	0.0	---
AT x N	41	1	2.4	3.0
TA x N	45	5	10.0	6.2
N x AT	43	0	0.0	---
N x TA	41	0	0.0	---
AT x HV	39	1	2.5	4.0
TA x HV	40	1	2.4	3.0
HV x AT	12	30	71.4	6.6
HV x TA	10	20	66.7	4.3
TA x HC	49	1	2.0	7.0
HC x TA	46	4	8.0	3.0
AT x JV	31	1	3.1	1.0
TA x JV	25	0	0.0	---
JV x AT	8	8	50.0	6.4
JV x TA	8	23	74.2	7.0
AT x JH	46	0	0.0	---
TA x JH	49	1	2.0	3.0
JH x AT	39	6	13.3	3.5
JH x TA	36	10	21.8	3.0
AT x AT	20	30	60.0	14.1
AT x TA	8	43	84.3	27.4
TA x AT	12	41	77.4	28.4
TA x TA	21	29	58.0	21.5
VH x A	6	94	94.0	3.8
HV x A	19	117	86.0	5.7
A x VH	44	0	0.0	---
A x HV	80	1	1.2	5.0
VH x T	5	54	91.5	4.8
HV x T	9	87	90.6	4.6
T x VH	40	0	0.0	---
T x HV	80	3	3.6	17.3
VJ x A	25	75	75.0	3.7
JV x A	4	40	90.9	8.1
A x JV	13	0	0.0	---
VJ x T	65	22	25.3	2.5
JV x T	12	24	66.7	8.1
T x JV	21	0	0.0	---
HJ x A	80	20	20.0	1.4
HJ x T	88	12	12.0	1.7
VC x A	12	60	83.3	4.0
CV x A	24	25	51.0	2.7
A x VC	68	5	7.0	1.0
JO x A	78	22	22.0	1.9
T x OJ	98	2	2.0	1.5

TABLE 4—Cont'd

Fertility

Cross ♀ x ♂	Number Sterile	Number Fertile	Per Cent Fertile	Average Per Tube
A x CJ	28	8	22.2	2.2
A x HJ	28	1	34.5	5.0
T x HJ	25	15	37.5	4.1
A x VJ	17	3	15.0	5.0
T x VC	22	21	48.9	11.4
VC x T	3	50	94.3	6.5
T x CJ	15	29	65.9	11.9
JN x A	22	12	35.3	2.2
T x JN	6	3	33.3	5.3
JN x T	21	17	44.8	1.6
CN x T	6	0	0.0	---
A x CN	12	0	0.0	---
A x JN	13	0	0.0	---
CN x A	19	4	17.4	1.2
CJ x T	32	5	13.5	1.0
T x VJ	14	14	50.0	7.6
CJ x A	40	2	4.7	1.0
JC x A	13	8	38.1	1.2

