TRANSLOCATIONS INVOLVING THE THIRD AND THE FOURTH CHROMOSOMES OF *DROSOPHILA MELANOGASTER**

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INTRODUCTION

The chromosomal aberrations known in Drosophila as well as in other animals and in plants may be divided in two classes. The first class comprises aberrations involving whole chromosomes or groups of whole chromosomes. Polyploidy and non-disjunction are the most frequently observed phenomena of this kind. The study of the chromosomal aberrations belonging to this class has given much information concerning the role of chromosomes in general and the role of individual chromosomes in particular in the transmission of hereditary characters. The most clear and convincing proof that the chromosomes are carriers of hereditary material is that secured by the study of non-disjunction of the first and of the fourth chromosomes of Drosophila (BRIDGES 1916, 1921). The aberrations involving sections of chromosomes constitute the second class. Here belong translocation, duplication, inversion and deficiency of section of a chromosome (see BRIDGES 1923). These phenomena are comparatively little known, although they furnish favorable material for the investigation of the distribution of hereditary material within the chromosomes. Translocations seem to be especially valuable for the study

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of the problem just mentioned because they are most likely to produce differences in the genetical behavior of some characters together with visible alterations of the chromosomes.

The term "translocation" was proposed by BRIDGES (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925) "for cases in which a section of chromosome is removed from its normal location but is present in an abnormal location." Theoretically, a section removed from its normal location in one chromosome may become attached either to a new locus in the same chromosome or to another chromosome. Both intrachromosomal and interchromosomal translocations are, therefore, possible. Most of the translocations described so far (BRIDGES 1923; MULLER and PAINTER 1929; PAINTER and MULLER 1929; also the translocations described in the present paper) are interchromosomal, but DUBININ (1929) recently found also an intrachromosomal translocation.

In the case of the "Pale" translocation described by BRIDGES (1919, 1923), a section of the second chromosome carrying the genes from plexus to speck was broken off and became permanently attached to the third chromosome near the gene ebony. Flies heterozygous for the translocation, that is, carrying one deficient second chromosome and one third chromosome with the section of the second attached to it, are nearly normal in appearance but give abnormal genetical results when crossed to flies free from the translocation. Four kinds of zygotes are produced in such a cross: (1) carrying both the deficient second chromosome and the third chromosome with the attached section, (2) the deficient second chromosome and the normal third, (3) a normal second and the third with the attached section, (4) normal second and third chromosomes. Zygotes 1 and 4, representing the parental combinations of the chromosomes, give flies heterozygous for the translocation and normal flies respectively. Zygotes 2 and 3 represent the recombinations of the parental chromosomes. Zygotes 2 die; zygotes 3 sometimes survive, but give rise to flies abnormal in appearance and weak in constitution because they carry a duplication for a section of the second chromosome. The elimination of zygotes 2 and the partial elimination of zygotes 3 result in an apparent linkage of the genes belonging to the second and the third linkage groups.

The apparent linkage observed in the case of the "Pale" translocation suggests a method of finding translocations in general. Each of the chromosomes of a fly may be marked by appropriate genes. If such a fly carries no translocation, all the possible recombinations of the "marking" genes must be present in its progeny because of the free recombination of the parental chromosomes in the gametogenesis. However, if a fly with

"marked" chromosomes carries a translocation involving any two of the chromosomes, a linkage of the genes localized in those chromosomes will be observed, Hence, some of the expected classes of the offspring will be absent, or will manifest somatic peculiarities due to the disturbance of the normal genic balance.

The "Pale" translocation arose spontaneously; the same is true in respect to a few other cases of translocations (see MORGAN, BRIDGES, STURTEVANT 1925, pp. 178–179). The frequency of the spontaneous origin of translocations seems to be, however, so low as to make an attempt to secure them at will impracticable. The production of translocations in comparatively large numbers became possible only when MULLER (1928a, 1928b) and WEINSTEIN (1928) discovered that the frequency of translocation is very considerably increased in the progeny of flies treated by X-rays.

Recently MULLER and PAINTER (1929) and PAINTER and MULLER (1929) have published a preliminary account of their cytological and genetical investigations on the translocations involving different chromosomes of *Drosophila melanogaster*. Most of their conclusions coincide with the conclusions drawn in the present paper on the basis of my own material.

The present work was done in 1928 and 1929 at the MARINE BIOLOGICAL LABORATORY, Woods Hole, and at the CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena. Preliminary accounts of the results have already been published (DOBZHANSKY 1929a and 1929b).

The author wishes to express his gratitude to Doctor T. H. MORGAN, Doctor A. H. STURTEVANT and Doctor C. B. BRIDGES for their invaluable advice, suggestions and criticism without which the present work could hardly have been done. The author is obliged to Miss E. M. WALLACE for the finishing of the drawings accompanying this paper.

ORIGIN OF THE TRANSLOCATIONS

Males heterozygous for the second-chromosome dominant gene Bristle (*B_l* - short bristles), and the third-chromosome dominant gene Dichaete (*D*-wings spread, anterior dorsocentral bristles missing) were treated by X-rays. The age of the flies at the moment of the treatment was not more than 14 hours from the emergence from the puparia. A glass vial was filled with cotton to within about $1\frac{1}{2}$ cm from the opening; flies were placed on this cotton, and the opening of the vial closed by a cotton stopper. The vial was placed vertically, the opening up, under the target of a broad-focus Coolidge tube, so that the flies were at 16 cm from the target. Between the vial and the tube an aluminum plate of about 1 mm thickness

was inserted. The tube was operated by a current of 50 peak kilovolts and 5 milliamperes during 48 minutes. As seen from these data, the dosage of X-rays used is approximately equal to that applied by MULLER (1928) in his experiments and called "t-4"

The treated males were crossed in individual cultures to untreated females having attached X-chromosomes homozygous for the sexlinked gene yellow (y-yellow body color) and homozygous for the fourth-chromosome recessive gene eyeless^2 (e_y^2-eyes small, occasionally absent). Of the 57 cultures in which the fathers were treated, 18 cultures gave no offspring. Of the 10 control cultures (in which untreated males and females of the same genetical constitution as in the treated series were bred) only one culture was sterile. The average number of offspring per culture in the treated series was markedly lower than in the control series. Evidently the dosage of X-rays was sufficient to produce a significant decrease of fertility in the treated males.

In the F_1 generation all the expected classes of offspring appeared in each of the cultures of the treated as well as of the control series. These classes are:

Females:	y,	yB_l	yD,	$y\ B_l\ D$
Males:	+,	B_l,	D,	$B_l\ D$.

Although no particular care was paid to the detection of mutations, some were found in the cultures of the treated series, but none in the control series. Two of the mutations found were extreme allelomorphs of forked, one was a dominant (lethal when homozygous) allelomorph of vestigial (phenotypically like Beaded), and one was an extreme Minute (locus not determined). Besides the mutations, there were found nine individuals which were mosaics having one-half or one-quarter of the body Haplo-IV eyeless. These mosaics were certainly due to the elimination of the treated fourth chromosome in the cleavage of the eggs from which the mosaic individuals developed. The grand total of flies examined in the treated series was 2420, and in the control series - 1067.

Let us consider the genotypical constitution of the $B_l\ D$ males which appeared in the cultures of the treated series. They received a treated X-chromosome, a treated second chromosome carrying B_l, a treated third chromosome carrying D, and a treated fourth chromosome carrying the normal allelomorph of e_y from their fathers. On the other hand, they received an untreated Y-chromosome, an untreated second and third chromosome carrying normal allelomorphs of B_l and D respectively, and an untreated fourth chromosome carrying e_y (The allelomorph of e_y

used throughout the present study is e_y^2. However, for sake of simplicity, the symbol e_y will be used from now on.) from their mothers. In other words, all the four pairs of chromosomes of these males are "marked" by different characters, namely: sex, B_l D and e_y.

From the treated series 153 B_l D males and from the control series 26 B_l D males were crossed in single-pair cultures to untreated females homozygous for e_y. The progeny of this cross normally consists of 16 classes of offspring representing all the recombinations of the four "marking" characters mentioned. These classes are:

Females: $+, B_l, D, e_y, B_l\ e_y, D\ e_y, B_l\ D, B_l\ D\ e_y,$
Males $+, B_l, D, e_y, B_l\ e_y, D\ e_y, B_l\ D, B_l\ D\ e_y.$

However, if a parental B_l D male carries a translocation involving any two chromosomes, the individuals of some of these 16 classes of offspring may be expected either to be missing, or to possess visible external abnormalities. For instance, if the male carries a translocation involving the treated second and third chromosomes, no D, B_l, $D\ e_y$ and $B_l\ e_y$ individuals of either sex are expected in his progeny. Likewise, if such a male carries a translocation involving the treated second and X-chromosomes, no B_l males and no non-B_l females are expected in his progeny (or, B_l males and non-B_l females may occur, but be abnormal in appearance).

Of the 144 cultures of the treated series, 23 cultures were sterile; of the 26 control cultures only 2 were sterile. Furthermore, 112 cultures of the treated series and 24 cultures of the control series produced normal progenies, that is, in the overhelming majority of these cultures all 16 expected classes of offspring were present in approximately equal numbers. Only two of these "normal" cultures produced too small a number of offspring, and failed, therefore, to show one of the expected classes. Table 17 presents the summary of the counts observed in the "normal" cultures of the treated series.

The results obtained in the remaining nine cultures of the treated series are presented in table 1. In five of these cultures (No. 1271, 1318, 1319, 1407,1425) eight of the expected classes, representing the recombination of D and e_y, are missing. This fact suggests that a translocation involving the third and the fourth chromosomes is present in each of these cultures. In cultures 1223, 1239, 1289 and 1401, eight classes which represent the recombinations of B_l and D are missing, suggesting the presence of translocations involving the second and third chromosomes.

The results obtained in these nine cultures are, indeed, so strikingly different from the results obtained in all the "normal" cultures, that no

TABLE 1

Cultures showing linkage of genes located in different chromosomes.

CULTURE NUMBER	STRAIN	TRANSLOCATION INVOLVING CHROMOSOMES	WILD TYPE		B_1		D		e_v		$B_1 D$		$D e_v$		$B_1 e_v$		$B_1 D e_v$	
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1223	A	II–III	28	28	..	..	..	..	24	26	22	24	..	..	..	..	15	21
1239	B	II–III	22	16	..	..	..	..	20	20	28	21	..	..	..	..	14	19
1289	C	II–III	4	5	..	..	..	..	8	4	9	14	..	..	..	..	9	6
1401	D	II–III	3	8	..	..	..	..	7	7	2	4	..	..	..	..	4	12
1271	a	III–IV	..	..	..	..	18	12	11	21	19	18	..	..	10	12	..	..
1318	b	III–IV	..	..	..	..	14	13	20	15	21	13	..	..	12	7	..	..
1319	c	III–IV	..	..	..	..	19	15	9	13	22	19	..	..	26	17	..	..
1407	d	III–IV	..	..	..	..	13	7	12	14	10	11	..	..	17	12	..	..
1425	e	III–IV	..	..	..	..	24	19	12	15	17	17	..	..	13	15	..	..

doubt is possible in classifying a given culture as belonging to the "normal" or to the "abnormal" class.

Each of the nine cultures just described served as the progenitor of a separate strain, in which the abnormal genetical situation present in these cultures has been maintained by appropriate breeding. These strains are designated by the characters of the alphabet, as shown in table 1. The present paper deals only with the results of the investigation of the translocations involving the third and the fourth chromosomes. The translocations involving the third and the second chromosomes will be described in a separate paper.

WORKING HYPOTHESES

The genes D and e_v are localized in the third and in the fourth chromosomes respectively. Nevertheless, there is observed a complete linkage between D and e_v in five cultures shown in table 1. The first question concerning the nature of this abnormality is whether the causes which prevented the free recombination of D and e_v in these five cultures are hereditary.

Dichaete non-eyeless males from each of these five cultures were crossed separately to their eyeless sisters. Only D and e_v (no wild-type and no De_v) offspring were produced (table 2). In the next generation D

males were crossed to unrelated eyeless females; again only *D* and e_y individuals were produced. The linkage of *D* and e_y is, therefore, permanent, and Dichaete individuals transmit the cause producing this linkage to their Dichaete progeny. This fact gives a simple method of maintaining stocks of the translocations: *D* males from the stock cultures of the translocations are crossed in each generation to their e_y sisters or to unrelated e_y females.

TABLE 2

Progeny of Dichaete-flies (carrying translocations) when crossed to eyeless.

TRANSLOCATION	EYELESS ♀ × DICHAETE ♂					DICHAETE ♀ × EYELESS ♂				
	D	+	De_y	e_y	TOTAL	*D*	+	De_y	e_y	TOTAL
a	480	..	..	424	904	1080	2	2	915	1999
b	349	..	..	324	673	818	1	2	689	1510
c	344	..	..	303	647	1140	98	61	901	2200
d	313	..	..	415	728	540	381	434	645	2000
e	306	..	..	275	581	1240	52	58	842	2192

TABLE 3

Bent ♀ × Dichaete ♂ (carrying translocations).

TRANSLOCATION	*D*	+	Db_t	b_t	TOTAL
a	286	—	—	245	531
b	320	—	—	314	634
c	250	—	—	285	535
d	209	—	—	251	460
e	261	2(?)	—	249	512

If *D* females carrying a translocation are crossed to e_y males, the progeny consists chiefly of *D* and e_y flies, but some wild-type and De_y individuals also appear (table 2). In males the linkage between *D* and e_y is complete, but in the females these genes may be separated from each other by crossing over. In other words, in the translocations the genes *D* and e_y behave as if they were localized in the same chromosome.

The frequency of crossing over between the loci occupied by *D* and e_y is very different in different translocations. In a- and in b-translocations only 0.2 percent of crossing over occurs between *D* and e_y; in c- and e-translocations the frequency of crossing over is 7.2 percent and 5.0 percent respectively; in d-translocation a frequency as high as 40.7 percent was observed.

Two hypotheses explaining the behavior of D and e_y in the translocations may be suggested. The first hypothesis is that one of the fourth chromosomes of the treated male carrying the normal allelomorph of e_y ha s become permanently attached to the D-carrying third chromosome. Hence, from such a male all the offspring receiving the D-carrying third chromosome receive at the same time the fourth chromosome containing the normal allelomorph of e_y, and consequently are Dichaete non-eyeless in appearance. However, in females carrying translocations, D and e_y may be separated by crossing over; hence, wild-type and $D\ e_y$ individuals appear along with D and e_y in the progeny. The frequency of the crossing over between D and e_y depends upon the distance between the locus of D and the point of attachment of the fourth chromosome to the third chromosome. Since the frequency of this crossing over is found to be very diverse in the five translocations studied, the fourth chromosome may be assumed to be attached to different parts of the third chromosome.

The second hypothesis assumes that a broken off section of the D-carrying third chromosome from the treated male is attached to the fourth chromosome carrying the normal allelomorph of e_y. In other words, the third chromosome was broken into two fragments, and one of these fragments became attached to the fourth chromosome. On this hypothesis males carrying a translocation produce two kinds of gametes in equal numbers (see figure 1). One kind of gametes contains both fragments of the third chromosome, one fragment being attached to the fourth chromosome. These gametes carry, therefore, the gene D and the normal allelomorph of e_y. The other kind of gametes contains the normal (that is, the unbroken) third chromosome and the free fourth chromosome. These gametes carry e_y but do not carry D. If such a male is crossed to a female homozygous for e_y but free from the translocation, only Dichaete non-eyeless and eyeless non-Dichaete individuals are produced (see figure 1). On the other hand, in females carrying a translocation both fragments of the third chromosome involved in the translocation may conjugate with the unbroken third chromosome. If crossing over occurs after such a synapsis, D and the normal allelomorph of e_y may be separated from each other. Consequently, $D\ e_y$ and wild-type individuals may appear in the progeny of a female carrying a translocation.

