GENETIC AND CYTOLOGICAL MAPS OF THE AUTOSOMES IN DROSOPHILA PSEUDOOBSCURA

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THE PROBLEM

Drosophila pseudoobscura presents considerable interest as an object for the study of a number of genetic problems, especially those connected with hybrid sterility and methods of race and species formation. As shown by Lancefield (1929), this species contains two races, race A and race B, which produce, when crossed, sterile male and fertile female hybrids. The two races are indistinguishable externally, but differ in a number of physiological characteristics (Shapiro 1932, Poulson 1934, Dobzhansky 1935). I have shown in a previous work (Tan 1935a,b) that the gene arrangement in the chromosomes of the two races is somewhat different, there being four inverted sections in the chromosomes of the hybrid.

An analysis of the cause of the sterility of the hybrids in this case (Dobzhansky and Boche 1933, Dobzhansky 1933a,b, 1934a, 1936) has shown that it is due to interaction of genetic factors rather than chromosome incompatibility between the two races. Lancefield (1929) and Koller (1932a) found that the factors responsible for the sterility of hybrids are located in the X-chromosome. Dobzhansky has further demonstrated that some of these factors are also present in the autosomes.

As a basis for these studies it is necessary to work out the genetic and the cytological maps of Drosophila pseudoobscura chromosomes. Especially, when the question of the distribution of the sterility factors in the chromosomes of the two races is raised, and their relation to interracial distinction in the chromosome structure is to be settled, such information is essential.

With the above aim in view, the present investigation is undertaken.

MATERIALS AND METHODS

Drosophila pseudoobscura Frolowa is a species native in the Pacific Coast of North America, which has for a long time been confused with the European Drosophila obscura Fallen; its description under this name can be found in Sturtevant's monograph of the genus Drosophila (Sturtevant 1921). Frolowa and Astauroff (1929) have shown the two forms to be really distinct species, and have given the name pseudoobscura for our object. Lancefield (1929) observed that the species pseudoobscura consists of two races, called A and B, which produce sterile hybrids when crossed. Tan (1935) and Koller (1936) found the two races to be different in gene arrangement - they differ in four inversions in as many chromosomes. Moreover, strains of the same race consisting of four different geographic regions may also differ in gene arrangement, and so it becomes necessary for the purposes of this work to use always a definite standard strain. Race A strain from Georgetown, Texas, was chosen.

The procedure adopted in breeding this species consists of keeping the flies to be crossed for three days in a vial, in which a small chip of food is placed, before transferring to a half pint culture bottle containing food. For linkage experiments all cultures were raised in an incubator at 25°C, for it was shown by Plough (1917) in Drosophila melanogaster that the amount of crossing over varies with temperature, though such effect has not yet been reported in our species. The apparatus used, such as incubator, etherizer, culture bottles, and the formula of food, were essentially the same as described by Bridges (1932). The counts from a bottle were continued for 12 to 14 days after the first day of hatching but not until the appearance of the succeeding generation.

All mutant stocks of race A Drosophila pseudoobscura kept in this laboratory were used for the purpose of constructing the genetic maps. Some of them were sent here from the Genetic Laboratory of Edinburgh University, Scotland, through the kindness of Doctor P. Koller, to whom the author wishes to express his indebtedness. In the course of the investigation a few new mutants were discovered by the author and some of them proved rather valuable because of their favorable locations. Professor Sturtevant also kindly let me use some of the new mutants he had found.

In constructing genetic maps, the distance between two nearest genes was determined by the percentage of crossing over between them. In a few cases where a big gap lies between two adjacent genes, the determination based on direct crossover values might not be accurate enough on account of double crossovers which have escaped detection.

In order to establish the cytological maps, twenty-two translocations involving different chromosomes (exclusive of X chromosome), were obtained with the aid of X-ray. Details regarding the origin of these translocations will be described later. For each translocation parallel genetic and cytological analysis was carried out. Genetic analysis of translocations consisted of testing for (1) viability and fertility of duplication and deficiency heterozygotes (2) viability of homozygous translocations, and (3) location of the breakage points. Translocations that give rise to viable hyperploid or hypoploid zygotes are more useful than those which do not; for in former cases tests for duplicating fragments usually give a more accurate information as to the whereabouts of the breakage loci than the crossing over tests do. Especially since there were not enough numbers of known genes properly distributed

in the autosomes of the species, the determination of the breakage points by means of comparing the crossover values in a translocation and that in the control was rather limited in value.

Individual salivary gland chromosomes can be identified in any nucleus, once one is acquainted with their distinctive characteristics. The correspondence between the respective chromosomes in the salivary gland cells and their counterparts among the linkage groups known in the species was previously established (Tan, 1935) by studying the inverted sections in the different chromosomes. Since each strand converging to the chromocenter represents the conjugated homologous chromosomes in structural homozygotes, and in case of heterozygotes (inversion, translocation, etc.) the section containing similar loci are paired, forcing the chromosomes to form sometimes very complex geometrical configurations, the breakage points of different chromosome aberrations were determined in the salivary gland cell of the hybrid larvae between standard strain and a particular translocation stock concerned. In cases of Y-translocations, only male hybrid larvae were heterozygous for translocations, and therefore only male larvae were selected for cytological study. As to the rest, the work was done mostly in female hybrids, since male hybrids were decidedly inferior to female for that purpose.

The aceto-carmine smear method for studying the salivary gland chromosomes has been applied as described by Painter (1934a,b). There are, however, a few points of improvements which may be mentioned here. Larvae selected for salivary chromosome study should not only be old, about ready to pupate, but also they must be well fed. As

soon as a good many larvae hatched in culture bottles, parent flies were removed, so as to prevent them from laying too many eggs, which would eventually hatch out to share the food. When the larvae were partly grown (about 7 days), about 1/8 cake of Fleishmann's yeast was put in on the surface of the food in each bottle. The culture bottles were then kept at low temperature, about 15° - 18°C, until the larvae became big enough to dissect. According to the advice of Drs. Bridges and Schultz, the dissection of the larvae and the fixation of the glands were made at a low temperature. For this purpose, a depression slide was supported by a paraffin mold on the two opposite edges of a rectangular copling jar, which could be filled with ice blocks. The thick slide was about half immersed in the ice water. The staining solution and the normal saline were chilled too. The larvae were transferred from culture bottle to the hollow depression of the slide. Dissection was performed on the cooled smooth surface of the slide. Glands were transferred by the tip of a needle to a small syracuse dish containing chilled staining solution. After the glands were left there for about 15 to 20 minutes, they were mounted up, usually 2 or 3 pairs on one slide, smashed gently and sealed up with paraffinmastic. Third, how to convert a temporary mount to a permanent one. Temporary mounts of salivary gland chromosomes could usually stay good for about a week. Drawings were made during that interval. In order to preserve desirable figures permanently, a simple technique was developed following the suggestion of Doctor Bridges to change temporary mounts into permanent ones. It consisted of scraping off the wax sealing by means of a knife and then dipping the slide with