TABLE 5

F_2 Cross ♀ X ♂	No. Sterile	No. Fertile	Per Cent Fertile	Average Per Tube	Pupa Color	Remarks
(VA)V X (VA)V	19	43	69.4	ca 40.0	black, or red and black	
V X (VA)V	31	52	62.7	62.2	black, or red and black	
(VA)V X V	3	45	93.7	45.3	black, or red and black	
V(VA) X V	1	50	98.0	54.2	red and black	
V X V(VA)	99	11	10.0	41.4	red and black	
(VA)A X (VA)A	7	14	66.7	30.0	red and black	
A X A(VA)	5	2	28.6	ca 25.0	red	
V X A(VA)	18	7	28.0	ca 25.0	red, or red and black	
(AV)V X V	1	34	97.1	45.5	black, or red and black	
V X (AV)V	16	21	56.8	61.4	black, or red and black	
(VT)V X V	0	47	100	73.4	black, or red and black	
V X (VT)V	10	70	87.5	57.4	black, or red and black	
V(VT) X V(VT)	92	31	25.2	ca 30.0	red and black	Part vials gave 1:1 R:b ratio, others 3:1
V(VT) X V	2	48	96.0	62.5	black, or red and black	
V X V(VT)	253	83	24.7	54.4	red and black	
T X V(VT)	12	3	20.0	20.0	red	
T(VT) X T(VT)	5	15	75.0	ca 30.0	red, or red and black	
V X T(VT)	5	35	87.5	ca 30.0	red, or red and black	
T X T(VT)	30	40	57.1	29.4	red	
(TV)V X V	1	48	98.0	41.9	black, or red and black	
V X (TV)V	18	64	78.0	39.7	black, or red and black	
V(TV) X V(TV)	34	68	66.7	ca 30.0	black, or red and black	
V X V(TV)	25	128	83.7	ca 40.0	black, or red and black	
V X (TV)T	5	6	54.5	ca 30.0	red, or red and black	
T(TV) X T(TV)	3	19	86.4	ca 30.0	red, or red and black	
T X T(TV)	12	0	0.0			
(HT)H X (HT)H	6	29	82.9	ca 30.0	black, or red and black	
H X (HT)H	15	0	0.0			
H(HT) X H(HT)	3	4	57.1	ca 30.0	red and black	
H(HT) X H	2	17	89.5	66.7	black, or red and black	
H X H(HT)	24	9	27.3	ca 30.0	red and black	
(HT)T X (HT)T	7	3	30.0	ca 30.0		
T X (HT)T	6	1	14.4	38.0	red	
T X T(HT)	8	0	0.0			
(TH)H X (TH)H	5	6	54.5	ca 40.0	black, or red and black	
(TH)H X H	2	45	95.7	64.8	black, or red and black	

TABLE 5—Cont'd

F_2 Cross	No. Sterile	No. Fertile	Per Cent Fertile	Average Per Tube	Pupa Color	Remarks
H X (TH)H	55	172	75.8	55.0	black, or red and black	
H(TH) X H(TH)	4	15	78.9	ca 30.0	black, or red and black	
H(TH) X H	1	4	80.0	46.5	black, or red and black	
H X H(TH)	59	140	70.4	57.3	black, or red and black	
T X H(TH)	60	19	24.1	31.2	red	
(OT)O X (OT)O	4	19	82.6	28.5	black, or red and black	
O X (OT)O	5	14	73.7	40.0	black, or red and black	
O(OT) X O(OT)	22	32	59.3	ca 30.0	red and black	
O X O(OT)	100	32	24.2	ca 30.0	red and black	
(OT)T X (OT)T	17	1	5.5	ca 20.0	(red)	
T X T(OT)	7	6	46.1	ca 20.0	red	
T(TA) X T(TA)	5	10	66.7	ca 20.0	red	
T X T(TA)	31	17	35.4	ca 20.0	red	
A X T(TA)	0	7	100	ca 20.0	red	
A(TA) X A(TA)	4	25	86.2	ca 20.0	red	
T X A(TA)	7	0	0			
A X A(TA)	23	33	58.9	ca 30.0	red	
(AT)T X (AT)T	6	3	33.3	ca 20.0	red	
T(AT) X T(AT)	21	33	61.1	ca 30.0	red	
T X T(AT)	47	16	25.4	28.1	red	
A X T(AT)	47	15	24.2	ca 30.0	red	
A X (AT)A	3	2	40.0	ca 30.0	red	
A(AT) X A(AT)	9	48	84.2	ca 30.0	red	
A X A(AT)	45	72	61.6	ca 30.0	red	
T X A(AT)	72	26	26.6	ca 30.0	red	