The two hypotheses just outlined are rather similar in nature. Both of them involve the assumption that in the translocations a union between the third and the fourth chromosomes occurred. According to both of them the frequency of crossing over between D and e_y indicates the distance between the locus of D and the locus of the attachment of the fourth chromo-

some. The only essential difference between these two hypotheses is that the first of them postulates the attachment of the fourth chromosome to the unbroken third chromosome, while the second hypothesis postulates a breakage of the third chromosome and an attachment of the fourth chromosome to one of the resulting fragments of the third. This difference is, however, important from the standpoint of terminology. If the fourth chromosome is attached to the unbroken third, the phenomenon should be

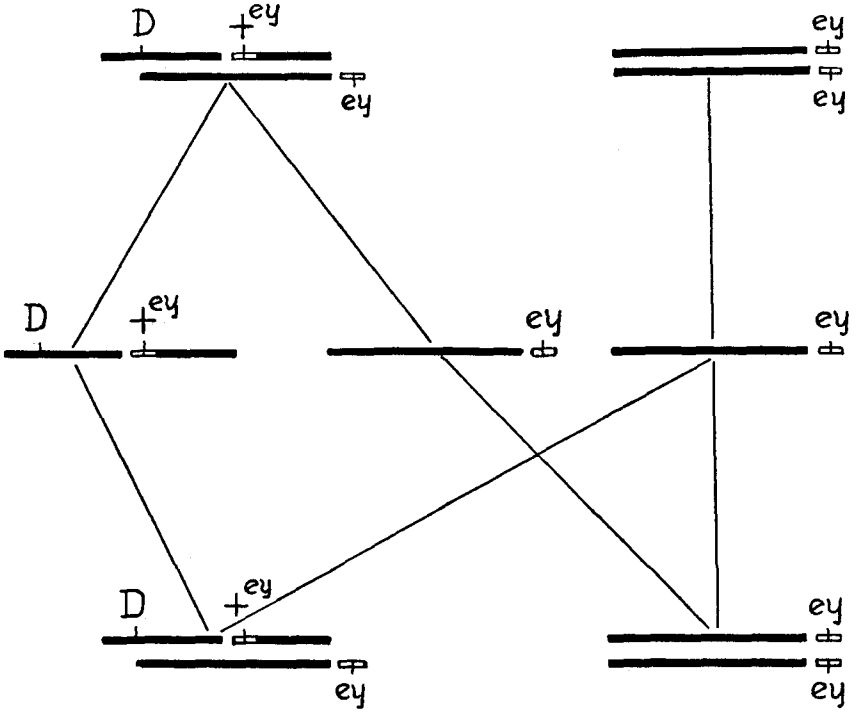


FIGURE 1.—Scheme of a mating of a fly heterozygous for a translocation to a fly free from translocations. Third chromosome—black; fourth chromosome—white. *D*—Dichaete; e_y—eyeless. Only gametes giving rise to viable zygotes are represented in this figure.

termed not a translocation but a compounding of chromosomes (see BRIDGES 1923). However, if a fragment of the third chromosome is attached to the fourth, we are dealing with a translocation of a section of the third chromosome on to the fourth.

The cytological investigation is, obviously, the most direct way to the solution of these alternatives. However, before presenting the results of the cytological investigation of the translocations, their genetical behavior may be examined in fuller detail.

BEHAVIOR OF THE GENES OF THE FOURTH LINKAGE GROUP IN THE TRANSLOCATIONS

The fourth chromosome of *Drosophila melanogaster* is much smaller in size than the other chromosomes of this species. In accordance with this smallness, the number of genes localized in the fourth chromosome is also much less than the number of the genes in other chromosomes. Furthermore, no (or very little) crossing over takes place between the known genes of the fourth chromosome (unpublished data of BRIDGES). It seems *a priori* very probable that in translocations the behavior of all the fourth chromosome genes is identical with the behavior of e_y which has been discussed above. Both hypotheses advanced in the preceding section assume that in all the translocations the whole fourth chromosome rather than a fragment of it is attached to the third chromosome. The extreme smallness and the absence of crossing over in the fourth chromosome make the contrary supposition improbable.

To test the validity of this point, *D* males carrying translocations were crossed to females homozygous for bent. Bent (b_t) is a fourth-chromosome recessive gene producing a shortening of the legs and a spreading of the wings of flies. From the F_1 of this cross *D* males were selected and backcrossed to homozygous b_t females. In the next generation only *D* and b_t (no wild-type and no *D* b_t) individuals appeared (table 3). The two wild-type flies shown in table 3 were probably genotypically b_t; their appearance is due to the fact that b_t some times overlaps wild type phenotypically. When *D* females from the F_1 were crossed to b_t males, some wild-type and *D* b_t individuals appeared along with *D* and b_t individuals. The behavior of b_t in translocations is, therefore, identical with behavior of e_y.

The presence of a linkage between *D* and the fourth-chromosome dominant gene Minute-IV in translocations was proved by a similar method. Dichaete males carrying translocations were crossed to females heterozygous for Minute-IV (Minute-IV is lethal when homozygous). In the F_1 generation Dichaete Minute-IV males were selected and crossed to wild-type females. Only *D* and Minute-IV (no wild type and no Dichaete Minute-IV) were produced in the progeny.

Although the two remaining genes located in the fourth chromosome (namely shaven and rotated abdomen) were not tested in the translocations, there can be no doubt that their behavior is identical with the behavior of e_y, b_t and Minute-IV.

CROSSING OVER IN THE THIRD CHROMOSOME INVOLVED IN THE TRANSLOCATIONS

A series of experiments was necessary to determine the exact loci in the third chromosome at which the fourth chromosome is attached in the different translocations. The exact determination of these loci is especially important, since, according to the second hypothesis outlined above, they may coincide (and as it will be proved later do coincide) with the breaking-points of the third chromosome in the translocations.

Dichaete males carrying translocations were crossed to females homozygous for e_y and homozygous for the third-chromosome recessive genes roughoid (r_u-rough eye-surface), hairy (h-hairs along the wing-veins), thread (t_h-unbranched aristae), scarlet (s_t-bright red eye-color), curled (c_u-wings curved upwards), stripe (s_r-dark longitudinal stripe on the thorax), sooty (e^s-dark body-color) and claret (c_a-purplish pink eye-color). Dichaete females were selected in the F_1 generation of this cross and backcrossed to $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males. The results obtained in the next generation are presented in tables 11-15 (appendix). (Flies homozygous for the allelomorph of e_y used, namely e_y^2, rarely have both eyes completely absent. In flies having no eyes the classification in respect to r_u, s_t and c_a is, of course, impossible. Such flies were disregarded and are not included in the counts presented in tables 11-15. The frequency of homozygous e_y^2 flies having no eyes is, however, so low that their omission can not influence appreciably the crossing over values obtained.)

The frequency of crossing over in an interval between two given genes depends to a certain extent upon the modifying genes present in the stock used. Therefore, it is not desirable to compare the frequency of crossing over in the third chromosome involved in the translocations directly with the frequency of crossing over in the normal third chromosome indicated by the standard map of this chromosome (for the standard map see figure 2, also MORGAN, BRIDGES, STURTEVANT 1925, p. 92). With the object of having a better standard of comparison a special control experiment was made. Dichaete males from the same stock of *D* flies from which the *D* males treated by X-rays were obtained (see above) were crossed to homozygous $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ females from the same stock which was used for the study of crossing over in the translocations. In the F_1 generation *D* females were selected and backcrossed to $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males. The results obtained in the next generation are recorded in table 16 (appendix).

The crossing over values were calculated from the data shown in tables 11-16. The calculated crossing over values are presented in table 4. As

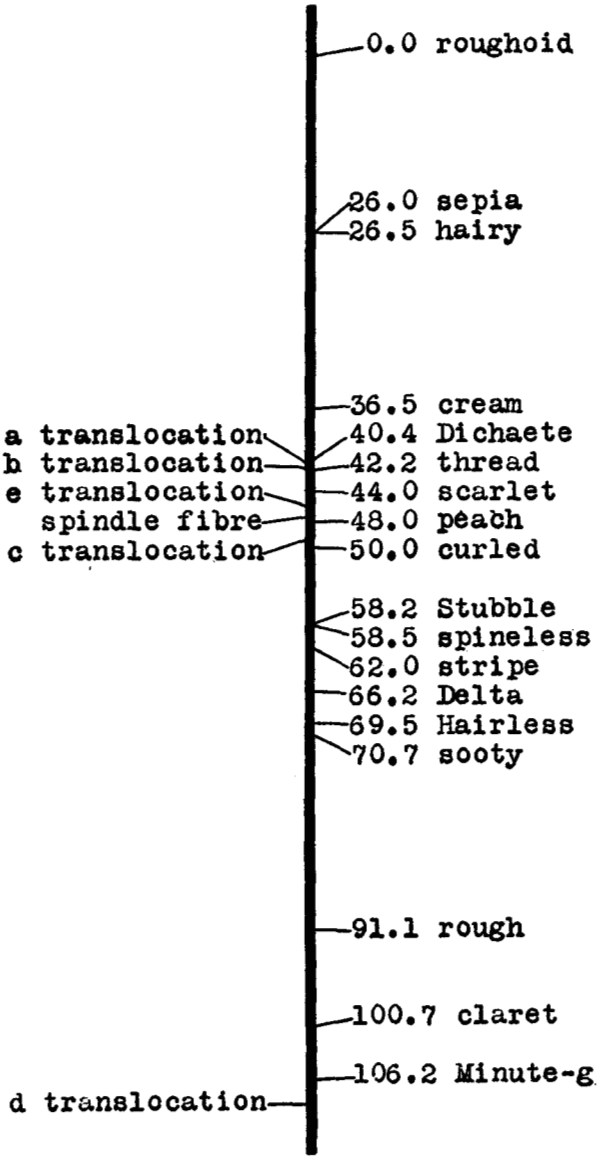


FIGURE 2.—Genetical map of the third chromosome of *Drosophila melanogaster*.

seen from table 4, the point of attachment of the fourth chromosome to the third (designated in table 4 by +) is not alike in the different translocations.

In a-translocation this point is located between the genes D and t_h. In b-translocation the point of attachment is nearly the same as in a-translocation but in b-translocation it seems to coincide with the locus of the gene t_h. In c-translocation and in e-translocation the fourth chromosome is attached near the middle of the length of the third chromosome, namely in the s_t-c_u interval. However, in e-translocation the point of attachment is located nearer to s_t than to c_u, whereas in c-translocation it is nearer to c_u than to s_t. Finally, in d-translocation the fourth chromosome is attached near the extreme right-hand end of the third chromosome, 5.1 units to the right of the gene c_a.

TABLE 4

Crossing over values for the intervals studied in the third chromosome.

				Translocation a.					
Interval	r_u-h	h-D	D-+	+-t_h	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^s	e^s-c_a
Value	17.2	2.9	.05	.1	.2	6.6	16.6	11.7	31.0
				Translocation b.					
Interval	r_u-h	h-D	D-+-t_h	t_h-s_t		s_t-c_u	c_u-s_r	s_r-e^s	e^s-c_a
Value	21.2	5.9	.2	.3		4.6	17.1	10.0	31.0
				Translocation c.					
Interval	r_u-h	h-D	D-t_h	t_h-s_t	s_t-+	+-c_u	c_u-s_r	s_r-e^s	e^s-c_a
Value	26.9	15.4	1.5	.9	3.8	.7	3.7	7.5	28.9
				Translocation d.					
Interval	r_u-h	h-D	D-t_h	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^s	e^s-c_a	c_a—+
Value	29.1	14.8	1.1	.9	5.9	12.6	8.9	19.2	5.1
				Translocation e.					
Interval	r_u-h	h-D	D-t_h	t_h-s_t	s_t-+	+-c_u	c_u-s_r	s_r-e^s	e^s-c_a
Value	19.5	11.6	1.1	.5	1.5	3.6	15.6	11.6	31.0
				Control					
Interval	r_u-h	h-D	D-t_h	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^s	e^s-c_a	
Value	23.7	13.7	1.1	.8	8.0	15.4	10.3	31.0	

Comparison of the crossing over values for the different intervals of the third chromosome observed in the translocation and in the control experiment reveals the presence of consistent differences between them (see table 5). In a, b- and in e-translocations the frequency of crossing over is markedly decreased to the left of the s_t-c_u interval but slightly increased to the right of this interval. On the other hand, in c- and d-translocations crossing over is decreased to the right of the s_t-c_u interval but increased to the left of it.

These regularities may be described also as follows. A point located approximately in the middle of the s_t-c_u interval divides the third chromosome into two limbs. If the fourth chromosome is attached to the third

chromosome to the left of this point (as is the case in a-, b- and e-translocations), the frequency of crossing over is decreased in the left limb but increased in the right limb of the third chromosome. If, however, the point of the attachment of the fourth chromosome lies to the right of the point mentioned above (as is the case in c- and d-translocations), the frequency of crossing over is decreased in the right limb of the third chromsome but increased in the left limb (see figure 2). (Following the practice established in the Drosophila literature, the end of the third chromosome at which the gene c_a is located is designated in this discussion as the right-hand end, and the end at which the gene r_u is located as the left-hand end of this chromosome.)

TABLE 5

Differences between the crossing-over values in the translocations and the corresponding values observed in the control experiment.

INTERVAL		TRANSLOCATION				
		a	b	c	d	e
Left of the spindle fibre	r_u-h	−6.5	−2.5	+3.2	+5.4	−4.2
	h-D	−10.8	−7.8	+1.7	+1.1	−2.1
	D-t_h	−.95	−.9	+.4	0.0	0.0
	t_h-s_t	−.6	−.5	+.1	+.1	−.3
Spindle fiber	s_t-c_u	−1.4	−3.4	−3.5	−2.1	−2.9
Right of the spindle fibre	c_u-s_r	+1.2	+1.7	−11.7	−2.8	+.2
	s_r-e^s	+1.4	−.3	−2.8	−1.4	+1.3
	e^s-c_a	0.0	0.0	−2.1	−11.8	0.0

The strongest reduction of the frequency of crossing over is observed in the intervals adjacent to the locus of the attachment of the fourth chromosome. Nevertheless, some reduction of crossing over takes place in all the intervals of the limb of the third chromosome in which the point of attachment of the fourth chromosome lies. For instance, in d-translocation the greatest decrease of the frequency of crossing over is observed in the e^s-c_a interval, while in the c_u-s_r interval the reduction is comparatively slight. In d-translocation the point of attachment of the fourth chromosome lies near the e^s-c_a interval. On the contrary, in c-translocation the frequency of crossing over is strongly decreased in the c_u-s_r interval, while in the e^s-c_a interval it is but slightly affected. In c-translocation the fourth chromosome is attached to the left of c_u. It is significant, however, that if the attachment of the fourth chromosome is near to the point dividing the third chromosome in two limbs (for example, the point lying half way between s_t and

c_u) the decrease of the crossing over frequency does not affect the adjacent intervals of both limbs (see c- and e-translocations). This fact suggests that the point dividing the two limbs of the chromosome plays some especially important role in the distribution of the crossing over along the third chromosome.