the cover glass on into 95% alcohol. Allow them to stay there over night. The cover glass usually came off on the next day. If they failed to do so, they could be easily teased off by putting two fingers at one edge of the cover to keep it from moving and using a fine forcep with the other hand to wedge the edge of the cover up. The tissue may adhere to the surface of either slide or cover glass or both. The detached slide and cover slip were then returned to 95% alcohol in separate vessels for 10 minutes before being mounted up with eupral. Not infrequently in the process of conversion from temporary to permanent mount, the smeared tissue might fall off from either the slide or cover, and get lost. Following the advice of Doctor H. Bauer, clean slides, on which tissues were going to be mounted, should first be smeared with a very thin film of 45% albumin solution and allowed to dry completely before using. When glands were mounted on such slides, they always got stuck on the surface of the slide, and hardly ever fell off in the process of changing to permanent mounts.

All drawings with two exceptions were made under the objective 120 and ocular 10 Zeiss, with the aid of a camera lucida. The two exceptional ones, figures 26 and 27 were made at a even higher magnification, objective 90 and ocular 20, for demonstrating the precise nature of a non-reciprocal translocation. The figures are of two kinds. Figures showing the composite maps of the three autosomes were copied from Dobzhansky and Tan (1936). All others were original drawings made from individual preparations of hybrid larvae. Since it rarely happens that a chromosome is clear in all its parts, in most drawings the banding was represented only in the clear sections, the remaining sections being represented by dotted contour lines.

As an aid for description, Dobzhansky and Tan (1936), following the example of Bridges (1935) in Drosophila melanogaster, subdivided the chromosomes of pseudoobscura into one hundred arbitrary sections, numbering them consecutively from the spindle fiber to the free end in each chromosome. The same numbering is adopted here. The breakage points of various translocations, which were determined by studying the homology of salivary chromosomes of heterozygous translocations, are indicated in those composite maps by lines above. Hence, the limits and nature of each translocation can easily be found at a glance simply by reading the sequence of the numbered section in the composite map.

No attempt was made to detect the precise point of breakage in every translocation with respect to the faint bands. There were, however, a few cases, which, as will be pointed out later, do clearly demonstrate the exact loci of breakage.

GENETIC MAPS

Introduction

The phenomenon of linkage of genetic factors was discovered in sweet pea (Lathyrus odoratus) by Bateson and Punnet (1905, 1911) who named it "gametic correlation" and spoke about "coupling" and "repulsion" of genes. The term linkage and the idea that this phenomenon is due to the location of linked genes in the same chromosome were proposed by Morgan (1911a,b). Sturtevant (1913, 1915), using the degree of linkage as a measure of the distance between the loci of the genes in a chromosome, has demonstrated that genes are

arranged in a linear series and has constructed the first genetic map of chromosomes (in Drosophila melanogaster).

The evidence for the theory of the linear order of genes, as well as the theory of genetic map construction has been adequately discussed by Sturtevant (1915, 1919), Muller (1916), Morgan and Bridges (1919) and others, and need not be presented here. Although most work of mapping the chromosomes has been done in Drosophila melanogaster, genetic maps are known at present also for maize (Emerson, Beadle and Fraser 1935), for several other species of Drosophila, and for a number of other organisms (Lathyrus, Pisum, Phorbitis, Gallus, Mus, Lepus and others). The fundamental principles of linkage proved to be alike for all these forms, and are probably universally valid.

One of the major requirements for producing an accurate genetic map is the possession of a large number of mutant genes in the linkage group in question. In Drosophila pseudoobscura the first mutant was reported by Sturtevant (1921). Metz (1916) reported three mutants. Lancefield (1919, 1922) gave an account of a very extensive series of sex-linked mutations in the same species. According to Morgan, Sturtevant and Bridges (1925) four autosomal groups of genes were also reported by Lancefield with 4, 6, 1, and 2 recognized loci respectively. These, together with the sex-linked group, made the total number of linkage groups 5, which equal to the haploid chromosome number of the species. In spite of the fact that the autosomal linkage groups reported by Lancefield were imperfect, some of his mutants have been found to be the same as some of the ones described below. For this reason, the numbering of II and III linkage groups still follow

the system of Lancefield. The numbering of the IV linkage group is justified on the basis of cytological evidence, which shows that he representative gene of this group is located in the rod-shaped chromosome and not in the chromosome now designated as the fifth, in which no gene is reported here. Whether Lancefield's 4th and 5th linkage groups corresponds to mine remains to be seen, because no mutant gene so far discovered in the 4th linkage group is certainly identical with any gene of either 4th or 5th group of Lancefield.

Description and Localization of Mutant Genes

Since the present investigation is confined to the autosomes of Drosophila pseudoobscura, only the autosomal mutants will be described below. In each chromosome mutants will be presented in an order more or less according to the time of their discovery.

The Second Chromosome

Bare (Ba)

Bare is dominant and homozygous when lethal. It was discovered by Professor A. H. Sturtevant (D.I.S. No. 1, p.41) in race A. It is characterized by the shortening of the macrocheatae. It was first shown by Doctor Shultz to be independent of all but the second chromosome of Lancefield. The character can be easily distinguished from the normal, and the viability and productivity of the heterozygous flies are excellent. Recently, Professor Sturtevant and the author have noticed that flies in the Bare stock may occasionally breed true, showing that homozygous Bare flies may sometimes be viable.