TABLE 6

Relation Between Mating and Fertility

Cross	Time After Mating Checked in Days	Survival		Condition of Male	Condition in Female					Remarks
		Dead Females	Dead Males		No Eggs and no Sperm	Eggs But no Sperm	No Eggs But Sperm	Both Eggs and Sperm: Gave no Progeny	Both Eggs and Sperm: Gave Progeny	
A X H	6	49	7							
T X A	6	9	4	dead	4	0	0	0	0	
				alive	5	0	2	0	5	
	30	1		alive	1	3	1	0	1	All males dissected had motile sperm present—12 tubes went so female not dissected
T X H	7	18	0	alive	11	1	0	0	0	
H X T	7	0	29	dead	7	6	0	0	0	
				alive	1	2	0	0	3	
H X A	7	0	30	dead	4	7	0	0	0	19 other females with dead males failed to go.
T X V	7	10	1	dead	1	0	0	0	0	
				alive	9	0	0	0	0	
	30			alive	10	8	2	1	2	All males dissected and had motile sperm—2 vials went before females checked
A X T	6	35	8	dead	5	0	1	0	0	
				alive	3	0	1	0	0	
V X T	7	4	5	dead	0	5	0	0	0	
				alive	0	3	0	0	6	
A X V	7	3	1	dead	1	0	0	0	0	
				alive	9	1	0	0	1	
V X A	6	2	5	dead	0	4	0	0	0	
				alive	0	0	0	1	5	
	30			alive	2*	0	0	0	0	*1 had with no normal sperm—28 tubes went so not checked

TABLE 7

Egg and Hatch Tests

Cross	Number of Pairs Tested	Number of Eggs	Number of Adults	Per Cent Hatch
V X V	13	870	798	92.0
T X T	13	1137	644	56.6
T X V	8	690	153	22.2
V X T*	13	1361	51	3.7
V X A*	8	1448	45	3.1
T X A	5	618	320	51.8
A X T	5	864	410	47.4
V X VT	11	1834	714	39.0
V X V(V(VT)	14	2366	0	0.0
	1	224	122	
	1	300	117	
	1	166	36	
	1	345	235	
	1	65	46	
	5	1100	556	50.0
V X TA	7	617	16	2.6
V(AV) X V	1	62	19	
	1	105	70	
	1	118	17	
	1	148	30	
	1	124	87	
	1	47	11	
	1	110	42	
	7	714	276	38.7
V(AV) X A	4	285	9	3.1
V X V(AV)	1	59	30	
	1	137	79	
	1	42	12	
	1	130	76	
	1	156	85	
	1	168	69	
	1	127	77	
	7	819	428	52.3

*From Table 8.

TABLE 8

Egg and Hatch Tests with Male Replacements

Cross	Continuous 24-Hour Intervals	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1	Eggs	Pupa Color R	Pupa Color B1
V X V	1	42	0	35																																		
	2				25	0	24							30	0	30																						
	3				31	0	28							36	0	36							44	0	39	31	0	30	20	0	20							
	4	44	0	36				53	0	52	32	0	29	34	0	31	37	0	35	40	0	38	50	0	44				49	0	49				58	0	54	31	0	27
V male replaced by T male																																						
	5				31	0	31				21	0	21				26	0	25	20	0	15	39	0	39	30	0	29	24	0	24	23	0	23						
	6																																	29	0	23			
	7																																						
	8	38	0	26				56	0	46							37	0	33	32	0	29												26	0	16			
	9	38	0	9	48	1	19				45	0	0	20	0	7	32	1	1	56	2	26	36	0	16	32	2	2	32	0	25	36	0	35						
	10										28	1	1										42	3	0	40	1	5	40	2	6								
	11				61	2	3										51	1	1	76	1	0	51	2	0				40	0	3	56	3	0	24	0	0	44	2	1
	12				45	1	0							22	1	0				23	1	0	38	0	0							37	2	0				38	3	0
	13	25	0	1	24	1	0	42	0	1				26	2	0				57	6	0	41	1	0							28	2	0	52	2	0	33	1	0
V X T	1							39	2	0	25	2	0				25	0	0				21	2	0	20	0	0				27	3	0						
	2							22	0	0	26	0	0				31	0	0																35	3	0			
	3	46	0	0				39	1	0	27	0	0	46	1	0	48	1	0				23	1	0	25	1	0	43	1	0				22	0	0	41	1	0
	4				32	1	0				27	1	0							35	1	0												34	2	0	52	5	0
	5																																						
	6	69	2	0	33	2	0	36	1	0	48	3	0	33	1	0	40	4	0	65	1	0				63	2	0	51	2	0				58	0	0	54	4	0
T male replaced by V male																																							
	7																			35	0	0							37	3	4	39	1	0						
	8																																						
	9							32	0	31										39	0	30				43	0	25	44	0	17							27	0	27
	10	37	0	29	41	0	37	42	0	36	32	0	17	52	0	44	50	0	27	42	0	34	38	0	32				23	0	19	49	0	35	41	0	39			
	11				34	0	32																			39	0	38										49	0	41
	12																												47	0	32									
V X A	1										53	0	0																										
	2	22	2	0													28	1	0	29	0	0	32	1	0														
	3																																						
	4				37	0	0	36	1	0	26	1	0				32	2	0	25	12	0	46	1	0														
	5							28	1	0																														
	6	31	0	0																																			
	7							33	1	0				43	0	0				40	2	0	39	1	0															
	8				38	0	0	50	4	0				45	3	0	39	0	0																			
	9	28	1	0				54	1	0	25	3	0				50	0	0				23	0	0														
	10	23	0	0	30	0	0	58	1	0	30	0	0	44	0	0	53	0	0	43	1	0																
	11	37	0	0	41	3	0	41	1	0				42	1	0	47	0	0	27	0	0																
	12																																						
A male replaced by V male																																							
	13				23	0	16										20	0	9				22	0	0														
	14	79	0	70	47	0	43	76	0	63	58	0	19	52	0	27	59	0	48	35	0	34	36	0	0														
	15	39	0	25	45	0	40	62	0	58	54	0	45	41	0	39	42	0	42	62	0	48	60	0	0														
	16	65	0	57	31	0	31	46	0	44	42	0	39	35	0	29				38	0	37	42	0	0														