Most of the differences between the crossing over values for the respective intervals observed in the translocations and in the control experiment are statistically significant. Furthermore, the consistency of the results obtained suggests that these differences are significant even in the cases where the value of the difference in question is below the limit of statistical certainty.

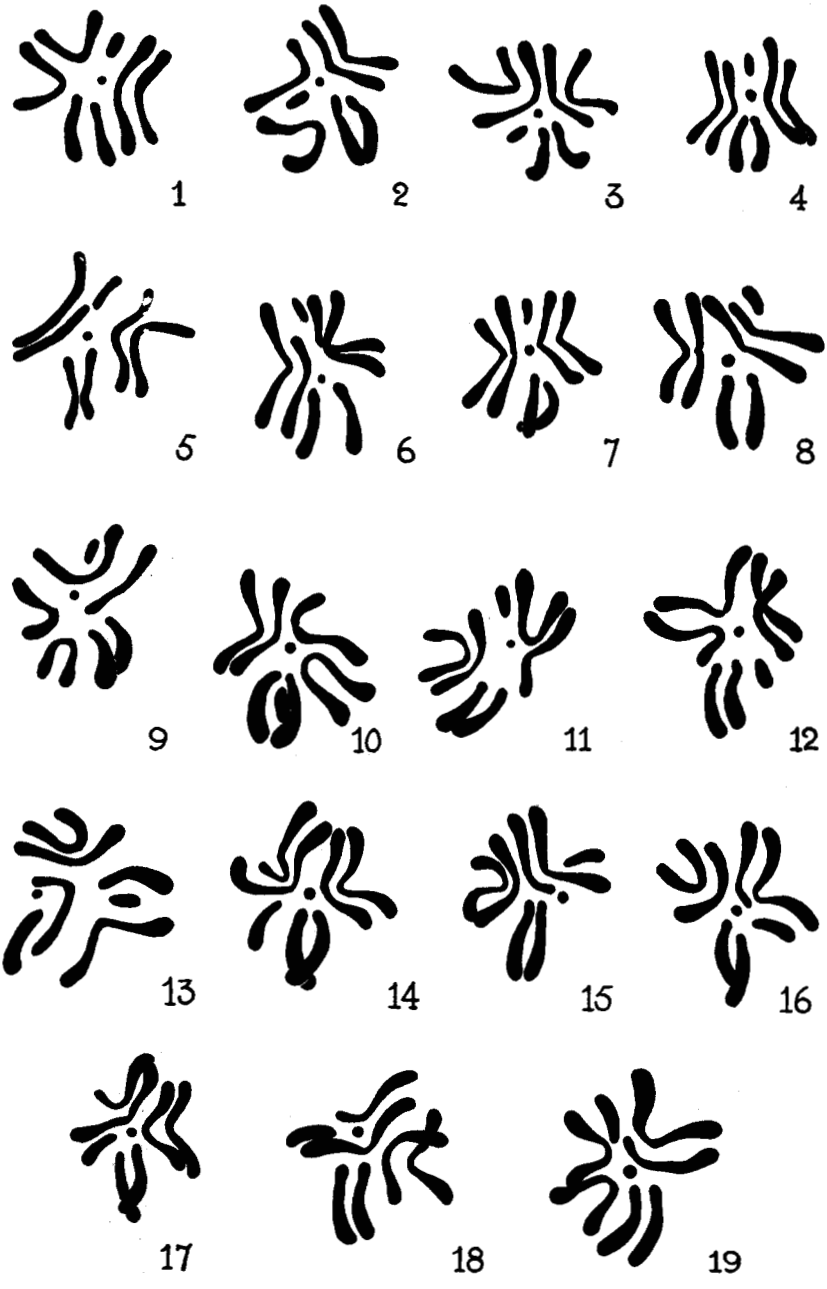
It might be supposed that differential viability of the various classes of crossover individuals is responsible for the alteration of the crossing over frequencies in the translocations. Since the point of attachment of the fourth chromosome to the third is not the same in different translocations the distribution of e_y among the crossovers is not alike in different cases. The gene e_y in homozygous condition causes some decrease of the viability of the flies. However, the difference between the crossing over values found in the different translocations can not be accounted for by the influence of e_y on the viability of the flies. Indeed in both *c*- and e-translocations the locus of the fourth chromosome attachment lies in the s_t - c_u interval. The distribution of e_y among the crossing-over individuals is, therefore, alike in c- and e-translocations. Nevertheless, in c-translocation there is observed a decrease of the frequency of crossing over in the right limb and an increase in the left limb of the chromosome, while in e-translocation a decrease takes place in the left limb and an increase in the right limb.

The third chromosome of *Drosophila melanogaster*, as seen in the cytological preparations, is V-shaped, and the point of attachment of the spindle fibre to the body of the chromosome lies at the middle of its length, dividing the chromosome into two limbs of equal size. It is very probable that the decrease of the frequency of crossing over in one part of the chromosome and the increase of its frequency in the other part of the same chromosome is due to the location of the point of the attachment of the fourth in relation to the spindle fibre. If the fourth chromosome is attached to the left of the locus of the attachment of the spindle fibre (in a-, b- and e-translocations) crossing over is reduced in the left limb of the chromosome and increased in the right limb. If, however, the fourth chromosome is attached to the right of the spindle fibre (as in c-and d-translocations), the crossing over frequency is decreased in the right limb but increased in

Explanation of Plates 1 and 2

The drawings represent chromosomes (oögonial plates) of the females carrying translocations in heterozygous (figures 1–33) or in homozygous (figures 34–38) condition. All the drawsings are done at the level of the work-table with the aid of camera lucida, under the Zeiss immers. obj. 1.5 and comp. oc. 30. The scale given in plate 2 below, common to all the drawings, represents 10 micra. As far as possible the figures were placed so that the X-chromosomes occupy the lower part of each figure.

Plate 1, figures 1–6 represent a-translocation; figures 7–13 b-translocation; figures 14–19 c-translocation (heterozygous); plate 2, figures 20–24 d-translocation; figures 25–33 e-translocation; figures 34–38 homozygous c-translocation.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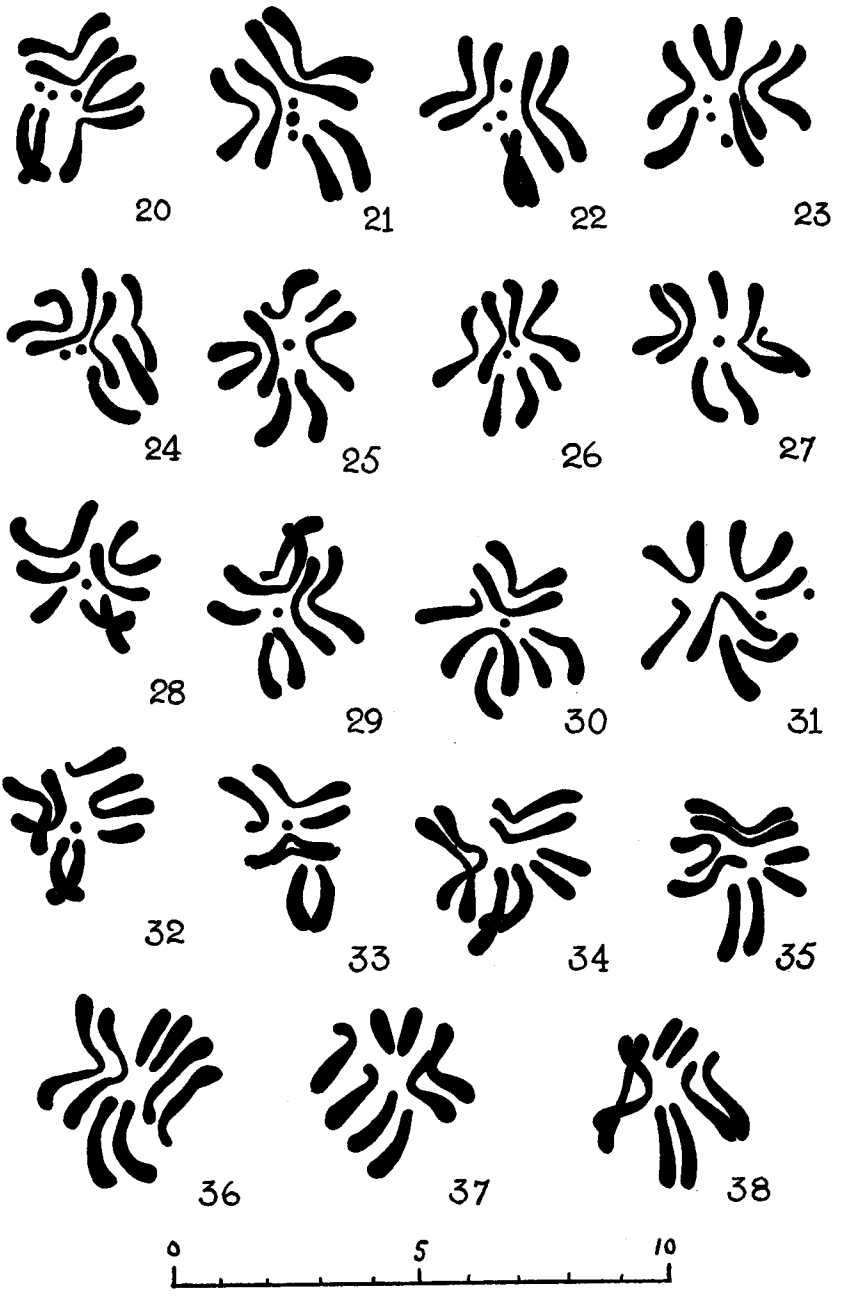
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the left limb of the chromosome. It is a plausible assumption that the point lying half way between s_t and c_u which divides the third chromosome in two "genetical limbs" (see above) is in reality the point of spindle-fibre attachment. The location of this point approximately in the middle of the length of the map of the third chromosome (see figure 2) is in good agreement with such an assumption, since the attachment of the spindle fibre is located cytologically also at the middle of the length of the chromosome.

It must be emphasized that the location of the point of spindle-fibre attachment between the loci of s_t and c_u was already probable on the basis of the data secured by quite independent methods. BRIDGES and MORGAN (1923) on the basis of the study of the distribution of double crossing over in the third chromosome came to the conclusion that the point of the spindle-fibre attachment lies between the loci of s_t and peach (the locus of peach is between s_t and c_u). STURTEVANT came to the same conclusion on the basis of the study of inverted sections (STURTEVANT and PLUNKETT 1926, also unpublished data). Recently REDFIELD (unpublished) obtained further evidence pointing in the same direction, from investigations of the crossing over frequency in the third chromosome of triploid females.

It has been shown above that in c-translocation the frequency of crossing over is decreased in the right limb and increased in the left limb of the third chromosome, while in e-translocation the conditions are reversed. It follows that the point of spindle-fibre attachment lies between the loci of attachment of the fourth chromosome in c- and in e-translocation. It is known, furthermore, that in both c- and e-translocations the fourth chromosome is attached to the third between the genes s_t and c_u. Therefore it becomes especially important to determine the location of the fourth-chromosome attachment in c- and e-translocations in respect to the gene peach (p-eye color) which lies within the s_t -c_u interval.

TABLE 6

Crossing over in $\frac{e_y \quad D}{\cdot\cdot \quad s_t\ p\ c_u}$ ♀ × $e_y\ D\ s_t\ p\ c_u$ ♂.

Translocation c.

Interval	D-s_t	s_t-p	p-+	+-c_u	Total flies examined
Value	2.2	3.4	.07	.2	1481

Translocation e.

Interval	D-s_t	s_t-+	+-p	p-c_u	Total flies examined
Value	.8	1.2	1.1	2.4	1288

Dichaete males carrying c- and e-translocations respectively were crossed to females homozygous for e_y, s_t, p and c_u. In the F_1 generation D females were selected and backcrossed to $e_y\ s_t\ p\ c_u$ males. The results

obtained in the next generation are summarized in table 6. As seen from table 6, in e-translocation the locus of attachment of the fourth chromosome lies in the s_t-p interval while in c-translocation it lies in the p-c_u interval. The frequency of crossing over in the D-s_t and the s_t-p intervals is considerably lower in e-translocation than in c-translocation. On the contrary, the frequency of crossing over in the p-c_u interval is lower in c-translocation than in e-translocation.

It may be concluded on the basis of this evidence that the point of the spindle-fibre attachment is located near the gene peach, probably slightly to the left of it. In other words, the spindle fibre is attached to the third chromosome between the loci 46 and 48 of the standard map (see figure 2).

CYTOLOGICAL CHANGES PRODUCED BY THE TRANSLOCATIONS

It has been pointed out above that the genetical behavior of the translocations may be explained on either of two assumptions. First, the fourth chromosome may have become permanently attached to the third chromosome. Second, a section of the third chromosome may have become broken off and attached to the fourth chromosome. The observed alterations of the frequency of crossing over in the the third chromosome might be produced, judging *a priori*, in either case, In other words, the genetical data so far presented give no evidence as to which of these two assumptions is correct. This, however, may be easily decided by the cytological investigation of the translocations. If the fourth chromosome in the translocations is attached to the unbroken third, only one instead of two fourth chromosomes must be present in the chromosomal plates. Except this, the chromosomes must look normal, since the point of the attachment of the fourth chromosome will be, probably, indistinguishable because of the extreme smallness of this chromosome, However, if a section of the third chromosome is broken off and attached to the fourth chromosome, one of the cytologically visible third chromosomes must be shortened, and one of the fourth chromosomes must be increased in size because of the addition of the material from the third chromosome.

Ovaries of freshly hatched females or of mature pupae were dissected and fixed successively in strong Flemming's fluid (1 hour) and in 1 percent solution of chromic acid (20 hours). Not less than 7 sufficiently clear chromosome plates were obtained for each of the five translocations. Most of them are presented in plates 1 and 2 (see also figures in DOBZHANSKY 1929a).

It is most convenient to begin the presentation of the results of the cytological investigation of the translocations with a description of the features in common to a-, b-, c- and e-translocations (plate 1, figures 1–19,

plate 2, figures 25–33). The overwhelming majority of the chromosomal plates studied in these translocations (52 plates out of 54 studied) contain only one free fourth chromosome of normal size. This fact is important, since it may be considered as a cytological proof of our basic assumption, namely that the observed linkage between the genes belonging to the third and to the fourth linkage groups is due to translocations involving these chromosomes. Individuals having only one fourth chromosome are known in *Drosophila melanogaster*, but they always have a complex of external peculiarities called "Haplo-IV" or "Diminished" (BRIDGES 1921). However, flies carrying translocations never possess the characteristics of Haplo-IV. This fact may be explained only on the assumption that the flies carrying translocations have in reality two fourth chromosomes, one free, and the other attached to the third chromosome or to a fragment of the latter. The few cases in which two free fourth chromosomes are found in the translocations are probably due to non-disjunction of the fourth chromosomes. In these cases the individuals have in reality three fourth chromosomes, two of them being free and one attached to the third chromosome. The plausibility of this explanation will be examined below.

The pair of X-chromosomes and the smaller pair of the V-shaped autosomes appear to be perfectly normal in all plates. However, the larger pair of the V-shaped autosomes consists of two partners distinctly unequal in length, although showing somatic pairing with each other in the majority of the plates studied. The longer partner is symmetrical, that is, it has both limbs equally long. The equality of the length of the limbs of the longer partner is especially clearly seen in the cases in which the median constriction of the chromosome is clearly pronounced (see plate 1, figures 6, 7, 8, 12, 16, 17, 18, plate 2, figures 27, 31). On the other hand, the shorter partner is asymmetrical, that is, it has one of the limbs markedly shorter than the other. The inequality of the two limbs of the asymmetrical partner is manifest in all plates studied, but it is especially striking in the case where the median constriction is pronounced (see plate 1, figures 3, 7, 10, 14, plate 2, figures 27, 29, 33).