Smoky (Sm)

Smoky, another dominant gene in this chromosome, was found by Miss Beers (D.I.S. No. 1, p. 41) in race B. The mutant gene causes the thickening and branching of wing veins, especially the second longitudinal vein. The minimum expression is the formation of small delta-like structure at the distal end of the second longitudinal vein. It is invariably lethal when homozygous. The mutant has been easily transferred from race B to race A standard strain. By crossing to Bare and backcrossing the F_1 Bare Smoky flies to wild type, it was found that Smoky is linked with Bare and the two are situated quite far away from each other, for the crossing over frequency was found to be more than 40%. Since the region between Bare and Smoky is long enough for double crossing over to occur freely, and since a double crossover could not be detected in a two point cross, the region must be longer than 40 units. The determination of the distance has, therefore, to wait until mutations will be obtained at intermediate loci. A Bare Smoky stock was established for the tests and localization of new genes in this chromosome.

glass (gl) and its interaction with orange (or)

Glass was discovered by Crew and Lamy (1935) . It is a recessive. The eye is reduced in size and surrounded by a smooth colorless rim. The irregular shape of the eye facets in the central area and numerous hairs pointing in all directions give the eye an appearance of extreme roughness. The pigment in the central space appears to be greatly reduced in amount and leaves only a pinkish or reddish hue. The character can be easily recognized with naked eye. The viability and fertility are about as good in wild type.

Crew and Lamy (1935) reported that glass gave free recombination with purple and short$_4$, representatives of the third and fourth linkage groups. With Stubble, a representative of second autosome, glass was found linked and observed to give about 47% of recombination. Since at the time when glass was introduced into this laboratory, the relations between the linkage groups short$_4$ and Stubby of Crew and Lamy and those of Bare-Smoky and Curly of this laboratory were not known, glass was immediately crossed to Bare-Smoky (second chromosome) and Curly (fourth chromosome), the third chromosome being represented by purple of Crew and Lamy needs not to be tested here. When F_1 Curly males were backcrossed to glass females, first counts of F_2 flies gave 25 Curly, 17 wild, 9 glass and 3 Curly glass flies. This indicates that glass is not located in the fourth chromosome. The result of backcross of Bare-Smoky males to glass females, however, gave only two kinds of flies, 80 glass and 91 Bare-Smoky. Hence, it may be concluded that glass must be located in the second chromosome. Since glass was found linked with Bare-Smoky, a full count of flies were made in a single culture of cross between $\frac{gl}{Sm\ Ba}$♀♀ × gl♂♂ to find the approximate location of glass in relation to Bare and Smoky. The figure given in table 1 gave

Table 1. Nature of the cross: $\frac{gl}{Sm\ Ba}$♀♀ × gl♂♂

Non-crossovers		single crossovers 1st region		single crossovers 2nd region		double c.o. 1, 2		
gl	Sm-Ba	Sm-gl	Ba	Sm-Ba-gl	+	Ba-gl	Sm	total
90	164	52	137	27	36	20	20	546

crossing over values of 42.0% between Bare and Smoky, 18.9% between Bare and glass, and 46.3 between Smoky and glass. Since the smallest number of flies were represented by Bare-glass and Smoky classes, which must be double cross-overs, the sequence of the three genes in the chromosome must be Smoky-Bare-glass, Bare being nearer to glass than to Smoky. A stock of Smoky-Bare-glass was then established.

As pointed out above, glass eyes are usually light reddish in color. However, in pure glass culture, occasionally some glass flies were found to be light gray in color. When such flies were mated to wild type, all F_1 individuals were of wild type. Among 164 F_2 flies, (Table 2) 43 were not glass but orange in eye color.

Table 2. Nature of the cross: $\frac{gl}{+}\ \frac{or}{+}\ ♀♀ \times \frac{gl}{+}\ \frac{or}{+}\ ♂♂$

	wild	orange	glass (light red)	glass (light gray)
Observed	164	43	34	13
Expected	141	48	48	16

Of 47 glass flies could be segregated into two classes, 34 light red and 13 light gray in approximately 3 to 1 ratio. The result can be explained on the assumption that glass with light gray color was due to the interaction of genes glass and orange. The appearance of glass orange flies in class stock might either be due to contamination or to a recurrent mutation of the locus orange in the original glass stock. Since the history of the stock was not very clear, it could not be ascertained which was the actual cause.

That glass with light gray color is due to the interaction of the genes glass and orange was confirmed by the results of following

matings. Glass flies with light reddish color were mated to orange. All F_1 progeny was of wild type, and in F_2, as shown in table 3, there appeared four types of flies, wild type, orange, glass with light red

Table 3. Nature of the cross: $\frac{gl}{+} \frac{+}{or}\,♀♀ \times \frac{gl}{+} \frac{+}{or}\,♂♂$

	wild	orange	glass (light red)	glass (light gray)
Observed	308	68	65	12
Expected	257	84	84	28

color and glass with light gray color, in approximately 9:3:3: 1 ratio. In separate tests, where glass with light red color flies were mated to wild type, only the two parental types appeared in F_2 in the ratio of about 3 to 1 (Table 4). The last and most conclusive test was when

Table 4. Nature of the cross: $\frac{gl}{+}\,♀♀ \times \frac{gl}{+}\,♂♂$

	wild	glass (light red)
Observed	235	51
Expected	215	71

light gray glass flies were mated to orange, all F_1 flies were orange.

bithorax (bi)

Bithorax was reported by Crew and Lamy (D.I.S. No. 1, p. 41) as an autosomal recessive mutant, causing an enlargement of the balancers. In extreme cases, the balancers may take the form of winglike organs. The mutant flies are relatively weak and viability is low. Sometimes the character may overlap normal. Bithorax flies were mated to Bare Curly, and F_1 Bare Curly males were backcrossed to bithorax females. The F_2 fly counts, as shown in table 5, demonstrate

Table 5. Nature of the cross: $\frac{Bi}{Ba}\ \frac{+}{cy}\ ♂♂ \times bi♀♀$

Ba Cy	Bi	Cy bi	Ba	Cy
15	18	23	20	9

that bithorax gives free recombination with Curly but not with Bare, indicating that bithorax belongs to the second chromosome. Some of bithorax flies listed in table 5 appeared like wild type. Since Bare bithorax flies were entirely absent, these wild type flies must actually be bithorax overlapping the normal. A cross of $\frac{bi}{Ba}♀♀ \times bi♂♂$ was also made. Table 6 shows that Bare and bithorax had crossed over

Table 6. Nature of the cross: $\frac{bi}{Ba}♀♀ \times bi♂♂$

Non-crossovers		crossovers		
bi	Ba	+	bi Ba	total
145	135	21	7	308

28 times in 308 individuals, the per cent of crossing over being 9.08%. Since Curly was found independent of bithorax, character Curly was entirely neglected in this count. In order to find out which side of Bare bithorax lies, a four point linkage experiment involving the genes Smoky, Bare, glass and bithorax was planned. As the backcrosses in this experiment would require bithorax glass flies, it was necessary first to establish a bithorax glass stock. To attain this aim, bithorax females were crossed to glass males, and in F_2 bithorax males were individually tested against glass females for heterozygosity of glass

as a possible result of crossing over. Out of 9 such matings, 6 cultures gave exclusively wild type flies and 3 cultures gave both wild type and glass in approximately 1 to 1 ratio. The glass flies thus obtained must be heterozygous for bithorax and so, by inbreeding, gave about 3/4 glass and 1/4 bithorax-glass flies. From these bithorax glass flies, stock of bi gl was established.