XII. HYBRIDIZATION IN THE MULLERI GROUP OF DROSOPHILA

J. T. PATTERSON AND J. F. CROW

INTRODUCTION

In connection with our studies on the wild populations of *Drosophila* in the Southwest and elsewhere we have discovered an interesting series of three forms which show different degrees of cross fertility. The forms belong to what may be called the "mulleri group." The members of this group will be fully described elsewhere, so that the following brief account must suffice for the purposes of this preliminary article.

1. *Drosophila mulleri mulleri* Sturtevant, 1921. This subspecies is evidently the form described by Sturtevant as *D. mulleri.* His description was based on specimens collected at Houston, Texas, by Dr. H. J. Muller. The characteristics of this subspecies, in contrast to those of the two forms which follow, are large bright-red eyes, a distinct and sharply defined abdominal color pattern, and yellowish testes. It breeds well under laboratory conditions.

2. *Drosophila mulleri mojavensis,* new subspecies (Patterson, in manuscript). A stock of this new subspecies was kindly given to us by Professor Warren P. Spencer, who collected the original flies at Mesquite Springs, Death Valley, California, and who suggested the subspecific name. When received the stock was labeled "mulleri-like." The general color tone of the fly is yellowish, in contrast to the grayish color of *D. mulleri mulleri.* It has small red eyes, a faded or ill-defined abdominal color pattern and yellowish testes. It also breeds well in the laboratory.

3. *Drosophila aldrichi,* new species (Patterson, in manuscript). This new species from Texas resembles *D. mulleri mulleri,* but has smaller eyes of vermilion-like color, a slightly different color pattern on the abdomen, and orange colored testes which turn to a deep rusty-red in old specimens. It breeds less well in the laboratory than either of the other two. At first it was not recognized as distinct from *D. mulleri mulleri,* and it was not until after a series of breeding tests had been carried out that its rank as a new species was definitely established.

In the remainder of the text we shall refer to these three forms as *mulleri, mojavensis,* and *aldrichi,* respectively. Their geographic distribution is a matter of interest and importance. From the records now available, *aldrichi* in Texas appears to be restricted to the south-central part of the State. Its area of distribution is roughly in the form of a broad triangle, with the apex located at or near Brownwood and the base extending along the Rio Grande River, from Brownsville to a point west of Eagle Pass.