Since the V-shaped autosomes in the chromosomal plates of the normal flies have both limbs equal in length, it is possible to conclude that the asymmetrical autosomes just described are the third chromosomes involved in the translocations.

The asymmetrical chromosome is the member of the larger pair of the V-shaped autosomes. The difference in length between the larger and the smaller pair of the V-shaped autosomes is clear enough in the majority of

the chromosomal plates studied. Among all the plates presented here perhaps only those in figure 5 (plate 1) and in figure 23 (plate 2) may be uncertain as to which of the two pairs of the V-shaped autosomes is the larger and which the smaller. Therefore, it is possible to conclude that the larger pair of the-V shaped autosomes of *Drosophila melanogaster* carries the third-chromosomal linkage group of genes, whereas the smaller pair carries the second-chromosome linkage group. In what follows we shall call the larger pair of the V-shaped autosomes the third chromosome, and the smaller pair the second chromosome.

This identification of the third and the second chromosomes is in disagreement with the identification given by BRIDGES (MORGAN, STURTEVANT, BRIDGES 1928), MULLER and PAINTER (1929) and PAINTER and MULLER (1929). Indeed, the authors mentioned identified the smaller pair of the V-shaped autosomes in *Drosophila melanogaster* as being the carrier of the third-chromosome linkage group, and the larger pair as the carrier of the second-chromosome linkage group of genes. The conclusions of BRIDGES were based on the cytological study of "Pale" translocation in which case only a very small fragment of the second chromosome has been broken off and attached to the third chromosome. The cytological differences observed were so slight, that no decisive conclusion could be drawn. In fact, BRIDGES himself previously identified the larger pair as the third chromosome and the smaller pair as the second chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 177) on the basis of the study of the same "Pale" translocation. Doctor C. B. BRIDGES has seen my preparations and agrees with my interpretation. MULLER'S and PAINTER'S material is quite dependable, since the translocations described by these authors involve large sections of the second and third chromosomes. But, as far as I can see, at least some of PAINTER'S own figures representing the cytological conditions in the translocations involving the second or the third chromosomes show the third chromosome longer than the second (see MULLER and PAINTER 1929, figures 1, 3, 5, 6, PAINTER and MULLER 1929, figure 29). As far as the length of the chromosomes is concerned there remains no doubt that the third chromosome is longer than the second.

Both ends of the normal V-shaped autosomes are thicker than the middle part of the same chromosomes. It is worth notice that the shorter limb of the asymmetrical third chromosome found in the translocations is always less thickened at the end than the longer limb of the same chromosome. The longer limb of the asymmetrical third chromosome is equal

in length and shape to either limb of the normal third chromosome present in the same plate.

Obviously a loss of some material normally located in the third chromosome is responsible for the shortening of one of the limbs of the asymmetrical third chromosome. This material is undoubtedly to be looked for in the small, rod-shaped, unpaired chromosome clearly visible in all the chromosomal plates in the translocations but not present at all in the normal chromosomal plate of *Drosophila melanogaster*. The length of the rod-shaped chromosome plus the length of the asymmetrical third chromosome is about equal to the length of the normal third chromosome present in the same plate. One of the ends of the new rod-shaped chromosome (that directed toward the periphery of the chromosomal plate) is in most cases thicker than the other end of the same chromosome (directed toward the center of the plate). The thickness of the thicker end of the rod-shaped chromosome is approximately equal to (or a little smaller than) the thickness of the ends of the normal third chromosome.

The interpretation of the chromosomal relations found in a-, b-, c- and e-translocations is obvious. In each of these translocations a third chromosome is broken into two fragments of unequal length. The shorter of these fragments is attached to a fourth chromosome. The resulting compound-chromosome (a fragment of the third chromosome plus the fourth chromosome) is seen cytologically as the unpaired rod-shaped chromosome just described. The longer of the fragments is represented by the asymmetrical third chromosome, having one limb markedly shorter than the other limb. The end of the shorter limb of the asymmetrical third chromosome corresponds to the point at which the third chromosome has been broken. Finally, since one of the fourth chromosomes is included in the unpaired rod-shaped chromosome, only, one free fourth chromosome is seen in most of the plates. In other words, the second of the two hypotheses suggested for the explanation of the genetical behavior of the translocations is confirmed by the cytological data.

According to the genetical data presented in the preceding section, the points at which the breakage of the third chromosome occured in a-, b,- c- and e-translocations are different (see figure 2). If the genetical map represents the real arrangement of the genes in the third chromosome, the broken off sections are of different lengths in different translocations. Consequently, the size of the asymmetrical third chromosome as well as the size of the unpaired rod-shaped chromosome must be differentiated in the different translocations studied.

In a- and b-translocations the third chromosome is broken between the loci of the genes D and t_h; in b-translocation the breakage occurred very slightly nearer to the middle of the chromosome than in a-translocation. The cytological conditions found in a- and b-translocations are nearly alike (a-translocation plate 1, figures 1–6, b-translocation plate 1, figures 7–13). The length of the unpaired rod-shaped chromosome equals about one-third the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb shorter than the other by approximately one-third of its length. In b-translocation the rod-shaped chromosome seems to be very slightly longer than in a-translocation, and correspondingly the asymmetrical third chromosome is slightly shorter in b-translocation than in a-translocation. According to the genetical data the rod-shaped chromosome in these cases must carry the genes from D to the left end of the third chromosome (including the gene r_u), and the asymmetrical third chromosome must carry the genes from t_h to right end of the third chromosome (including c_a).

In e-translocation the third chromosome is broken between the locus of s_t and the hypothetical locus of the spindle-fibre attachment. Consequently, the rod-shaped chromosome in this case contains the genes from s_t to r_u, and therefore must be longer than the rod-shaped chromosome in a- and b-translocations (compare figure 2). The asymmetrical third chromosome in e-translocation carries the genes from p to c_a, and, therefore, its shorter limb must be considerably shorter than in a- and b-translocations. This expectation is confirmed by the cytological investigation of e-translocation (plate 2, figures 25–33). The length of the rod-shaped chromosome equals five-sixths or four-fifths the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb very short. It is clearly visible, in cases in which the median constriction is pronounced, that the third chromosome is broken very near its middle, that is, near the point of attachment of the spindle fibre (see figures 27, 29, 33).

In c-translocation the third chromosome is broken between the genes p and c_u, that is to the other side of the spindle-fibre attachment as compared with e-translocation. The rod-shaped chromosome in this case contains the genes from c_u to c_a, and the asymmetrical third chromosome carries the genes from p to r_u. Cytologically (plate 1, figures 14–19) the conditions found in c-translocation are similar to those in e-translocation, but here the rod-shaped chromosome is slightly shorter and the asymmetrical third chromosome slightly longer than in e-translocation. This fact indicates

that the locus of attachment of the spindle fibre is cytologically nearer to the point at which the third chromosome is broken in e-translocation than to the point of breakage in c-translocation.

The chromosomes in d-translocation are at first glance like those of a normal fly. Both pairs of V-shaped autosomes as well as the X-chromosomes appear to be normal. However, one of the fourth chromosomes is clearly increased in size, being roughly from $1\frac{1}{2}$ to twice as large as the other fourth chromosomes present in the same plates. Only in a single plate (figure 24) is the increase in size of one of the fourth chromosomes not distinct. There are present three free fourth chromosomes in most plates in d-translocation, indicating the high frequency of non-disjunction of the fourth chromosomes in this translocation.

The increase in size of one of the fourth chromosomes indicates that the nature of d-translocation is the same as the nature of the other translocations studied, namely that here also a section of the third chromosome is broken off and attached to the fourth chromosome. However, in D-translocation the section of the third chromosome removed from its normal location is so small that its loss is not noticeable in the cytologically visible third chromosome because the third chromosome itself is relatively very large. Nevertheless, the addition of this section to the fourth chromosome is visible cytologically because of the extreme smallness of the latter chromosome.

This explanation agrees very nicely with the genetical data on d-translocation. According to the genetical data the point of breakage of the third chromosome in d-translocation is located 5.1 units to the right of c_a, that is, near the extreme right-hand end of the third chromosome. The map of the third chromosome shows the gene Minute-g located 5.5 units to the right of c_a (figure 2). However, in d-translocation the break in the third chromosome is probably to the right of Minute-g, for the crossing over value for the e^s-c_a interval is 19.2 percent in d-translocation instead of 31.0 units observed in the control. Since in d-translocation the frequency of crossing over is considerably decreased in the right limb of the chromosome, the claret - Minute-g interval is probably considerably shorter than 5.5 units. Correspondingly, the point of the breakage of the third chromosome is located to the right of Minute-g, in the unknown part of the extreme right-hand end of the third chromosome. The size of the section of the third chromosome which has been broken off and attached to the fourth chromosome must be very small in d-translocation.

It is a remarkable fact that in all five cases of translocations studied there is observed a breakage of the third chromosome and an attachment

of the broken off section to the fourth chromosome. In not a single case is the fourth chromosome attached to the unbroken third chromosome. Furthermore, the fragment of the third chromosome attached to the fourth never includes the region of the third chromosome in which the spindle-fibre attachment lies. On the contrary, the fragment of the third chromosome remaining free always includes the region of the spindle-fibre attachment.

These relations suggest the possible manner of origin of the translocation described. The chromosomes treated by X-rays are likely to stick together because of the increase of ionization produced by the rays. An attachment of the fourth chromosome to the third may occur. However, the resulting compound has two spindle fibres (one contributed by the third chromosome and the other by the fourth chromosome). The two spindle fibres may pull the compound chromosome to opposite poles of the spindle during mitosis, producing the breakage of the chromosome, or else the rate of contraction of the two spindle fibres may be different, in which event the breakage of the compound-chromosome also may take place. Both resulting fragments have one and only one spindle fibre, and therefore behave during mitosis like normal chromosomes. It seems very probable that a fragment of a chromosome can be normally transmitted from one cell-generation to the other only when it has its own spindle fibre, or acquires a spindle fibre by an attachment to another chromosome or to a fragment of a chromosome possessing a spindle fibre.

The points of breakage of the third chromosome observed in the translocations do not show a tendency to coincide with the constrictions in the chromosome described by BRIDGES (1927). According to BRIDGES, the third chromosome of *Drosophila melanogaster* has a strong median constriction at the point of the spindle-fibre attachment, and two other less pronounced constrictions in each of the two limbs. One of the latter constrictions, the submedian, lies at about one-fifth of the limb-length from the median constriction; another, still less pronounced, lies at about one-quarter of the limb-length from the end of the chromosome. None of the observed breakages of the third chromosome coincide with the median constriction. The other two constrictions are so inconspicuous that it is difficult to decide whether in a given case the point of breakage coincides with one of them. However, the breakage point in both c- and e-translocations can not coincide with the submedian constriction. Indeed, in c-translocation the point of breakage of the chromosome is located further from the median constriction than in e-translocation, whereas

the submedian constrictions in both limbs of the chromosome are equidistant from the median constriction. Likewise, the point of breakage either in a- or in b-translocation may be supposed to coincide with the constriction located at one-fourth of the limb-length from the end of the chromosome. However, at least in one of these cases the point of breakage certainly does not coincide with the constriction, since, as the genetical data show, the breakages in a- and b-translocations are not located at the same point. Finally, the breakage observed in d-translocation is located in a region of the third chromosome in which no constrictions are known.

GENETICAL CONSEQUENCES OF THE BREAKAGE OF THE THIRD CHROMOSOME IN THE TRANSLOCATIONS

In each of the translocations the locus in the third chromosome at which the fourth chromosome is attached was determined by studying the linkage relations of the genes of the third and fourth linkage groups. It has been pointed out above that this method gave no evidence on the question whether the fourth chromosome is attached to the unbroken third, or a section of the third chromosome has been broken off and attached to the fourth chromosome. This question was solved by the cytological investigation of the translocations. Indeed, in each of the five translocations studied the third chromosome was observed to be broken into two fragments and the shorter of these fragments is attached to the fourth chromosome. It is obviously desirable to check the results of the cytological investigation by an *experimentum crucis*, that is, to demonstrate by purely genetical methods the presence of a break of the third chromosome in the translocations.

As shown by the cytological study, one normal (unbroken) third chromosome, one third chromosome broken into two fragments and one free fourth chromosome are present in each of the cells of the flies carrying translocations. Another fourth chromosome is also present in each of the cells, but it is attached to the shorter fragment of the third chromosome, and therefore is not distinguishable cytologically (see figures 1 and 3). Let us consider the distribution of these chromosomes at the reduction during the gametogenesis.

The unbroken third chromosome and the free fourth chromosome might go to one pole of the spindle at the reduction division, and the two fragments of the broken third chromosome might go together with the attached fourth chromosome to the other pole (figures 1 and 3). Gametes 1 and 2 (figure 3) will be formed in this case. However, either of the frag-

ments of the broken third chromosome might go together with the unbroken third chromosome to the same pole of the spindle. As a result of such a distribution of the chromosome at the reduction division some of the gametes formed will carry a complete third chromosome plus a fragment of the other third chromosome (hyperploid gametes, figure 3, gametes 3 and 5). The other gametes will carry only one of the fragments of the third chromosome but will be deficient in respect to the other fragment of the same chromosome (hypoploid gametes, figure 3, gametes 4 and 6).

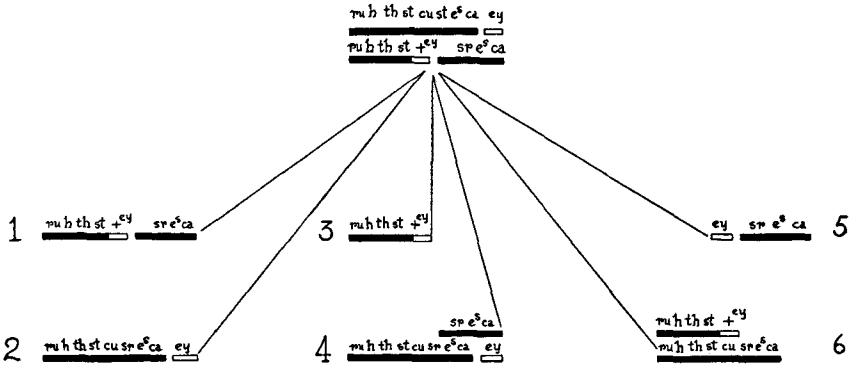

FIGURE 3.—Six kinds of gametes produced by a fly heterozygous for a translocation. Third chromosome—black; fourth chromosome—white.

The gametes 1 and 2 (figure 3) may be called "regular" gametes, whereas gametes 3 to 6 are "non-disjunctional" for one or the other section of the third chromosome. If a fly carrying a translocation is crossed to a fly free from translocation the regular gametes will give rise to zygotes carrying translocation and to normal zygotes respectively (figure 1). The non-disjunctional gametes will give rise to zygotes carrying a duplication or a deficiency for a section of the third chromosome. Flies developing from the latter zygotes must be expected to possess external peculiarities due to the genic unbalance. No such flies have been found in the cultures of any of the translocations here described. Hence, the non-disjunctional gametes are either not produced, or, more probably, the somatic changes in the zygotes, due to the changes in genic balance, are so great that the zygotes are unable to survive.