Bithorax glass females were now mated to Smoky Bare males. Among the F_1 progeny, Sm Ba females were selected and backcrossed to bi gl males. Four such cultures were made and the results observed in F_2 are summarized in table 7. The calculated crossing over values in the different regions are as follows:

Sm/bi	bi/Ba	Ba/gl	Sm/gl	Sm/Ba	bi/gl
43.6%	9.4%	22.7%	46.7%	43.2%	28.9%

The order of the genes can be best determined by comparing the values bi/Ba, bi/gl and Ba/gl. The fact that the percentage of crossing over between glass and bithorax, 28.9%, was significantly higher than that of either between Bare and glass, 22.7%, or between bithorax and Bare, 9.4%, indicated that bithorax must be localized to the left of the locus Bare. The sequence of the four genes is, therefore, Sm-bi-Ba-gl. As shown in table 7, the number of triple crossovers is unexpectedly equal to the double crossovers of the regions 2 and 3. This fact could be accounted for by the great length of the region 1, in which crossing over might have reached randomness. The same explanation also applies to the result that the crossing over values between Smoky and bithorax and between Smoky and Bare were about equal. The possibility that

Table 7. Nature of the cross: $\frac{\text{bi}}{\text{Sm}}\ \frac{\text{gl}}{\text{Ba}}$ ♀♀ x bi gl ♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers						triple crossovers		+	total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3		1,2,3			
	bi-gl	Sm-Ba	Sm-bi-gl	Ba	bi-Ba	Sm-gl	bi	Sm-Ba-gl	Sm-bi-Ba	gl	Sm-bi	Ba-gl	bi-Ba-gl	Sm	Sm-bi-Ba-gl			
1	26	42	12	42	2	2	13	7	4	3	8	12	0	2	0	2		177
2	63	67	56	65	5	11	22	13	2	8	9	21	1	3	0	3		349
3	42	39	12	22	3	1	13	5	2	5	5	10	0	0	1	2		162
4	55	62	20	70	7	6	30	20	5	12	6	17	0	2	0	0		312
	186	210	100	199	17	20	78	45	13	28	28	60	1	7	1	7		1000

either poor viability of bithorax flies or overlapping expression of the gene bithorax might also play a part in both of the above discrepancies is also not excluded.

pink (p) and its interaction with orange (or)

Pink eye was found in a cross of La Grande-2 strain to or-Sc-pr by Prof. Sturtevant in 1934 (D.I.S. No. 3, p. 43). It is a recessive gene located near Bare. Pink eye color itself is variable and sometimes overlaps wild type. However, in combination with orange, a third chromosome gene, it can be easily classified. As a matter of fact, orange alone gives a bright red eye color (like vermillion) while in combination with pink it gives a true orange color.

According to Prof. Sturtevant pink is located close to Bare and the two gave only few per cent of crossing over. An experiment involving three genes relatively near to each other, like bithorax, pink and Bare, would be very useful to determine the order of these loci. Before this experiment could be carried out, a homozygous multiple recessive stock of orange bithorax pink should first be raised. Essentially the same method as described in the previous section for the obtaining of the bi gl stock was followed here, except that a large number of individual tests was needed here to get crossovers between bithorax and pink, for it was known that Bare, which is close to pink, gives only about 9% of recombination with bithorax. Out of some thirty test cultures, only two were crossovers between bithorax and pink. Those flies helped to build up the or bi p stock. Orange Bare flies were then mated to orange bithorax pink females. F_1 orange females were backcrossed to the

multiple recessives. Table 8 represents the summary of the result from

Table 8. Nature of the cross: $\frac{or\ bi\ p}{or} \frac{}{Ba}$ ♀♀ × or bi p♂♂

Culture No.	non-c.o.		single crossovers				double c.o.		total
			Reg.1		Reg.2		1, 2		
	bi-p	Ba	p	bi-Ba	bi-p-Ba	+	p-Ba	bi	
1	110	106	6	2	3	3	2	1	233
2	96	120	14	7	2	4	2	3	248
	206	226	20	9	5	7	4	4	481

two separate cultures. As an aid for the distinction of pink from wild type, the gene orange was introduced in the cross to take place of the wild type. Since every class of fly shown in table 8 is homozygous for orange, the designation for orange was omitted there. Crossing over frequencies between bithorax and pink amount to 7.7% and that between pink and Bare 4.2%. The sum of the two being 11.9% is somewhat higher than the value, 9.2% that was directly obtained between bithorax and Bare as has been shown previously (Table 6). Despite the unexpectedly high number of observed double crossovers, the reason for that being unknown, it is justifiable to put the gene pink between bithorax and Bare.

pauciseta (ps^1) and its allellomorph (ps^2)

Pauciseta[1] was discovered by Miss Groscurth (D.I.S. No. 3, p. 43) in Chehalis-4 strain, several generations after this strain had been derived from a single female caught in nature. The mutant is recessive; and is characterized by the absence of some bristles, especially the anterior dorso-centrals and the scutellars. In some cases, however, all bristles may be present, but one or both anterior dorso-central become

somewhat more slender than normal. Unless the bristles are carefully examined, pauciseta can be overlooked.

Pauciseta^2 was found by the author as a single female in the progeny of a cross between $\frac{ct\ y\ m\ sn\ v\ se}{sh^2}$♀♀ x ct y m sn v se ♂♂ in March, 1936. The fly had some bristles missing on both thorax and scutellum. It was mated to some wild type males and produced exclusively wild type flies in F_1, which, inter se, produced both wild type and pauciseta. The result of the counts in two separate cultures is shown in table 9 and indicates that pauciseta^2 is an autosomal recessive.