Mulleri is also found over this same triangular area, but its distribution extends beyond the limits of this area and covers a considerable portion of the State. It has not been found in the extreme western tip of the State, a region known as the Trans-Pecos area. Moreover, in the northern and northeastern parts of the State it occurs very rarely, as only a few

specimens were found among many thousands of flies captured in these regions. *Mulleri* and *aldrichi* together constitute the third largest population group of *Drosophila* in Texas.

Professor Spencer states (personal letter) that *mojavensis* is very common in the California deserts. He found it breeding on rotting barrel cactus *(Echinocactus acanthodes)* in the Providence Mountains and the nearby desert, in a region lying between the Colorado and Mojave deserts. Beyond this, we have no knowledge of the exact limits of its distribution area. We do know, however, that it does not occur in Texas, and it must therefore be widely isolated from *mulleri* and *aldrichi*.

Breeding Tests

Cross breeding tests have brought out an interesting and significant relationship between these three forms. The crosses between *mulleri* and *aldrichi* have been much more extensively carried out than have those involving *mojavensis*. If *mulleri* females are crossed to *aldrichi* males a few F_1 flies are produced, but these are completely sterile both in inbred and backcross tests. Dissections of these hybrids made by Mr. L. E. Rosenblad show that the gonads of both sexes are underdeveloped and that those of the male are somewhat degenerate. Numerous attempts to obtain hybrids from the reciprocal cross have so far failed.

Although the tests with *majavensis* have been somewhat limited, yet we believe that they are sufficient to justify the following statement. *Mulleri* females crossed to *mojavensis* males are slightly fertile. Only a few individuals appear in cultures in which hundreds of eggs have been laid. The F_1 hybrid females from this cross are also only slightly fertile when backcrossed to *mulleri* males, but are quite fertile in the backcross to *mojavensis* males. The F_1 males were found to be sterile in the inbred and both backcrosses. The reciprocal cross is sterile. Offspring from crosses between *mojavensis* and *aldrichi* are also difficult to obtain, but we have secured a few sterile female hybrids when *aldrichi* females were mated to *mojavensis* males.

The hybrids obtained from the use of *mojavensis* resemble this subspecies in color much more closely than they do either of the other parents, suggesting the presence of dominant factors in *mojavensis*. Further tests are being carried out by the junior author.

Cytological Results

The metaphase plates of these three forms, as seen in brain smears, are practically indistinguishable from one another. In each form the plate shows five pairs of rod-shaped and one pair of dot-shaped chromosomes in the female. The largest pair of rods represents the sex-chromosomes. The same configuration is present in the male, except that the two sex-chromosomes are of unequal length, the Y being shorter than the X and of about the same length as the rod-shaped autosomes.

The salivary-gland chromosomes of the hybrid larvae have been examined and were found to show the following conditions: In the cross

between *mulleri* and *aldrichi* the homologues of these chromosomes show a very strong tendency to remain unsynapsed. No large inversions have been detected. In the cross between *mulleri* and *mojavensis* synapsis of the homologues is very much better and there is some evidence for large rearrangements. Finally, in the cross between *aldrichi* and *mojavensis* the homologues synapse rather poorly and there are some rearrangements present.

Discussion

The three members of the *mulleri* group represent a most interesting case in speciation. *Mulleri* and *aldrichi* may be considered first. In phenotype they are strikingly alike, but *aldrichi* requires about three days longer for its development. Both species have a fairly dense population, occupy a common area and have acquired genetic isolation. This isolation is seen not only in the complete sterility of the hybrids, but also in the fact that the cross goes but one way. Reproductive effort is thus conserved and there is no opportunity for an exchange of genes between the two species. They can therefore follow their respective courses in evolution while living side by side. It is interesting to note that we have found a few F_1 male hybrids from this cross in nature. They were discovered in three different localities, and in each place the proportion of *aldrichi* was high.