It is possible to test for the production of the non-disjunctional gametes by a genetical method. If two flies both carrying the same translocation are crossed to each other, gamete 3 from one parent united with gamete 4 from the other parent will give a normal zygote, since these gametes are com-

plementary to each other. Likewise, gamete 5 from one parent united with gamete 6 from the other parent must also give a normal zygote for the same reason. It is easy to make the zygotes produced by the union of the non-disjunctional gametes distinguishable in appearance from the zygotes produced by the union of the regular gametes. For this purpose it is necessary to introduce some recessive mutant genes into the chromosomes involved in the translocations.

Flies carrying translocations and at the same time homozygous in respect to various third-chromosome recessive genes may be easily obtained. In fact, many such flies have been observed during the course of the experiments planned for the purpose of studying the frequency of crossing over in the third chromosome (all the non-eyeless flies shown in tables 11–15 in the appendix). The appearance of such flies is due to crossing over between the fragments of the third chromosome involved in the translocations and the unbroken third chromosome carrying third-chromosome recessive genes. The following stocks of flies carrying translocations and homozygous with respect to certain third-chromosome recessive genes were established:

$$\text{a-translocation:}\quad \frac{r_u\ h\ D}{r_u\ h} \;\;\frac{s_r\ e^s\ c_a}{t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$$

$$\text{c-translocation:}\quad \frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\;\frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}$$

$$\text{e-translocation:}\quad \frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\;\frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}$$

Using these stocks and the regular stocks of the translocations carrying no third-chromosome recessive genes the following crosses were made:

$$\text{a-translocation:}\quad \frac{r_u\ h\ D}{r_u\ h} \;\;\frac{s_r\ e^s\ c_a}{t_h\ s_t\ c_u\ s_r\ e^s\ c_a}\ \text{♀} \times \frac{D}{\quad}\ \text{♂}$$

$$\text{c-translocation:}\quad \frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\;\frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ \text{♀} \times \frac{D}{\quad}\ \text{♂}$$

$$\text{e-translocation:}\quad \frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\;\frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ \text{♀} \times \frac{D}{\quad}\ \text{♂}$$

If each of the parents in these crosses produces the six kinds of gametes shown in figure 3, thirty-six classes of zygotes must be produced (see table 7). In c- and e-translocations twenty-eight of these thirty-six classes must die since they carry either a deficiency or a duplication for a section of the third chromosome. The surviving classes are (table 7): Dichaete,

wild type, $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$. If the assortment of the fragments of the third chromosome in translocations takes place at random, all the six classes of gametes must be produced in equal numbers. Hence, the frequency of the surviving classes of zygotes must be: $3D$: 3 wild type: 1 $D\ s_r\ e^s\ c_a$: 1 $r_u\ h\ t_h\ s_t$. If no non-disjunctional gametes are produced no $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$ individuals will appear. If, finally, the non-disjunctional gametes are produced less frequently than the regular gametes, the frequency of $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$ individuals will be lower than indicated above.

TABLE 7

	(1) $r_u\ h\ t_h\ s_t$ / $s_r\ e^s\ c_a$	(2) $r_u\ h\ t_h\ s_t\ c_u$ / $s_r\ e^s\ c_a$	(3) $r_u\ h\ t_h\ s_t$	(4) $r_u\ h\ t_h\ s_t\ c_u$ / $s_r\ e^s\ c_a$	(5) $s_r\ e^s\ c_a$	(6) $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ / $r_u\ h\ t_h\ s_t$
(1) D	*Dichaete*	*Dichaete*	dies	dies	dies	dies
(2)	*Wild type*	*Wild type*	dies	dies	dies	dies
(3) D	dies	dies	dies	$D\ s_r\ e^s\ c_a$	dies	dies
(4)	dies	dies	*Wild type*	dies	dies	dies
(5)	dies	dies	dies	dies	dies	$r_u\ h\ t_h\ s_t$
(6) D	dies	dies	dies	dies	*Dichaete*	dies

The results actually obtained in experiments prove the last assumption, that is, they show that the non-disjunctional gametes are produced, but their frequency is much lower than the frequency of the regular gametes. Indeed, in e-translocation only 29 $r_u\ h\ t_h\ s_t$ and 2 $D\ s_r\ e^s\ c_a$ individuals are observed among 672 flies (table 8). The appearance of the $r_u\ h\ t_h\ s_t$ individuals is due to non-disjunction of the left limb of the third chromosome, whereas the appearance of the $D\ s_r\ e^s\ c_a$ individuals depends upon non-

disjunction of the right limb of the third chromosome (see figure 3 and table 7). In e-translocation the third chromosome is broken to the left of the spindle fibre, that is, the breakage point lies in the left limb of the third chromosome. The higher frequency of $r_u\ h\ t_h\ s_t$ individuals as compared with the $D\ s_r\ e^s\ c_a$ indicates that non-disjunction of the shorter fragment of the third chromosome (which is attached to the fourth chromosome) takes place more frequently than non-disjunction of the longer fragment of the third chromosome (which has retained its own spindle fibre).

In c-translocation the third chromosome is broken to the right of the spindle fibre. By analogy with e-translocation, non-disjunction of the right limb of the third chromosome must take place more frequently than the non-disjunction of the left limb in c-translocation. In other words, $D\ s_r\ e^s\ c_a$ individuals must be more frequent than $r_u\ h\ t_h\ s_t$ individuals. Indeed, 18 $D\ s_r\ e^s\ c_a$ and only 5 $r_u\ h\ t_h\ s_t$ individuals were observed in c-translocation (table 8).

TABLE 8

Non-disjunction of sections of the third chromosome in translocations.

Translocation a.

$$\frac{r_u\ h\ D \quad\quad s_r\ e^s\ c_a}{r_u\ h \quad\quad t_h\ s_t\ c_u\ s_r\ e^s\ c_a} \text{ ♀} \times \frac{D \quad\quad}{\quad\quad\quad\quad} \text{ ♂}$$

Class	Dichaete	Wild type	$r_u\ h\ D$	$D\ s_r\ e^s\ c_a$	Total
Number	196	79	31	2	308

Translocation e.

$$\frac{r_u\ h\ t_h\ s_t \quad\quad s_r\ e^s\ c_a}{r_u\ h\ t_h\ s_t \quad\quad c_u\ s_r\ e^s\ c_a} \text{ ♀} \times \frac{D \quad\quad}{\quad\quad\quad\quad} \text{ ♂}$$

Class	Dichaete	Wild type	$r_u\ h\ t_h\ s_t$	$D\ s_r\ e^s\ c_a$	Total
Number	305	336	29	2	672

Translocation c.

$$\frac{r_u\ h\ t_h\ s_t \quad\quad s_r\ e^s\ c_a}{r_u\ h\ t_h\ s_t \quad\quad c_u\ s_r\ e^s\ c_a} \text{ ♀} \times \frac{D \quad\quad}{\quad\quad\quad\quad} \text{ ♂}$$

Class	Dichaete	Wild type	$r_u\ h\ t_h\ s_t$	$D\ s_r\ e^s\ c_a$	Total
Number	309	374	5	18	706

If two individuals both carrying a-translocation and carrying the third chromosomal genes indicated in table 8 are crossed to each other, twenty nine out of the thirty-six possible classes of offspring die. The remaining classes are: 4 D : 1 wild type: 1 $r_u\ h\ D$: 1 $D\ s_r\ e^s\ c_a$ (the indicated ratio holds true, of course, only in case all six possible classes of the gametes are produced in equal numbers). Actually the ratio 196 D : 79 wild type : 31 $r_u\ h\ D$: 1 $D\ s_r\ e^s\ c_a$ is observed (table 8). It follows that the non-

disjunctional classes of gametes are produced in a-translocation less frequently than regular gametes. In a-translocations the breakage of the third chomosome occurred at a point located slightly to the right of *D*, that is, in the left limb of the chromosome. Consequently the higher frequency of r_u *h D* individuals as compared with *D* s_r e^s c_a individuals indicates that non-disjunction of the fragment of the third chromosome attached to the fourth takes place more frequently than the non-disjunction of the other fragment of the third chromosome which has retained its own spindle fibre.

Taken as a whole, the results of the experiments just described are important in two respects. First, these results may be explained only on the assumption that the third chromosome in the translocations is broken into two fragments. In other words, the most important result of the cytological investigation of the translocations is proved by independent genetical evidence. Second, the experiments with non-disjunction of the sections of the third chromosome definitely prove that the different fragments of the third chromosome observed in the chromosomal plates of the translocations contain different blocks of genes corresponding to definite sections of the map of the third chromosome.

NON-DISJUNCTION OF THE FOURTH CHROMOSOMES IN THE TRANSLOCATIONS

Only one free fourth chromosome is seen in most of the chromosomal plates in the translocations. Since the flies carrying translocations are identical with normal flies in appearance and do not possess the characteristics of Haplo-IV, the presence of one more fourth chromosome in their cells is postulated. This fourth chromosome is evidently attached to one of the fragments of the third chromosome. The resulting compound appears in most of the translocations as an unpaired, rod-shaped chromosome in which no dividing line between the fourth chromosome and the fragment of the third is noticeable.

The conjugation of the fourth chromosomes in the translocations must take place in rather unusual conditions, since only one of these chromosomes is free, and the other is permanently attached to the fragment of the third chromosome. Nevertheless, as shown by the genetical behavior of the translocations, the reduction division proceeds more or less normally, and most of the gametes produced carry only one fourth chromosome. That is to say, the free fourth chromosome goes together with the unbroken third chromosome to one pole, and the attached fourth chromosome goes together with both fragments of the broken third chromosome to another pole of the spindle (figure 1). However, the cytological investigation revealed the occurrence of individuals having two free fourth chromosomes

in their cells. Such individuals are especially common in d-translocation, but they are also found in e-translocation. The appearance of such individuals may be explained on the assumption that the non-disjunction of the fourth chromosomes occurs rather frequently in the translocations. The free fourth chromosome might go sometimes together with the attached fourth chromosome and with both fragments of the third chromosome to the same pole of the spindle at the reduction division. Gametes carrying two fourth chromosomes may be produced in this way. Such gametes will give rise to zygotes having three fourth chromosomes, two of the latter being free and one attached to the third chromosome.

Individuals having three fourth chromosomes (triplo-IV) are so little different from normal individuals (diplo-IV) in appearance that their identification on the basis of external characters is impracticable. Nevertheless, the presence of a certain percentage of triplo-IV individuals in the stocks of the translocations may be proved by genetical methods. Two genetical methods were devised to distinguish the triplo-IV individuals from the diplo-IV ones in the translocations.

The first of these methods consists in crossing flies carrying translocations to those carrying the fourth-chromosome gene Minute-IV. Minute-IV is known to be dominant over one dose of the normal allelomorph but recessive to two doses of the normal allelomorph (SCHULTZ 1929). Hence, in the progeny of such a cross, the flies carrying translocations can be at the same time Minute-IV only if they have only two fourth chromosomes (one free and another attached to the third). If the flies carrying translocations have three fourth chromosomes (two free and one attached to the third chromosome), no characteristics of Minute-IV will be manifest.

The second method is based on the fact that in the flies from stocks of the translocations the fourth chromosome attached to the third carries the normal allelomorph of e_y, whereas the free fourth chromosome (or the free fourth chromosomes) carry e_y. It is clear that if such a fly is crossed to a wild-type fly, and its offspring are then crossed to a fly homozygous for e_y, no e_y individuals will appear in the next generation unless the initial fly was a triplo-IV individual.

Both methods just described were used for testing the flies from the stocks of the translocations. The number of the diplo-IV and the triplo-IV flies found is shown in the right-hand column in table 9. The number of the diplo-IV and triplo-IV flies found cytologically is presented in the middle column of table 9.

As seen from table 9, the triplo-IV flies are most frequent in the stock of d-translocation and comparatively rare in the other translocations. The agreement of the results received by the genetical and the cytological methods is satisfactory.

BEHAVIOR OF THE TRANSLOCATIONS IN HOMOZYGOUS FORM

In the preceding sections we have been dealing with the behavior of the heterozygous translocations. As shown by the cytological data, one of the third chromosomes in the heterozygous translocations is broken into two

TABLE 9

Number of diplo-IV and triplo-IV flies found in the stocks of the translocations.

	CYTOLOGICALLY		GENETICALLY	
	DIPLO-IV	TRIPLO-IV	DIPLO-IV	TRIPLO-IV
a-translocation	15	..	12	1
b-translocation	15	..	12	1
c-translocation	11	..	10	2
d-translocation	4	8	6	14
e-translocation	11	2	16	2

fragments, but the other third chromosome is completely normal. In homozygous translocations two pairs of the fragments of the third chromosome and no normal, unbroken third chromosome must be present. Since some interesting genetical phenomena may arise as a result of such a condition, experiments were planned to secure some of the translocations in homozygous form.

In d-translocation the section of the third chromosome lying to the right of the gene c_a is known to be broken off and attached to the fourth chromosome. Males carrying in the third chromosome involved in the translocation the genes r_u, h, t_h, s_t, c_u, s_r *and* e^s and carrying in the normal third chromosome the genes r_u, h, t_h, s_t, c_u, s_r, e^s and c_a (that is of the constitution $\frac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s}{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$) were crossed to regular (*i.e.*, carrying no translocation) females having in one of their third chromosomes the dominant gene Deformed (D_f), the recessive c_a and the crossing over suppressors $C_{III\ R}$ and $C_{III\ L}$. In the F_1 generation of this cross Deformed non-claret individuals were selected and inbred. These individuals are heterozygous for the translocation and have the constitution: $\frac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s}{C_{III\ L}\ D_f\ C_{III\ R}\ c_a}$. In the next generation (F_2) D_f and $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ individuals may be expected to appear

in the ratio 2:1 (the gene D_f is lethal when homozygous). The $r_u\ h\ t_h\ s_t$ $c_u\ s_r\ e^s$ individuals must be homozygous for the translocation. However only D_f individuals were received in F_2 (about 1000 flies were examined). In other words, d-translocation is lethal when homozygous.

In e-translocation the third chromosome is broken between the loci of the genes s_t and p, and the section containing the genes from r_u to s_t is attached to the fourth chromosome (see figure 2). Males of the constitution $\frac{r_u\ h\ t_h\ s_t \quad s_r\ e^s\ c_a}{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$ were crossed to regular females carrying in one of their third chromosomes the dominant genes D and S_b and the recessives s_t, c_u and e^s. In the F_1 generation the individuals manifesting the characters of D, s_t, S_b and e^s were selected and inbred. These individuals have the constitution: $\frac{r_u\ h\ t_h\ s_t \quad s_r\ e^s\ c_a}{D \quad s_t\ c_u\ S_b \quad e^s}$. In the F_2 generation some $r_u\ h\ t_h\ s_t\ s_r\ e^s\ c_a$ individuals appeared; they must be homozygous for e-translocation.

Individuals homozygous for e-translocation are normal in appearance and have a good viability. However, the females have rudimentary ovaries resembling those of the *Drosophila melanogaster—D. simulans* hybrids (STURTEVANT 1920). Therefore, they are completely sterile when crossed either to males homozygous for e-translocation or to wild-type males. Males homozygous for e-translocation are fertile, though they produce markedly fewer offspring than the wild-type males or males heterozygous for e-translocation.