Table 9. Nature of the cross: $\frac{ps^2}{+}$♀♀ × $\frac{ps^2}{+}$♂♂

Culture No.	wild ♀	wild ♂	ps^2 ♀	ps^2 ♂	total
1	83	113	23	22	241
2	96	104	25	21	246
Observed	396		91		487
Expected	365		122		487

The viability of the mutant flies is somewhat lower than normal. When some pauciseta^2 males were mated to bithorax glass pauciseta^1 females, the progeny all showed the pauciseta character, indicating that the new pauciseta is an allel to the old one. Since the two are indistinguishable from one another, the new pauciseta may be designated as ps^2.

To localize the gene pauciseta in the second chromosome, two sets of experiments were carried out. They were $\frac{or\ bi\ p\ ps}{or}$♀♀ × $\frac{bi\ p\ ps}{+}$ or bi p ps ♂♂ and $\frac{bi\ gl\ ps}{+}$♀♀ × bi gl ps ♂♂ crosses. The results are

summarized in tables 10 and 11 respectively. As calculated from the

Table 10. Nature of the cross: $\frac{or\ bi\ p\ ps}{or\ +}$ ♀♀ × or bi p ps ♂♂

	non-crossovers		single crossovers Reg.1		single crossovers Reg.2		double c.o. 1, 2		
Culture No.	bi-p-ps	+	p-ps	bi	bi-p	ps	p	bi-ps	total
1	70	112	3	5	52	77	5	3	327
2	68	111	7	4	37	88	2	1	318
	138	223	10	9	89	165	7	4	645

data given in table 10, crossing over value between bithorax and pink a amounts to 4.7%, which is lower than 7.7% for the same region obtained before (table 8). Crossing over between pink and pausciseta is 41.4%, indicating that the two genes are quite far apart. Considering bi ps and p as the double crossover classes, and comparing the data presented with those given in table 7, it may be safely concluded that the gene pauciseta lies on the right end of the second chromosome, in which the left end is represented by the gene Smoky.

Table 11 gives the result of crossing over between bithorax

Table 11. Nature of the cross: $\frac{bi\ gl\ ps}{+}$ ♀♀ × bi gl ps♂♂

non-crossovers		single crossovers Reg.1		single crossovers Reg. 2		double c.o. 1, 2		
+	bi-gl-ps	bi	gl-ps	bi-gl	ps	bi-ps	gl	total
119	21	21	44	10	24	5	6	250

and glass 31% and that between glass and pauciseta 18%. This enables us to localize the gene glass between bithorax and pauciseta.

The Third Chromosome

orange (or)

Orange eye color mutant was originally found by Lancefield and later described by Crew and Lamy (1934). It is recessive and can not be distinguished from the sex-linked vermillion eye color. The mutant is easily classifiable even in combination with most other eye colors. The viability and productivity of the mutant fly are as good as in normal.

purple (or)

Purple, another recessive eye color mutant, was found by Crew and Lamy (1932). It is a translucent color ranging from yellowish brown to chestnut. In case of males, the testicular sheath appears colorless. When it is combined with orange, it becomes grayish white in color. Crew and Lamy (1934) reported three different allels of the purple locus, whose coloration differs from each other in rather slight degree. However, they can be distinguished one from another, when each is separately combined with vermillion. The percentage of crossing over between purple and orange was reported by Crew and Lamy ('34) to be 47.2.

Scute (Sc)

Scute, a dominant gene causing the absence of most bristles on the thorax and the head, was also reported by Crew and Lamy (1934). The homozygous forms of Scute can be easily distinguished from the heterzygotes by the rough eyes and the absence of some microchaetae. Moreover, both homozygous and heterozygous Scute flies do not show any lowering of viability or productivity. According to Crew and Lamy (1934)

Scute is located about half way between orange and purple, each of which gives about 25% of crossing over with Scute. Due to its favorable location and its clear cut expression, Scute has proved to be an extremely valuable mutant in this group. The combined stock, or Sc pr, was built up to represent the third chromosome for standard tests of various kinds.

crossveinless (cv)

Crossveinless was reported by Crew and Lamy (D.I.S. No. 1, p. 41) as an autosomal recessive, which causes the absence of the posterior crossvein. The anterior crossvein may either be absent or incomplete. The mutant is easily distinguishable, if the wing is not damaged. Its viability is about normal; so is its productivity.

Following the report of Crew and Lamy that crossveinless gave 10% of recombination with the autosomal-short, which, as will be described later, is located in the Curly linkage group, the author started the genetic analysis by mating crossveinless to Curly with a view to find out the spatial relations between these two genes. Table 12 represents

Table 12. Nature of the cross: $\frac{+}{Ba}\ \frac{+}{Cy}\ \frac{cv}{+}$♂♂ × cv♀♀

cv	Ba-Cy	Ba-cv	Cy	Ba	cv-Cy	wild	Ba-cv-Cy
23	18	18	42	34	43	35	29

the fly counts of a cross between $\frac{+}{Ba}\ \frac{+}{Cy}\ \frac{cv}{+}$♀♀ × cv♂♂. The result shows, contrary to expectation, that crossveinless gives free recombination with both Bare and Curly, second and fourth chromosome genes respectively. In the meantime fly counts of the reciprocal cross, as shown in table 13 indicate 46.2% of recombination between Curly and crossveinless, that

Table 13. Nature of the cross: $\frac{+}{Cy}\ \frac{cv}{+}$ ♀♀ × cv♂♂

Culture No.	non-crossovers cv	non-crossovers Cy	crossovers cv Cy	crossovers +	total
1	34	46	31	39	150
2	27	25	22	21	95
	61	71	53	60	245

in the light of the result of the other cross (Table 12) would mean independent assortment of Curly and crossveinless rather than possible linkage of the two gene with long distance apart.

So far it has been shown that crossveinless is located neither in the fourth chromosome as reported by Crew and Lamy nor in the second chromosome. Since it is an autosomal gene, it may lie either in the third or in the fifth chromosome. Crossveinless flies were mated to Curly orange Scute purple, and the F_1 Curly Scute flies were selected and reciprocally backcrossed to crossveinless. Fly counts of this mating show only 4 kinds of flies present, 36 Cy cv, 28 cv, 32 Cy Sc and 26+. The presence of Curly crossveinless recombination flies at random and the entire absence of Scute crossveinless flies indicates that crossveinless is definitely linked in the or Sc pr group. The reciprocal cross, shown in table 14, gave 45.7% of recombination between Scute and

Table 14. Nature of the cross: $\frac{Cy\ Sc}{+\quad cv}$ ♀♀ × cv♂♂

Culture No.	Non-crossovers Sc Cy	Non-crossovers Sc +	Non-crossovers cv Cy	Non-crossovers cv +	crossovers Sc cv Cy	crossovers Sc cv +	crossovers + Cy	crossovers + +	total
1	62	53	78	40	45	50	62	40	430
2	38	22	33	30	29	34	25	14	225
	100	75	111	70	74	84	87	54	655

and crossveinless, which indicates that the locus of crossveinless is quite far away from Scute.