The *mulleri-aldrichi* relationship may be compared with certain cases of hybridization found among other species of *Drosophila.* The most obvious one is the *melanogaster-simulans* case which was worked out by Sturtevant (1919, 1920). This case resembles the *mulleri-aldrichi* combination, in that its members have a dense population, frequently live together and exhibit genetic isolation. However, the case differs in certain other respects, particularly in producing hybrids by reciprocal crosses. Nevertheless, the cross goes better when *melanogaster* is used as the female parent. On the basis of these facts, one may assume that the members of the *mulleri-aldrichi* pair have reached a slightly higher level of isolation than is found in the *melanogaster-simulans* combination.

If the third member, *mojavensis,* be considered, further comparisons are possible. This subspecies shows the following differences in comparison with *mulleri* and *aldrichi:* (1) it is geographically isolated from the other two; (2) differs more widely in phenotype; (3) hybrid larvae show greater chromosomal rearrangements; and (4) produces fertile females in at least one cross *(mulleri* ♀ x *mojanvensis* ♂*).*

In some ways this case is very similar to the *pseudoobscura-miranda* group (Dobzhansky, 1939). *D. pseudoobscura A* and *B* are cross-fertile and produce hybrids, the females of which are fertile, but when either *A* or *B* is crossed with *D. miranda* the offspring are almost completely sterile (Macknight, 1939). In the *mulleri* series there is a similar grouping. There are two members of the group, *mulleri* and *mojavensis,* which when crossed give fertile female hybrids, but the hybrids produced by crossing either of these two to *aldrichi* are sterile. Thus in both groups there are three members, two of which give fertile hybrids when crossed

to each other, but sterile hybrids when crossed to the third member of the group.

Pseudoobscura A and *B* occur in overlapping ranges so that there is a possibility for interchange of genes between the two races. The fact that the F_1 flies are fertile only in backcrosses makes it possible for gene transfer to occur without the formation of intermediate forms, although the probability for the occurrence of this depends on a number of factors, such as for example sexual selection. In *mulleri,* however, the two groups which produce fertile hybrids are geographically isolated and gene transfer could not occur, unless there is an overlapping of the populations at some unknown point. These two groups are easily distinguishable phenotypically, the desert living form *D. mojavensis* being much lighter in color, while *pseudoobscura A* and *B* are very similar in appearance.

D. pseudoobscura A and *B,* which produce fertile female hybrids, differ by four inversions and these differences are about of the same order of magnitude as those between *mulleri* and *mojavensis,* so that in this respect the two are alike. *D. pseudoobscura A* and *B* differ in a number of rearrangements (from 40 to 80) from *D. miranda,* with which they produce almost all sterile hybrids. In contrast to this, *mulleri* and *aldrichi,* which produce sterile hybrids, differ in no major rearrangements, although there is a definite tendency for the chromosome not to synapse. This last condition is similar to that in *melanogaster-simulans* hybrids described by Kerkis (1936).

In wild stocks of either race of *pseudoobscura* numerous inversions are found so that the inversions between *A* and *B* are not of much greater magnitude than are found in individuals within either race. Furthermore, as numerous as the inversions are in the population, none has been found which shows any marked selective advantage or disadvantage, indicating that they are not associated with breakage effects which may be lethal or detrimental. Numerous lethals have been found in *D. pseudoobscura* populations but none of these is associated with the chromosome rearrangements. The fact that the population is large means that chromosomal changes, especially those which have no particular selective advantage, probably would not become fixed in the population but would fluctuate. It has been demonstrated by Dobzhansky and Queal (1938) and Koller (1939) that the *pseudoobscura* population is broken up into smaller breeding populations with some migration from point to point. This allows some possibility of shift in the chromosomal type of local populations, but there must be some deterioration in heterozygosity if there is any definite tendency to change. In *D. miranda,* due to the fact that the populations are small and indigenous, it is most probable that, as in *D. virilis,* certain rearrangements have become irreversibly fixed in the population so that other changes would proceed from that point. The *D. miranda* population would be expected to show, as it does, a great number of rearrangements.