In c-translocation the third chromosome is broken between the genes p and c_u, and the section of the third chromosome containing the genes from c_u to c_a (see figure 2) is attached to the fourth chromosome. Males of the constitution $\frac{r_u\ h\ t_h\ s_t \quad e^s\ c_a}{r_u\ h\ t_h\ s_t \quad c_u\ s_r\ e^s\ c_a}$ were crossed to regular females carrying in one of their third chromosomes the genes D, s_t, c_u, S_b and e^s. In the F_1 generation $D\ s_t\ S_b\ e^s$ individuals were selected and inbred. They have the constitution $\frac{r_u\ h\ t_h\ s_t \quad e^s\ c_a}{D \quad s_t \quad c_u\ S_b\ e^s}$. In the F_2 some $r_u\ h\ t_h\ s_t\ e^s\ c_a$ individuals appeared; they are homozygous for c-translocation. Flies homozygous for c-translocation appear normal somatically, and are fertile in both sexes, though females produce markedly fewer offspring than do wild-type females or females heterozygous for c-translocation.

The behavior of the different translocations in homozygous condition is, therefore, diverse: d-translocation is lethal when homozygous, e-translocation survives but is sterile in one sex, and c-translocation survives and is

fertile in both sexes. (The behavior of a- and b-translocations in homozygous form was not studied because in both of them the third chromosome involved in the translocation carries the dominant gene *D* which is lethal when homozygous. The locus of *D* is so close to the point at which the breakage of the third chromosome occurred in these translocations that it is very difficult to obtain them without the gene *D*.)

The causes which may hinder the viability of the homozygous translocations are obscure. Theoretically, flies heterozygous as well as homozygous for the translocations must have the same genic balance that the normal flies have, and, therefore, must be viable, normal in appearance and in fertility. Indeed, most of the known translocations are normal in heterozygous condition, but, so far as known, only the c-translocation described here approaches the expectation when homozygous.

"Pale" translocation described by BRIDGES is lethal when homozygous (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925). This fact has been explained by BRIDGES as due to a loss of a fragment of the translocated section during the process of transfer. In other words, the flies homozygous for "Pale" translocation are, according to BRIDGES, homozygous also for a deficiency of a small section of one of the chromosomes involved in the translocation. This explanation is supported by the fact that if the individuals heterozygous for "Pale" translocation are homozygous for the sex-linked gene eosin (an eosin-like eye-color), the resulting color of the eyes is somewhat diluted. Thus, "Pale" translocation has an abnormal genic balance even in heterozygous condition.

Three explanations of the lethal effect (and of the disturbance of the genic balance in general) produced by some of the translocations in homozygous condition may be suggested. First, a lethal or semilethal mutation might arise in one of the chromosomes involved in the translocation. Second, the breakage of the chromosome necessary to produce a translocation might cause some injury to the chromosome involved. For instance, some genes neighboring to the point at which the breakage occurred might be lost or destroyed (see BRIDGES' explanation). Third, it is possible to suppose that the breakage of a chromosome may occur only at a point which has been previously injured by some agent, for instance by the X-ray treatment. The data at present available give no evidence as to which of these explanations is true in the cases of d- and e-translocations.

Females homozygous for c-translocation were studied cytologically. All the chromosomal plates found (plate 2, figures 34–38) contain four pairs of chromosomes. Two pairs out of these four are rod-shaped; one of them is

markedly shorter than the other. Moreover, one pair is V-shaped, and one pair is J-shaped. The J-shaped chromosomes have a subterminal spindle-fibre attachment. Taken as a whole, the chromosomal complex in the homozygous c-translocation is more like that of *Drosophila immigrans* Sturt. (see METZ and MOSES 1923, figure 2 D) or that of *Drosophila melanica* Sturt. (see METZ and MOSES 1923, figure 2 E) than like that of normal *Drosophila melanogaster*.

Nevertheless, the interpretation of the cytological features found in the homozygous translocation is easy if the conditions found in heterozygous c-translocation are taken into account (see plate 1, figures 14–19). The longer pair of the rod-shaped chromosomes found in homozygous translocation are obviously the X-chromosomes. The pair of V-shaped chromosomes are the second chromosomes. The shorter pair of rod-shaped chromosomes and the pair of J-shaped chromosomes are the fragments of the third chromosome. No free fourth chromosome is found in any of the plates studied in homozygous translocation. Obviously, the shorter pair of rod shaped chromosomes consists of the fragments of the third chromosome attached to the fourth chromosomes. Indeed, since only one free fourth chromosome is present in the plates studied in heterozygous c-translocation, no free fourth chromosome must be expected in the chromosomal complex of the homozygous c-translocation.

This interpretation may be tested genetically. If the third chromosome is broken into two separate chromosomes (the rod-shaped and the J-shaped), the genes localized in one of them must show no linkage with the genes localized in the other. In other words, the third-chromosome linkage group of *Drosophila melanogaster* must be broken into two separate linkage groups in the homozygous translocation. The point of the breakage of the third chromosome is located in c-translocation to the right of the spindle fibre, between the genes peach and curled. Hence, the rod-shaped chromosome corresponds to the section of the genetical map of the third chromosome extending from peach to claret; the J-shaped chromosome corresponds to the section extending from scarlet to roughoid (figure 2). The sections just mentioned must behave in the homozygous translocation as two independent linkage groups.

Females homozygous for c-translocation and having the constitution $\frac{r_u\ h\ t_h\ s_t \quad e^s\ c_a}{r_u\ h\ t_h\ s_t \quad e^s\ c_a}$ were crossed to males heterozygous for c-translocation and having the constitution $\frac{D}{\quad\quad}$. In the F_1 generation D males were selected and crossed to females homozygous for r_u, h, t_h, s_t, c_u, s_r, e^s, c_a and free from

the translocation. These D-males must have the constitution $\frac{D}{r_u\ h\ t_h\ s_t}\ \frac{}{e^s\ c_a}$, that is, they are homozygous for the translocation but heterozygous for the third-chromosomal genes indicated. Should the third chromosomes in the homozygous translocation not be broken, only D and $r_u\ h\ t_h\ s_t\ e^s\ c_t$ individuals would appear in the offspring of this cross. However, if the third-chromosome linkage group, following the breakage of the third chromosome, is also broken into two independent linkage groups, D, $r_u\ h\ t_h\ s_t\ e^s\ c_a$, $r_u\ h\ t_h\ s_t$ and $D\ e^s\ c_a$ individuals will appear in the ratio 1:1:1:1. The results obtained in the experiment are:

D	$r_u\ h\ t_h\ s_t$	$D\ e^s\ c_a$	$r_u\ h\ t_h\ s_t\ e^s\ c_a$	Total
232	243	238	227	940

Hence, two "new" linkage groups of genes replace, in the homozygous translocation, the original third-chromosome linkage group of *Drosophila melanogaster*. If females homozygous for c-translocation and having the constitution $\frac{D}{r_u\ h\ t_h\ s_t}\ \frac{}{e^s\ c_a}$ are crossed to homozygous $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males free from the translocation, the two "new" linkage groups remain independent from each other, but some crossing over occurs within each of them. The frequency of crossing over in the "new" linkage groups is shown in table 10 which is based on the counts of 752 flies.

TABLE 10

Frequency of crossing over in the "new" linkage groups in the homozygous translocation.

	INTERVAL	FREQUENCY IN HOMOZYGOUS TRANSLOCATION—PERCENT	STANDARD FREQUENCY PERCENT	DIFFERENCE
The J-shaped chromosome	r_u-h	27.8	26.5	+1.3
	h-D	14.4	13.9	+0.5
	D-t_h	1.2	1.8	−0.6
	t_h-s_t	1.5	1.8	−0.3
	s_t-e^s	46.5	26.7	+19.8
The rod-shaped chromosome	e^s-c_a	31.1	30.0	+1.1

It may be concluded that the frequencies of crossing over in the J-shaped chromosome are approximately equal to the frequencies of crossing over in the corresponding section of the normal, unbroken, third chromosome. The same is true in respect to the frequency of crossing over in the

e^s–c_a interval in the rod-shaped chromosome. Whether the frequency of crossing over in the whole rod-shaped chromosome is also equal to that in the corresponding section of the normal third chromosome, can not be decided on the basis of the data at hand. It may conceivably be different, since the rod-shaped chromosome in c-translocation contains not only the material corresponding to a section of the third chromosome, but also that corresponding to the fourth chromosome of the normal fly. The presence of the fourth-chromosome material in the rod-shaped chromosome might alter the frequency of crossing over in the neighboring part of the third chromosome as compared with the normal condition. This question needs further study.

In any case, the data presented here show that the classical assumption, according to which each linkage group corresponds to a separate chromosome, is correct.

DISCUSSION OF THE RESULTS

The cytological study of the translocations has shown that in each of them the third chromosome is broken into two fragments of unequal length and that the shorter fragment is attached to the fourth chromosome. The non-disjunction of the sections of the third chromosome in the translocations furnishes a genetical proof of the breakage of the third chromosomes. Finally, the results of the observations on the behavior of the homozygous translocation must be evaluated as the most convincing evidence in favor of our interpretation of the translocations.

The loci at which the breakage of the third chromosome took place in each of the translocations are determined by two completely independent methods. The first of these methods is a purely genetical one: the study of the linkage relations of the genes belonging to the third and to the fourth linkage groups. The location of the break is determined by this method in terms of units of map-distance, by essentially the same procedure as that by which the location of a mutant gene in the third chromosome of Drosophila is usually studied. The relative distances between the breaking-points in the different translocations may be compared in the same way in which the relative distances between the genes located in the third chromosome may be compared (figure 2). In doing so we proceed on the same basic assumption on which the genetical "map" of the third chromosome or the genetical map of any other chromosome is built. That is the assumption that the genes within a chromosome are arranged in linear order, and that the frequency of crossing over between any two genes

located in the same chromosome is a function of the distance between these genes.

The second method of determination of the locus at which the breakage of the chromosome took place is the direct investigation of the length of the fragments of the chromosome in cytological preparations. Since the shape of the chromosome during the metaphase stage is relatively constant, the location of the breakages may be expressed in terms of their distance from the ends of the chromosome, from the spindle fibre, from the constrictions, and from each other.

Assuming that the theory of linear arrangement of the genes within the chromosome is correct, the cytological conditions may be predicted on the basis of the knowledge of the genetical location of the breaking-point and vice versa. It has been shown in the preceding sections that such a prediction is really possible. In a- and b-translocations the loci of breakage as determined genetically nearly coincide with each other (figure 2). The chromosomes of a-translocation are nearly indistinguishable from those of b-translocation. In e-translocation the locus of the breakage on the basis of the genetical data must be supposed to lie between that observed in a- and b-translocations and the spindle fibre. This is exactly what has been found cytologically in e-translocation. The genetical data show that only a very small piece of the third chromosome may be attached to the fourth in d-translocation. Correspondingly, only a slight increase of one of the fourth chromosomes is observed cytologically. On the basis of the genetical evidence the third chromosome is broken in c-translocation at approximately the same distance from the spindle fibre as in e-translocation. The fragments of the third chromosome observed cytologically in c-translocation are but slightly different in size from those observed in e-translocation.

There is no escape from the conclusion that the sequence of the third-chromosomal genes as represented by the genetical map of this chromosome is the same as their sequence in the chromosome studied cytologically. In other words the genetical map represents the actual sequence of genes within the chromosome. Thus, the theory of the linear arrangement of the genes receives a strong basis of cytological evidence.

If the question about the reality of the linear order of genes within the chromosome is answered in the affirmative, it becomes possible to raise another question. Are the relative distances between the genes correctly represented by the genetical map of the third chromosome, or does the

genetical map give a somewhat distorted image of the actual spatial relations of the genes?

The cytologically observed points at which the third chromosome is broken in different translocations form a skeleton of a "cytological map" of the third chromosome. Furthermore, since certain genes are known to be adjacent to the points at which the breakage of the chromosome has occurred in different translocations, it seems to be justified to conclude that these genes are located in the chromosome in the neighborhood of the observed breakage-points. Thus, the "cytological map" represents the loci of the observed breakages, and, by inference, the loci of some of the third-chromosomal genes. The genetical and the cytological maps of the third chromosome, drawn to the same scale, are shown in figure 4.

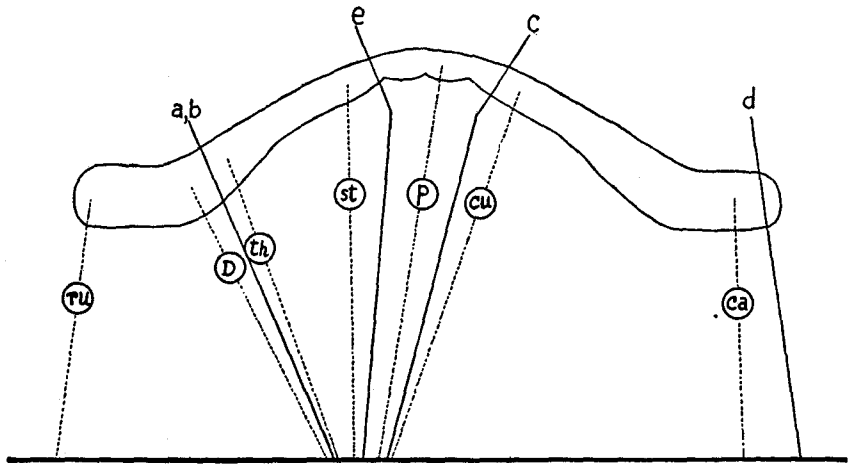

FIGURE 4.—Comparison of a genetical (below) and a cytological (above) map of the third chromosome of *Drosophila melanogaster*. a, b, c, d and e—the observed breaking-points. r_u—roughoid; *D*—Dichaete; t_h—thread; s_t—scarlet; *p*—peach; c_u—curled; c_a—claret.

It must be emphasized, that the cytological map presented in figure 4 is far less exact than the standard genetical map. First of all, the exact measurement of the fragments of the chromosomes of Drosophila is exceedingly difficult because of their extreme smallness. Furthermore, the presence of the fourth chromosome attached to one of the fragments of the third chromosome makes the estimation of the size of the latter still less exact. A certain alteration of the shape of the fragments of the third chromosome after breakage is also possible. Finally, the number of breakages of the third chromosome so far observed is too small to allow the construction of a detailed cytological map. Nevertheless, the relative distances

between the genes as represented by the genetical and the cytological map are so widely different, that certain conclusions may be drawn even on the basis of study of the cytological map available at present.

The distance between the locus of the breakage observed in a-translocation and the left-hand end of the third chromosome (gene roughoid) equals (at least) 41 map-units. The distance between the breakage observed in a-translocation and that observed in e-translocation equals only about 4 map-units. In other words, the distance between the left end of the third chromosome and the breakage observed in a-translocation is at least ten times longer than the distance between the breakages observed in a- and in e-translocations. That is the determination based on the genetical data. However, the cytological map shows that the latter distance is actually longer than the former. If studied genetically, the distance between the breakages observed in e- and c-translocations equals about 5 map-units, as compared with the 40 (or more) units distance between the breaking-point observed in a-translocation and the left-hand end of the third chromosome. Cytologically these distances are about equal in length. The distance between the point of the breakage observed in c-translocation and the right-hand end of the third chromosome is at least 60 units long; the distance between the points of breakage observed in e- and in c-translocations is about 5 units long. Cytologically, the former distance is only about twice as long as the latter (see figure 4).

PAINTER and MULLER (1929) have shown that the section of the third chromosome extending from sooty to the right-hand end of the third chromosome (this section must be at least 35 units long) is actually so small that it becomes scarcely visible if attached to the second chromosome.