As has been shown by Crew and Lamy (1934), Scute gave about 25% crossing over with either orange or purple. Since crossveinless gave 45.7% of crossing over with Scute, crossveinless must lie at one of the extreme ends of the third chromosome. Before the tests could be made an or pr cy stock must be raised. By the time when the stock was ready, another third chromosome dominant gene, Jagged, was found. Consequently, a four point cross, involving orange, purple, crossveinless and Jagged, was carried out to localize both crossveinless and Jagged. As the result has bearing also on the new mutant, Jagged, it seems more desirable to discuss that after the description of the latter. Suffice it here to mention, however, that crossveinless was found located over at the purple end.

Jagged (Ja)

Jagged wing is a dominant mutation which was recently found by the author. Usually, only the inner margin of the wing becomes notched. In extreme cases, the whole wing may become strap-like and bear notches on all margins. Three such flies, two females and one male, appeared in one of these cultures (No. 4 of Table 7) of the cross between $\frac{\text{bi}}{\text{Sm}}\ \frac{\text{gl}}{\text{Ba}}$ ♀♀ and bi gl ♂♂ . A single Bare Jagged female and a single bithorax glass Smoky Jagged male were obtained in December, 1936. Each was immediately mated to wild type in separate vials, but both matings failed to produce any offspring. Fortunately, a single Smoky Bare Jagged female obtained on December 28 was successfully crossed to 3 wild type

males. The fly counts of the cross is shown in table 15. The fact that

Table 15. Nature of the cross: $\frac{+ \quad + \quad Ja}{Sm \quad Ba \quad +}$ ♀♀ × + ♂♂

Jagged								non-Jagged							
♀				♂				♀				♂			
Sm-Ba	Sm	Ba	+	Sm-Ba	Sm	Ba	+	Sm-Ba	Sm	Ba	+	Sm-Ba	Sm	Ba	+
11	2	6	6	4	1	7	15	5	3	4	16	10	5	9	11
25				27				28				35			
52								63							

approximately half of the offspring were Jagged, and that they were equally distributed among the two sexes indicates that the mutant is an autosomal dominant. Table 15 further suggests that the Jagged parent was heterozygous in constitution. Since Jagged flies occurred in F_2 equally frequently in combination with the two non-crossover classes, Jagged seems not to be linked with the Smoky Bare group. This was soon confirmed by several subsequent crosses between $\frac{Sm \quad Ba \quad Ja}{bi \quad gl}$ ♂♂ and bi gl ♀♀ as shown in table 16.

Table 16. Nature of the cross: $\frac{Sm \quad Ba \quad Ja}{bi \quad gl \quad +}$ ♂♂ × bi gl ♀♀

Culture No.	Sm-Ba-Ja	bi-gl	bi-gl-Ja	Sm-Ba
1	7	10	5	8
2	5	2	1	3
3	20	6	8	18

To test for the viability of the flies homozygous for Jagged wing, Jagged flies were crossed to wild type, and in F_1 flies heterozygous for Jagged were selected and intercrossed. There appeared in F_2 131 Jagged flies against 99 wild type. The result can be explained,

as shown in table 17, on the basis of 2:1 ratio, which indicates that

Table 17. Nature of the cross: $\frac{Ja}{+}\varphi\varphi \times \frac{Ja}{+}\sigma\sigma$

	Jagged	wild
Observed	131	99
Expected on the basis of 3:1 ratio	172.3	59.7
Expected on the basis of 2:1 ratio	153	77

homozygous Jagged flies do not exist. Indeed, when Jagged flies were inbred even for several generations, they never bred true.

As has already been shown in table 16, Jagged is not linked in the Smoky Bare group. Jagged flies were then mated to or pr cv and in j tg^3, representatives of the third and the fourth linkage groups respectively. In each case, Ja flies were selected in F_1 and reciprocally backcrossed to their respective multiple recessives. The result of backcross to multiple recessive females would immediately show whether or not Ja is linked with the particular group; if positive, the counts of the backcross to the recessive males would localize the new gene in relation to other genes in the same group. Tables 18 and 19 show the fly counts of $\frac{Ja}{+}\ \frac{+}{in\ j\ tg^3}\sigma\sigma \times$ in j $tg^3\varphi\varphi$ and $\frac{Ja}{or\ pr\ cv}\sigma\sigma \times$ or pr cv$\varphi\varphi$ respectively.

Table 18. Nature of the cross: $\frac{Ja}{+}\ \frac{+}{in\ j\ tg^3}\sigma\sigma \times$ in j $tg^3\varphi\varphi$

Culture No.	Ja	in-j-tg^3	Ja-in-j-tg^3	+
1	17	11	15	21
2	19	12	14	13

Table 19. Nature of the cross: $\frac{Ja}{or \quad pr \; cv}$ ♂♂ × or pr cv♀♀

Culture No.	Ja	or-pr-cv
1	68	26
2	61	42

A comparison of the results in two cases clearly indicates that Jagged is a member of the or pr cv group. Full counts in the reciprocal cross, $\frac{Ja}{cv \quad pr \; cv}$♀♀ × or pr cv, was then made. The results are summarized in table 20. In this four point cross, the two classes, crossveinless and or Ja pr, which include only the smallest number of flies, are evidently those which represent the two complementary classes of the triple crossovers, an assumption which places Jagged between purple and orange. The frequencies of crossing over between successive gene loci were then calculated. They were 13.2% between or and Ja, 23.8% between Ja and pr, and 18.0% between pr and cv. The sequence of the four genes is therefore or-Ja-pr-cv, in which Ja is nearer to or than to pr. Since Scute is equidistant between orange and purple, Ja can be inferred from the present data to lie between orange and Scute. This was confirmed by another four point cross between $\frac{or \quad Sc \; pr}{Ja}$♀♀ and or pr ♂♂, the result of which is given in table 21. From this experiment, crossing over values were found to be 15.5% between or and Ja, 6.8% between Ja and Sc, and 20.5% between Sc and pr. Jagged may, therefore, be safely put in between Sc and or. It is, however, nearer to Sc than to or.