We would presume from the evidence of lack of correlation of gene changes and chromosomal rearrangements in *D. pseudoobscura* that there is no reason to assume that the changes in gene sequence in *D. miranda*

have been accompanied by changes in gene function. Although the numerous rearrangements present in *pseudoobscura-miranda* hybrids would prevent free recombination, the fact remains that the sterility is not conditioned by this reason but is genetic, as is indeed the sterility of *pseudoobscura A* and *B* males. Consequently it remains an open question as to the importance of the rearrangements in the evolution in genotype of the *pseudoobscura* group.

In *melanogaster* and *simulans*, and in the *mulleri group*, there are very few large chromosome changes found in the populations. In populations the size of these, one would not expect to find a great number of permanent changes in a single group, as was the case in *miranda*, but, if inversions occur at the same rate and are of the same type (that is, are of equal selective value) as in *D. pseudoobscura*, they should be found quite frequently in the population, although fluctuating. There are so many different arrangements in chromosome 3 in *pseudoobscura* as to make it impossible to establish a standard strain, but such changes are not so common in most other species studied, for example in *D. melanogaster*. Experimental work on *D. melanogaster* has shown that a consederable number of chromosomal rearrangements are accompanied by breakage effects, such as visible and lethal mutants. Rearrangements of this type would tend to be reduced to a minimum in the population. It is possible, then, that there is a much higher percentage of breakage effects accompanying chromosome changes in *melanogaster* and *mulleri* than in *pseudoobscura*. Whether or not this is true could be determined by experimental production of chromosomal changes and the analysis of accompanying mutations in *pseudoobscura* and comparing the results to those of *D. melanogaster*.

The fact that there are few chromosomal differences between *D. mulleri* and *aldrichi* would seem to indicate that the two species have become genetically isolated by other than chromosomal rearrangements, and that this isolation became complete only after both species had developed fairly dense populations. The similarities between the two species does not necessarily mean that the isolation was recent, since the population is dense enough to be evolving quite slowly (contrasted to, say, the *D. virilis* group) and the same environmental factors are at work on both species.

It is legitimate to speculate on a possible origin of the members of the mulleri group. On the basis that they have evolved from a common remote ancestor, such a form should have occupied a region located farther south than either Texas or California, perhaps at some point in Central America. This would seem to follow from the fact that members of this group survive best in a warm, or even hot, climate. The present available data on the distribution of *mulleri* in Texas indicate that the range of its distribution does not extend much beyond the northern boundary of the state. The records show that the density of its population increases from north to south and reaches its highest level at the Texas-Mexico boundary. We also know that its distribution area extends on down into Mexico.

The reason why *mulleri* has not been able to extend its range into the northern parts of the continent is probably due to the effects of low temperature during the winter months; certainly it cannot be due to physical barriers such as mountain ranges. The exact east-west limits of its distribution area in Texas has not as yet been definitely established. But we know that it is rarely found in the eastern and northeastern parts of the state, and so far it has not been taken in Louisiana. It has not been captured in the extreme western tip of the state, and if present there at all, it must be extremely rare. All of this amounts to saying that *mulleri* is distributed in those parts of Texas which are least subject to severe low temperatures during the winter, and is absent or extremely rare in those parts in which such climatic conditions do occur, including all regions of relatively high altitude.

The eastern and western ridges of the Rocky Mountain System extend down through Mexico, with the high Mexican Plateau lying between them. On either side of this high region there is a coastal plain which extends from lower Mexico to the United States. The high central area should form an effective barrier against the migration of forms like *mulleri* between the two coastal plains, except at the southern tip, while the plains themselves could form natural pathways for the northern spread of such species. If the common ancestral species lived at some point lying below the southern limits of the high central area (Sturtevant, 1921 records *D. mulleri* from Honduras), its descendants could have split into two groups, one passing along the eastern plain into Texas where the modern species of *D. mulleri mulleri* and *D. aldrichi* occur, the other moving up the west coast into California where *D. mulleri mojavensis* is now found. This of course is but a suggestion, but it is a good working hypothesis for future studies.

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