Thus, the discrepancies between the length of the different sections of the third chromosome estimated on the basis of the genetical data and their length actually observed are very great. All these discrepancies may, however, be generalized in a form which discloses a very interesting regularity. The distances between the loci of the breakages or of the genes lying in the middle part of the chromosome are longer cytologically than they might be expected to be on the basis of the genetical map. On the other hand, the distances between the loci lying near the ends of the chromosome are shorter cytologically than they might be expected to be on the basis of the genetical data. The genetical map represents the genes located in the middle part of the chromosome (near the spindle fibre) relatively too close to each other, and the genes located at the ends of the chromosomes relatively too far apart.

In the metaphase stage the ends of the third chromosome appear to be considerably thicker than the middle part of the chromosome. This is partly due to a stronger condensation of the material located near the ends of the chromosome as compared with the material located near its middle, and partly to a premature splitting of the ends of the chromosome (compare figure 5, plate 1, representing the chromosome in the late prophase stage with the other figures representing chromosomes in the metaphase stage). This may suggest that the distances between the genes located near the ends of the chromosome are relatively shorter when the chromosome is in the metaphase stage than they are in the stage when crossing over takes place. However, the discrepancies between the relative distances of the genes from each other shown by the genetical and the cytological maps are too great to be accounted for by the different diameter of the different parts of the third chromosome.

The distances between the genes on the genetical map represent nothing but the frequency of crossing over between these genes. There is, however, no evidence that the frequency of crossing over between any two genes must be necessarily proportional to the absolute distance between these genes in the chromosome. In fact, there exists some evidence against such an assumption. The genes are not distributed at random along the genetical maps of the chromosomes but are crowded in some regions and sparse in others. This fact leads to the hypothesis according to which the frequency of crossing over per unit of the absolute distance may be different in different regions of the same chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 93). The map of the third chromosome shows that the genes are crowded in the region neighboring to the spindle-fibre attachment but both ends of the map have few genes and long intervals in which no genes are known (figure 2).

If we suppose that the frequency of crossing over per unit of the absolute distance is much higher near the ends of the third chromosome than near the spindle fibre, the observed discrepancies between the genetical and the cytological maps of this chromosome find a simple explanation. The genetical map of the third chromosome represents correctly the actual sequence of the genes within the chromosome. However, the distances between the genes shown by the genetical map are functions of the actual distance between them in the chromosome as well as of the regional differences in the frequency of crossing over. In its turn, the frequency of crossing over per unit of the absolute distance in a given region seems to be a function of the distance between this region and the locus of the spindle-fibre attachment.

A similar conclusion is reached by PAINTER and MULLER (1929) on the basis of study of the deletions in the X-chromosome of *Drosophila melanogaster*. In this case the cytological data indicate that the actual distances between the genes located in the left-hand end of the X-chromosome are relatively longer than the distances between these genes suggested by the genetical map of this chromosome. The genetical map of the X-chromosome shows that the genes are strongly crowded at the left-hand end of the chromosome.

It is fairly certain on the basis of the data at hand that the genes are distributed at random along the genetical map of the third chromosome of *Drosophila melanogaster*, or at least much more nearly so than on the genetical map. Both the genetical and the cytological maps show only those genes which have given mutations. It follows that the process of mutation affects all the parts of the chromosome more or less at random.

SUMMARY

1. Five cases of translocation involving the third and the fourth chromosomes were observed in the progeny of flies treated by X-rays. In each of the five cases a section of the third chromosome became broken off and attached to the fourth chromosome.

2. Flies carrying translocations in heterozygous form are apparently normal in appearance and in viability, and nearly normal in fertility.

3. The loci at which the breakage of the third chromosome took place in each of the translocations were determined by studying the linkage between the genes of the third and those of the fourth chromosome linkage groups, which in the translocations behave as if they were localized in the same chromosome. The loci of breakage of the third chromosome are different in different translocations.

4. The frequency of crossing over is markedly decreased in the limb of the third chromosome in which the breakage has occurred, and slightly increased in the opposite limb. The point dividing the two limbs (the point of the spindle-fibre attachment) is slightly to the left of the locus of peach.

5. Non-disjunction of the sections of the third chromosome occurs in the translocations and can be detected by a suitable genetical method (see text). The study of the non-disjunction of the sections of the third chromosome gives a proof of the presence of the breakages of the third chromosome in the translocations.

6. Non-disjunction of the fourth chromosomes also occurs in the translocations.

7. The cytological investigation has shown that the longer pair of the V-shaped chromosomes is that affected by the translocations. It may be concluded that the longer pair of the V-shaped autosomes of *Drosophila melanogaster* carries the third-chromosome linkage group, and the shorter pair of the V-shaped autosomes carries the second-chromosome linkage group of the genes.

8. One of the third chromosomes is found cytologically to be broken into fragments in the translocations. One of the fragments is apparently attached to the fourth chromosome, since only one free fourth chromosome is present in most plates studied.

9. The length of the fragments of the third chromosome observed in cytological preparations is roughly proportional to their length suggested by the genetical data. This fact may be considered as a cytological proof of the theory of linear arrangement of the genes within the chromosomes.

10. A "cytological map" of the third chromosome is constructed on the basis of the cytological study of the translocations (see figure 4 in text). The comparison of the cytological map with the regular genetical map of the third chromosome shows that the distances between the genes located in the middle part of the chromosome are larger cytologically than they might be expected to be on the basis of the genetical map. This is apparently due to the low frequency of crossing over per unit of absolute distance in the middle part of the third chromosome, and to the high frequency of crossing over near the ends of the chromosome.

11. Two of the translocations were obtained in homozygous form. Individuals homozygous for the translocations are normal in appearance but sometimes sterile in the female.

12. Cytological study of the homozygous translocation showed, according to expectation, that both third chromosomes are broken into two fragments. No free fourth chromosome is found in homozygous translocation. Hence, the homozygous translocation possesses four pairs of chromosomes but two of them are very different in appearance from the chromosomes found in the normal fly.

13. The third-chromosome linkage group of *Drosophila melanogaster* is broken in homozygous translocation into two independent linkage groups of genes corresponding to the different sections of the normal third chromosome. This is, undoubtedly, due to the breakage of the third chromosome.

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APPENDIX—TABLES 11–17.

TABLE 11

Translocation a.

$$\frac{e_y}{\cdot\cdot}\ \frac{r_u\ h\,2\ 3\ 4\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}{1\ D+\ 5\ 6\ 7\ 8\ 9} \times e_y\, r_u\, h\, t_h\, s_t\, c_u\, s_r\, e^s\, c_a\ \delta.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 D	508	8, 9 $D\,e^s$	17
0 $e_y\, r_u\, h\, t_h\, s_t\, c_u\, s_r\, e^s\, c_a$	143	8, 9 $e_y\, r_u\, h\, t_h\, s_t\, c_u\, s_r\, c_a$	3
Total non-crossovers	651	1, 8 $r_u\, D\, e^s\, c_a$	26
		1, 8 $e_y\, h\, t_h\, s_t\, c_u\, s_r$	4
1 $D\, r_u$	140	2, 8 $r_u\, h\, D\, e^s\, c_a$	2
1 $e_y\, h\, t_h\, s_t\, c_u\, s_r\, e^s\, c_a$	33	2, 8 $e_y\, t_h\, s_t\, c_u\, s_r$	1
2 $D\, r_u\, h$	24	4, 8 $D\, t_h\, s_t\, c_u\, s_r$	1
2 $e_y\, t_h\, s_t\, c_u\, s_r\, e^s\, c_a$	11	6, 8 $D\, c_u\, s_r$	9
3 $e_y\, D\, t_h\, s_t\, c_u\, s_r\, e^s\, c_a$	1	6, 8 $e_y\, r_u\, h\, t_h\, s_t\, e^s\, c_a$	4
4 $e_y\, r_u\, h$	1	1, 7 $r_u\, D\, s_r\, e^s\, c_a$	22
5 $D\, s_t\, c_u\, s_r\, e^s\, c_a$	1	1, 7 $e_y\, h\, t_h\, s_t\, c_u$	14
5 $e_y\, r_u\, h\, t_h$	2	2, 7 $r_u\, h\, D\, s_r\, e^s\, c_a$	4
6 $D\, c_u\, s_r\, e^s\, c_a$	55	2, 7 $e_y\, t_h\, s_t\, c_u$	4
6 $e_y\, r_u\, h\, t_h\, s_t$	25	6, 7 $D\, c_u$	2
7 $D\, s_r\, e^s\, c_a$	156	6, 7 $e_y\, r_u\, h\, t_h\, s_t\, s_r\, e^s\, c_a$	3
7 $e_y\, r_u\, h\, t_h\, s_t\, c_u$	56	5, 7 $e_y\, r_u\, h\, t_h\, s_r\, e^s\, c^s$	2
8 $D\, e^s\, c_a$	120	1, 6 $r_u\, D\, c_u\, s_r\, e^s\, c_a$	5
8 $e_y\, r_u\, h\, t_h\, s_t\, c_u\, s_r$	54	1, 6 $e_y\, h\, t_h\, s_t$	1
9 $D\, c_a$	318	2, 6 $r_u\, h\, D\, c_u\, s_r\, e^s\, c_a$	1
9 $e_y\, r_u\, h\, t_h\, s_t\, c_u\, s_r\, e^s$	83		
Total single-crossovers	1080	Total double crossovers	332
1, 9 $r_u\, D\, c_a$	82	1, 7, 9 $r_u\, D\, s_r\, e^s$	8
1, 9 $e_y\, h\, t_h\, s_t\, c_u\, s_r\, e^s$	13	1, 7, 9 $e_y\, h\, t_h\, s_t\, c_u\, c_a$	4
2, 9 $r_u\, h\, D\, c_a$	7	1, 6, 9 $r_u\, D\, c_u\, s_r\, e^s$	3
2, 9 $e_y\, t_h\, s_t\, c_u\, s_r\, e^s$	7	1, 6, 9 $e_y\, h\, t_h\, s_t\, c_a$	1
6, 9 $D\, c_u\, s_r\, e^s$	12	1, 6, 8 $r_u\, D\, c_u\, s_r$	1
6, 9 $e_y\, r_u\, h\, t_h\, s_t\, c_a$	15	1, 8, 9 $r_u\, D\, e^s$	1
7, 9 $D\, s_r\, e^s$	52	Total triple crossovers	18
7, 9 $e_y\, r_u\, h\, t_h\, s_t\, c_u\, c_a$	19	Grand total	2081

TABLE 12

Translocation b.

$e_y\ r_u\ h\ 2\ 3\ 4\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$

$\cdot\cdot\ \ 1\ \ D+\ \ 5\ 6\ 7\ 8\ 9$ ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 D	304	6,9 $D\ c_u\ s_r\ e^s$	7
0 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	110	6,9 $e_y\ r_u\ h\ t_h\ s_t\ c_a$	9
Total non-crossovers	414		
1 $r_u\ D$	129	7,9 $D\ s_r\ e^s$	22
1 $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	22	7,9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ c_a$	12
2 $r_u\ h\ D$	27	8,9 $D\ e^s$	9
2 $e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	18	8,9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ c_a$	1
3 $r_u\ h$	1	1,8 $r_u\ D\ e^s\ c_a$	7
3 $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	1	1,8 $e_y\ h\ t_h\ s_t\ c_u\ s_r$	4
5 $D\ s_t\ c_u\ s_r\ e^s\ c_a$	1	2,8 $r_u\ h\ D\ e^s\ c_a$	5
5 $e_y\ r_u\ h\ t_h$	4	2,8 $e_y\ t_h\ s_t\ c_u\ s_r$	1
6 $D\ c_u\ s_r\ e^s\ c_a$	20	6,8 $D\ c_u\ s_r$	5
6 $e_y\ r_u\ h\ t_h\ s_t$	17	6,8 $e_y\ r_u\ h\ t_h\ s_t\ e^s\ c_a$	3
7 $D\ s_r\ e^s\ c_a$	114	1,7 $D\ r_u\ s_r\ e^s\ c_a$	36
7 $e_y\ r_u\ h\ t_h\ s_t\ c_u$	35	1,7 $e_y\ h\ t_h\ s_t\ c_u$	10
8 $D\ e^s\ c_a$	69	2,7 $r_u\ h\ D\ s_r\ e^s\ c_a$	8
8 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r$	37	2,7 $e_y\ t_h\ s_t\ c_u$	3
9 $D\ c_a$	202	1,6 $r_u\ D\ c_u\ s_r\ e^s\ c_a$	5
9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	71	2,6 $r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$	1
		1,2 $h\ D$	1
Total single crossovers	768	Total double crossovers	255
1,9 $r_u\ D\ c_a$	45	1,7,9 $r_u\ D\ s_r\ e^s$	5
1,9 $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	40	2,7,9 $r_u\ h\ D\ s_r\ e^s$	1
2,9 $r_u\ h\ D\ c_a$	16	1,8,9 $r_u\ D\ e^s$	2
2,9 $e_y\ t_h\ s_t\ c_u\ s_r\ e^s$	4	7,8,9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ e^s$	1
3,9 $r_u\ h\ c_a$	1	1,2,7 $e_y\ r_u\ t_h\ s_t\ c_u$	1
		Total triple crossovers	10
		Grand total	1447

TABLE 13

Translocation c.

$e_y\ r_u\ h\ 2\ 3\ t_h\ s_t\ 5\ 6\ c_u\ s_r\ e^s\ c_a$

$\frac{\text{..}\ 1\ D\ 4\ +\ 7\ 8\ 9}{}$ ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

CLASSES OF THE OFFSPRING		NUMBER OBSERVED
0	D	675
	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	240
Total non-crossovers		915
1	$r_u\ D$	396
	$e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	143
2	$r_u\ h\ D$	198
	$e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	116
3	$r_u\ h$	18
	$e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	5
4	$r_u\ h\ t_h$	11
	$e_y\ D\ s_t\ c_u\ s_r\ e^s\ c_a$	11
5	$r_u\ h\ t_h\ s_t$	37
	$e_y\ D\ c_u\ s_r\ e^s\ c_a$	26
6	$e_y\ r_u\ h\ t_h\ s_t$	8
	$D\ c_u\ s_r\ e^s\ c_a$	12
7	$e_y\ r_u\ h\ t_h\ s_t\ c_u$	12
	$D\ s_r\ e^s\ c_a$	50
8	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r$	51
	$D\ e^s\ c_a$	96
9	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	133
	$D\ c_a$	384
Total single crossovers		1707
1, 9	$r_u\ D\ c_a$	169
	$e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	48
2, 9	$r_u\ h\ D\ c_a$	79
	$e_y\ t_h\ s_t\ c_u\ s_r\ e^s$	35
3, 9	$r_u\ h\ c_a$	15
	$e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s$	2
4, 9	$r_u\ h\ t_h\ c_a$	2
5, 9	$e_y\ D\ c_u\ s_r\ e^s$	4
	$r_u\ h\ t_h\ s_t\ c_a$	23
6, 9	$D\ c_u\ s_r\ e^s$	1
7, 9	$D\ s_r\ e^s$	8
	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ c_a$	4
8, 9	$D\ e^s$	6
	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ c_a$	1
1, 8	$r_u\ D\ e^s\ c_a$	40
	$e_y\ h\ t_h\ s_t\ c_u\ s_r$	6
2, 8	$r_u\ h\ D\ e^s\ c_a$	16
	$e_y\ t_h\ s_t\ c_u\ s_r$	11
3, 8	$r_u\ h\ e^s\ c_a$	3
4, 8	$r_u\ h\ t_h\ e^s\ c_a$	1
5, 8	$e_y\ D\ c_u\ s_r$	2
	$r_u\ h\ t_h\ s_t\ e^s\ c_a$	4
6, 8	$D\ c_u\ s_r$	1
1, 7	$r_u\ D\ s_r\ e^s\ c_a$	22
	$e_y\ h\ t_h\ s_t\ c_u$	1
2, 7	$r_u\ h\ D\ s_r\ e^s\ c_a$	10
	$e_y\ t_h\ s_t\ c_u$	2
3, 7	$r_u\ h\ s_r\ e^s\ c_a$	2
4, 7	$r_u\ h\ t_h\ s_r\ e^s\ c_a$	3
1, 5	$e_y\ r_u\ D\ c_u\ s_r\ e^s\ c_a$	4
	$h\ t_h\ s_t$	10
2, 5	$e_y\ r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$	1
	$t_h\ s_t$	5
3, 5	$D\ t_h\ s_t$	2
1, 2	$h\ D$	9
	$e_y\ r_u\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	1
Total double crossovers		553
1, 2, 9	$h\ D\ c_a$	3
2, 7, 9	$r_u\ h\ D\ s_r\ e^s$	1
1, 7, 9	$r_u\ D\ s_r\ e^s$	1
1, 5, 9	$h\ t_h\ s_t\ c_a$	2
2, 5, 9	$t_h\ s_t\ c_a$	1
2, 6, 8	$r_u\ h\ D\ c_u\ s_r$	1
1, 5, 7	$h\ t_h\ s_t\ s_r\ e^s\ c_a$	1
Total triple crossovers		10
Grand total		3185

TABLE 14

Translocation d.