abrupt (ab)

Abrupt longitudinal vein is a new recessive mutant, which was discovered by the author. It produces a shortening of the fourth longitudinal vein. In extreme cases, the vein may abruptly stop at the

Table 20. Nature of the cross: $\frac{or}{Ja}\ \frac{pr\ cv}{}$ ♀♀ × or pr cv♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers								triple crossovers		total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3				1,2,3,		
	or-pr-cv	Ja	or-Ja	pr-cv	or	Ja-pr-cv	or-pr	Ja-cv	+	or-Ja-pr-cv	or-Ja-cv	pr	or-cv	Ja-pr			or-Ja-pr	cv	
1	74	133	15	14	44	33	26	21	5	4	1	8	3	4			0	2	367
2	25	22	4	7	11	14	12	10	4	1	0	4	3	1			0	0	118
3	103	138	21	18	43	38	30	28	3	2	3	5	2	2			1	0	437
	202	373	40	39	98	85	68	59	12	7	4	17	8	7			1	2	922

Table 21. Nature of the cross: $\frac{or\ Sc\ pr}{Ja}$ ♀♀ × or pr ♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers						triple crossovers			total
			reg.1		reg.2		reg.3		1,2		1,3		2,3		1,2,3			
	or-Sc-pr	Ja	or-Ja	Sc-pr	or	Ja-Sc-pr	or-Sc	Ja-pr	or-Ja-Sc-pr	+	or-Ja-pr	Sc	or-pr	Ja-Sc	pr	or-Ja-Sc		
1	94	135	23	19	8	8	30	22	1	0	3	2	2	3	0	5		355
2	58	70	14	17	2	7	24	20	0	1	2	3	1	1	0	0		220
	152	205	37	36	10	15	54	42	1	1	5	5	3	4	0	5		575

place just below the posterior crossvein. The mutant flies can not be distinguished from either shortened, a sex-linked gene causing the shortening of the third and fourth longitudinal vein, or short-4 which was described by Crew and Lamy to produce the shortening of the fourth and fifth longitudinal veins. In order to avoid the confusion of terminology, separate names are now proposed to designate different genes for more or less similar characters. Short (sh) remains to denote the sex-linked one. The fourth chromosome one, originally known as sh_4, which will be described later, is now given the name incomplete (in). The name, abrupt (ab), applies to the one in the third chromosome, which is now under discussion.

The origin of abrupt can be traced back to the or pr stock. In three cultures of $\frac{na}{or\ Sc\ pr}$ ♀♀ × or pr ♂♂, there appeared several males whose wings were indented at their inner margin. These were found to be allelomorphs of beaded originally described by Lancefield (1922). They are known as bd^2. From a cross between or bd^2 and or sibs, three or pr females appeared to have their fourth longitudinal veins slightly shortened. By mating them to their wild type ribs, a good many abrupt orange purple flies of both sex were obtained. Since all abrupt flies had orange purple eye color, the mutant must have originated in or pr stock. When or ab pr flies were mater to incomplete (in), all F_1 individuals were wild type, indicating that abrupt and incomplete are not allels. An or ab pr stock was soon established. At the beginning some abrupt flies appeared to overlap normals. But after several generations of selection and inbreeding, the character became more pronounced and at same time bred true.

To start out genetic analysis of this new mutant, or ab pr flies were mated to Bare Curly flies. In F_1, Ba Cy flies were selected and reciprocally backcrossed to or ab pr flies. Table 22 gives the result of a cross between $\frac{\text{or ab pr}}{+}\ \frac{+}{\text{Ba}}\ \frac{+}{\text{Cy}}$ ♂♂ and or ab pr ♀♀.

Table 22. Nature of the cross: $\frac{\text{or ab pr}}{+}\ \frac{+}{\text{Ba}}\ \frac{+}{\text{Cy}}$♂♂ × or ab pr♀♀

or ab pr				non or ab pr			
Ba Cy	Ba	Cy	+	Ba Cy	Ba	Cy	+
12	12	10	9	19	20	20	22

Since abrupt, as shown in the table, gives random recombination with both Bare and Curly, and since all abrupt flies are orange purple in eye color, it may be considered established that abrupt is linked with the or pr group. The results of the reciprocal cross are summarized in table 23. Three cultures gave a total of 736 flies, out of which crossing

Table 23. Nature of the cross: $\frac{\text{or ab pr}}{+}$♀♀ × or ab pr♂♂

	Non-crossover		single crossovers				double c.o.		
			Reg. 1		Reg. 2		1, 2		
Culture No.	or ab pr	+	or	ab pr	or ab	pr	or pr	ab	total
1	39	79	10	1	60	42	4	1	236
2	49	109	14	4	71	33	6	2	288
3	58	63	5	4	19	51	8	4	212
	146	251	29	9	94	182	18	7	736

over between orange and abrupt is 8.6% that between abrupt and purple is 41.0%, and that between orange and purple is 42.4%. It shows that gene abrupt is located near to orange. The logical sequence of the three genes appeared to be or-ab-pr. Because orange is so far away from purple

as to make the difference of the crossing over percentages between or/pr and ab/pr insignificantly small, the result could hardly be considered conclusive to ascertain which side of orange does abrupt lie, unless another intervening gene between orange and purple is available for test. For this purpose, orange abrupt purple flies were mated to Jagged, and F_1 Jagged females were backcrossed to or ab pr males. Table 24 shows the result of counts in three separate cultures. The crossing over values are calculated to be as follows: 10.8% between or and ab, 12.3% between ab and Ja, and 23.2% between Ja and pr. The result supports the conclusion drawn from table 23 that the gene abrupt is located between Jagged and orange.

One may notice from tables 22 to 24 that abrupt flies occur in much smaller number from the non-abrupt ones. This is most probably due to the low viability caused by the presence of the gene abrupt. Since this effect was observed in all classes involving abrupt, the possibility that it may affect the accuracy of crossing over percentage computation may not be very serious. Hence, this is neglected in the calculations. Indeed, if one calculates the percentage of crossing overs in those cases on the basis of abrupt flies alone, one would find the resulting values not too much different from those calculated otherwise.