$e_y\ r_u\ h\ 2\ 3\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a\ 9$

$\frac{\cdot\cdot\quad 1\quad D\quad 4\ 5\ 6\ 7\ 8\ +}{}$ ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

CLASSES OF THE OFFSPRING		NUMBER OBSERVED
0	D	289
	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	152
Total non-crossovers		441
1	$D\ r_u$	185
	$e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	99
2	$D\ r_u\ h$	77
	$e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	81
3	$r_u\ h$	6
	$e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	5
4	$r_u\ h\ t_h$	3
	$e_y\ D\ s_t\ c_u\ s_r\ e^s\ c_a$	7
5	$r_u\ h\ t_h\ s_t$	29
	$e_y\ D\ c_u\ s_r\ e^s\ c_a$	28
6	$r_u\ h\ t_h\ s_t\ c_u$	41
	$e_y\ D\ s_r\ e^s\ c_a$	72
7	$r_u\ h\ t_h\ s_t\ c_u\ s_r$	37
	$e_y\ D\ e^s\ c_a$	48
8	$r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	82
	$e_y\ D\ c_a$	79
9	$r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	8
	$e_y\ D$	21
Total single crossovers		908
1, 9	$e_y\ r_u\ D$	17
	$h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	4
2, 9	$e_y\ r_u\ h\ D$	6
	$t_h\ s_t\ c_u\ s_r\ e^s\ c_a$	1
4, 9	$D\ s_t\ c_u\ s_r\ e^s\ c_a$	1
5, 9	$D\ c_u\ s_r\ e^s\ c_a$	2
	$e_y\ r_u\ h\ t_h\ s_t$	1
6, 9	$D\ s_r\ e^s\ c_a$	8
8, 9	$D\ c_a$	5
	$e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	9
1, 8	$h\ t_h\ s_t\ c_u\ s_r\ e^s$	31
	$e_y\ r_u\ D\ c_a$	51
2, 8	$t_h\ s_t\ c_u\ s_r\ e^s$	26
	$e_y\ r_u\ h\ D\ c_a$	13
3, 8	$D\ t_h\ s_t\ c_u\ s_r\ e^s$	2
	$e_y\ r_u\ h\ c_a$	1
4, 8	$D\ s_t\ c_u\ s_r\ e^s$	4
5, 8	$D\ c_u\ s_r\ e^s$	8
5, 8	$e_y\ r_u\ h\ t_h\ s_t\ c_a$	3
6, 8	$D\ s_r\ e^s$	7
7, 8	$D\ e^s$	1
1, 7	$e_y\ r_u\ D\ e^s\ c_a$	32
	$h\ t_h\ s_t\ c_u\ s_r$	11
2, 7	$e_y\ r_u\ h\ D\ e^s\ c_a$	10
	$t_h\ s_t\ c_u\ s_r$	4
5, 7	$D\ c_u\ s_r$	2
	$e_y\ r_u\ h\ t_h\ s_t\ e^s\ c_a$	3
1, 6	$h\ t_h\ s_t\ c_u$	14
	$e_y\ r_u\ D\ s_r\ e^s\ c_a$	38
2, 6	$t_h\ s_t\ c_u$	13
	$e_y\ r_u\ h\ D\ s_r\ e^s\ c_a$	14
3, 6	$D\ t_h\ s_t\ c_u$	1
	$e_y\ r_u\ h\ s_r\ e^s\ c_a$	3
4, 6	$e_y\ r_u\ h\ t_h\ s_r\ e^s\ c_a$	1
5, 6	$e_y\ r_u\ h\ t_h\ s_t\ s_r\ e^s\ c_a$	1
1, 5	$h\ t_h\ s_t$	6
	$e_y\ r_u\ D\ c_u\ s_r\ e^s\ c_a$	6
2, 5	$t_h\ s_t$	4
	$e_y\ r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$	1
3, 5	$D\ t_h\ s_t$	1
1, 2	$h\ D$	1
Total double crossovers		367
1, 8, 9	$e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$	1
1, 5, 9	$e_y\ h\ t_h\ s_t$	1
5, 8, 9	$e_y\ D\ c_u\ s_r\ e^s$	1
7, 8, 9	$e_y\ D\ e^s$	1
1, 2, 8	$r_u\ t_h\ s_t\ c_u\ s_r\ e^s$	1
1, 6, 8	$r_u\ D\ s_r\ e^s$	1
2, 6, 8	$r_u\ h\ D\ s_r\ e^s$	4
1, 2, 7	$r_u\ t_h\ s_t\ c_u\ s_r$	1
1, 5, 7	$r_u\ D\ c_u\ s_r$	3
	$e_y\ h\ t_h\ s_t\ e^s\ c_a$	1
2, 5, 7	$r_u\ h\ D\ s_r$	1
Total triple crossovers		16
1, 5, 8, 9	$h\ t_h\ s_t\ c_a$	1
Total quadruple crossovers		1
Grand total		1733

TABLE 15

Translocation e.

e_y r_u h 2 3 t_h s_t 5 6 c_u s_r e^s c_a

—— ——————————— ♀ × e_y r_u h t_h s_t c_u s_r e^s c_a ♂.

.. 1 D 4 + 7 8 9

CLASSES OF THE OFFSPRING		NUMBER OBSERVED
0	D	285
	e_y r_u h t_h s_t c_u s_r e^s c_a	81
Total non-crossovers		366
1	r_u D	135
	e_y h t_h s_t c_u s_r e^s c_a	24
2	r_u h D	49
	e_y t_h s_t c_u s_r e^s c_a	43
3	e_y D t_h s_t c_u s_r e^s c_a	1
	r_u h	6
4	e_y D s_t c_u s_r e^s c_a	2
	r_u h t_h	2
5	e_y D c_u s_r e^s c_a	6
	r_u h t_h s_t	6
6	D c_u s_r e^s c_a	20
	e_y r_u h t_h s_t	14
7	D s_r e^s c_a	117
	e_y r_u h t_h s_t c_u	27
8	D e^s c_a	85
	e_y r_u h t_h s_t c_u s_r	33
9	D c_a	199
	e_y r_u h t_h s_t c_u s_r e^s	67
Total single crossovers		836
1, 9	r_u D c_a	57
	e_y h t_h s_t c_u s_r e^s	24
2, 9	r_u h D c_a	39
	e_y t_h s_t c_u s_r e^s	7
3, 9	r_u h c_a	1
	e_y D t_h s_t c_u s_r e^s	1
4, 9	r_u h t_h c_a	1
	e_y D s_t c_u s_r e^s	
5, 9	r_u h t_h s_t c_a	1
6, 9	e_y r_u h t_h s_t c_a	1
	D c_u s_r e^s	7
		5
7, 9	D s_r e^s	26
	e_y r_u h t_h s_t s_u c_a	6
8, 9	D e^s	5
	e_y r_u h t_h s_t c_u s_r c_a	5
1, 8	r_u D e^s c_a	17
2, 8	r_u h D e^s c_a	10
3, 8	r_u h e^s c_a	6
5, 8	r_u h t_h s_t e^s c_a	6
6, 8	D c_u s_r	3
	e_y r_u h t_h s_t e^s c_a	1
1, 7	r_u D s_r e^s c_a	20
	e_y h t_h s_t c_u	10
2, 7	r_u h D s_r e^s c_a	11
	e_y t_h s_t c_u	7
3, 7	r_u h s_r e^s c_a	1
4, 7	r_u h t_h s_r e^s c_a	1
2, 6	r_u h D c_u s_r e^s c_a	2
2, 5	t_h s_t	1
Total double crossovers		282
1, 8, 9	r_u D e^s	1
1, 7, 9	r_u D s_r e^s	1
1, 6, 9	e_y h t_h s_t c_a	1
5, 7, 9	r_u h t_h s_t s_r e^s	2
2, 8, 9	e_y t_h s_t c_u s_r c_a	1
1, 2, 9	e_y r_u t_h s_t c_u s_r e^s	1
6, 7, 9	e_y r_u h t_h s_t s_r e^s	1
Total triple-crossovers		8
1, 2, 7, 9	e_y r_u t_h s_t c_u c_a	1
	h D s_r e^s	3
Total quadruple crossovers		4
Grand total		1496

TABLE 16

Crossing over in the normal third chromosome—control experiment.

$$\frac{e_y \quad r_u \; h \; 2 \; 3 \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a}{+ \quad 1 \quad D \quad 4 \; 5 \; 6 \; 7 \; 8} \; \text{♀} \times e_y \, r_u \, h \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a \, \text{♂}.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 D	354
0 $r_u \, h \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a$	165
Total non-crossovers	519
1 $r_u \, D$	144
1 $h \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a$	56
2 $r_u \, h \, D$	76
2 $t_h \, s_t \, c_u \, s_r \, e^s \, c_a$	46
3 $r_u \, h$	6
3 $D \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a$	6
4 $r_u \, h \, t_h$	11
4 $D \, s_t \, c_u \, s_r \, e^s \, c_a$	2
5 $r_u \, h \, t_h \, s_t$	44
5 $D \, c_u \, s_r \, e^s \, c_a$	28
6 $r_u \, h \, t_h \, s_t \, c_u$	46
6 $D \, s_r \, e^s \, c_a$	87
7 $r_u \, h \, t_h \, s_t \, c_u \, s_r$	41
7 $D \, e^s \, c_a$	57
8 $r_u \, h \, t_h \, s_t \, c_u \, s_r \, e^s$	77
8 $D \, c_a$	218
Total single crossovers	945
1, 8 $r_u \, D \, c_a$	110
1, 8 $h \, t_h \, s_t \, c_u \, s_r \, e^s$	23
2, 8 $r_u \, h \, D \, c_a$	53
2, 8 $t_h \, s_t \, c_u \, s_r \, e^s$	23
3, 8 $r_u \, h \, c_a$	3
3, 8 $D \, t_h \, s_t \, c_u \, s_r \, e^s$	1
4, 8 $D \, s_t \, c_u \, s_r \, e^s$	2
5, 8 $r_u \, h \, t_h \, s_t \, c_a$	27
5, 8 $D \, c_u \, s_r \, e^s$	7
6, 8 $r_u \, h \, t_h \, s_t \, c_u \, c_a$	13
6, 8 $D \, s_r \, e^s$	26
7, 8 $D \, e^s$	13
7, 8 $r_u \, h \, t_h \, st \, c_u \, s_r \, c_a$	3
1, 7 $r_u \, D \, e^s \, c_a$	28
1, 7 $h \, t_h \, s_t \, c_u \, s_r$	18
2, 7 $r_u \, h \, D \, e^s \, c_a$	12
2, 7 $t_h \, s_t \, c_u \, s_r$	9
3, 7 $r_u \, h \, e^s \, c_a$	2
3, 7 $D \, t_h \, s_t \, c_u \, s_r$	1
4, 7 $D \, s_t \, c_u \, s_r$	1
5, 7 $D \, c_u \, s_r$	1
5, 7 $r_u \, h \, t_h \, s_t \, e^s \, c_a$	10
7, 6 $D \, s_r$	1
7, 6 $r_u \, h \, t_h \, s_t \, c_u \, e^s \, c_a$	6
1, 6 $r_u \, D \, s_r \, e^s \, c_a$	41
1, 6 $h \, t_h \, s_t \, c_u$	19
2, 6 $r_u \, h \, D \, s_r \, e^s \, c_a$	17
2, 6 $t_h \, s_t \, c_u$	15
3, 6 $r_u \, h \, s_r \, e^s \, c_a$	3
3, 6 $D \, t_h \, s_t \, c_u$	1
5, 6 $r_u \, h \, t_h \, s_t \, s_r \, e^s \, c_a$	11
5, 6 $D \, c_u$	5
1, 5 $r_u \, D \, c_u \, s_r \, e^s \, c_a$	9
1, 5 $h \, t_h \, s_t$	6
2, 5 $t_h \, s_t$	2
1, 2 $h \, D$	6
1, 2 $r_u \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a$	1
Total double crossovers	528
1, 6, 8 $r_u \, D \, s_r \, e^s$	7
1, 6, 8 $h \, t_h \, s_t \, c_u \, c_a$	1
2, 6, 8 $r_u \, h \, D \, s_r \, e^s$	4
2, 6, 8 $t_h \, s_t \, c_u \, c_a$	4
1, 7, 8 $h \, t_h \, s_t \, c_u \, s_r \, c_a$	1
2, 7, 8 $t_h \, s_t \, c_u \, s_r \, c_a$	2
1, 5, 8 $r_u \, D \, c_u \, s_r \, e^s$	3
1, 5, 8 $h \, t_h \, s_t \, c_a$	3
2, 5, 8 $t_h \, s_t \, c_a$	2
5, 6, 8 $r_u \, h \, t_h \, s_t \, s_r \, e^s$	1
5, 7, 8 $D \, c_u \, s_r \, c_a$	1
1, 2, 8 $h \, D \, c_a$	1
2, 5, 7 $r_u \, h \, D \, c_u \, s_r$	2
1, 5, 6 $h \, t_h \, s_t \, s_r \, e^s \, c_a$	2
2, 5, 6 $t_h \, s_t \, s_r \, e^s \, c_a$	1
1, 2, 6 $h \, D \, s_r \, e^s \, c_a$	1
Total triple crossovers	36
1, 4, 6, 8 $h \, t_h \, s_r \, e^s$	1
Total quadruple crossovers	1
Grand total	2029

TABLE 17

$\frac{B_l\ D\ +}{+\ +\ e_y}$ ♂ × $\frac{+\ +\ e_y}{+\ +\ e_y}$. *Summary of counts in the "normal" cultures.*

	WILD TYPE	B_l	D	e_y	$B_l D$	$D e_y$	$B_l e_y$	$B_l D e_y$
Females	1152	1145	1163	1052	1191	986	1023	1016
Males	1044	1038	1083	1015	1133	911	1117	975
Total	2196	2183	2246	2067	2324	1897	2140	1991

Grand total 17044