Blade (Bl)

Blade wing is a dominant mutation only recently discovered by Prof. Sturtevant. The wing assumes a blade-like shape. It is easily classifiable and has normal viability and productivity. Prof. Sturtevant crossed Blade Scute /or females to or males, and found Blade giving 7.7%

Table 24 Nature of the cross: $\frac{\text{or ab}}{\text{Ja}}\ \frac{\text{pr}}{\text{pr}}$ ♀♀ × or ab pr ♂♂

cult. No.	Non-crossovers		single crossovers reg. 1		single crossovers reg. 2		single crossovers reg. 3		double crossovers 1,2		double crossovers 1,3		double crossovers 2,3		triple crossovers 1,2,3		total
	or-ab-pr	Ja	ab-pr	or-Ja	or-ab-Ja	pr	or-ab	Ja-pr	ab-Ja	or-pr	ab	or-Ja-pr	or-ab-Ja-pr	+	ab-Ja-pr	or	
1	49	95	4	15	7	17	12	29	1	1	1	1	2	3	0	1	238
2	48	103	4	16	8	13	8	33	2	1	2	2	0	0	0	1	241
3	49	88	6	12	9	14	25	46	1	6	1	1	0	3	1	0	262
	146	286	14	43	24	44	45	108	4	8	4	4	2	6	1	2	741

of crossing over with orange and 17.7% with Scute. With his permission the result of this cross is tabulated in table 25.

Table 25. Nature of the cross: $\frac{Bl\ Sc}{or}$ ♀♀ × or ♂♂

Culture No.	Non-crossovers		single crossovers				double c.o.		
	Bl-Sc	or	Reg. 1		Reg. 2		1,2		
			+	or-Bl-Sc	Bl	or-Sc	Sc	or-Bl	tot.
1	160	175	19	13	34	39	1	1	442

narrow (na) and plexus (px)

Narrow (na) and plexus (px) were discovered by Miss Groscurth in two separate strains of race A, whose third chromosome differs from the standard sequence by an inverted section. This inversion has been cytologically demonstrated and will be discussed later. Both narrow and plexus are recessive and affect wing characters. In the case of narrow, the shape of wing is visibly narrower than normal and the tip of the wing appears somewhat pointed. Plexus is characterized by remarkable entanglement of longitudinal veins on the wings, especially between the second and the third, and between the fourth and the fifth. Both mutants can be easily distinguished from the wild type.

The association of narrow and plexus with an inversion was first found by Prof. Sturtevant. This was soon confirmed by the author's data given in tables 26 and 27. In the cross between $\frac{na}{or\ Sc\ pr}$ ♀♀

Table 26. Nature of the cross: $\frac{na}{or\ Sc\ pr}$ ♀♀ × or pr ♂♂

Culture No.	Non-crossovers		single crossovers				double c.o.		
			Reg. 1		Reg. 2		1,2		
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	or-pr	Sc	total
1	107	98	6	4	0	0	0	0	215
2	121	97	7	3	0	0	3	0	231
3	107	113	3	4	0	0	1	0	228
	335	308	16	11	0	0	4	0	674

Table 27. Nature of the cross: $\frac{px}{or\ Sc\ Pr}$ ♀♀ × or pr ♂♂

Culture No.	Non-crossovers		single crossovers Reg.1		single crossovers Reg.2		double c.o. 1,2		total
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	or-pr	Sc	
1	167	122	14	7	0	0	2	1	313
2	102	82	2	1	0	0	2	0	189
	269	204	16	8	0	0	4	1	502

and or pr ♂♂, crossing over between or and Sc was found to be 5.78% and that between Sc and pr only 1.0%. In the cross between $\frac{px}{or\ Sc\ pr}$ ♀♀ and or pr ♂♂, crossing over between or and Scute was found to be 4.6% and that between Sc and pr 1.0%. Since the normal frequency of crossing over between or and Sc and that between Sc and pr was about 25% each, it is apparent in the present two cases that the crossing over in the sequence or-Sc-pr has been greatly reduced, especially in the second region. The fact that the degree of reduction of crossing over is approximately equal in the two cases indicates that the inverted sections carried in the two strains are probably the same. To test the validity of the above statement orange plexus was crossed to narrow, and orange narrow was crossed to plexus. The results of the backcrosses are shown in tables 28 and 29.

Table 28. Nature of the cross: $\frac{or\ na}{px}$ ♀♀ × or na ♂♂

Culture No.	Non-crossovers na-or	Non-crossovers +	crossovers na	crossovers or	total
1	77	51	20	26	174
2	93	79	28	27	227
	170	130	48	53	401

Table 29. Nature of the cross: $\frac{or\ px}{na}$ ♀♀ × or px ♂♂

Culture No.	Non-crossovers px-or	Non-crossovers +	crossovers px	crossovers or	total
1	139	124	32	42	337
2	150	143	51	51	395
	289	267	83	93	732

It seems evident from comparing the data given above and crossing over frequencies deduced from that (25.2% between orange and narrow and 24.0% between orange and plexus) that the strain from which narrow mutation was originated carried the same inverted section in the third chromosome as the one which gave rise to the plexus mutant. This was later checked to be true from the study of salivary gland chromosomes of the hybrids between na and px. Moreover, comparison of crossing over values further suggests that the loci narrow and plexus probably lie very near to each other.

Crossing over frequencies between or and na and that between or and px were once more tested against the standard strain in a different kind of cross. Orange narrow and orange plexus were separately crossed to wild type flies which represent the standard sequence, and the F_1 females were backcrossed to multiple recessives. The results of counts are shown in tables 30 and 31.

Table 30. Nature of the cross: $\frac{or\ na}{+}$ ♀♀ × or na ♂♂

Culture No.	Non-crossovers or-na	Non-crossovers +	crossovers na	crossovers or	total
1	124	185	5	8	322
2	134	146	4	8	292
	258	331	9	16	614

Table 31. Nature of the cross: $\frac{or\ px}{+}$ ♀♀ × or px ♂♂

Culture No.	Non-crossovers or-px	Non-crossovers +	crossovers px	crossovers or	total
1	155	164	9	18	346
2	131	146	8	10	295
	286	310	17	28	641

Calculated from the data given above, crossing over has been reduced from 25.2% to 4.07% between orange and narrow, and from 24.0% to 7.01% between orange and plexus.

Attempts were made to transfer genes narrow and plexus to the standard sequence. They have so far failed, due to the close association of these genes with one of the ends of the inverted region. Since direct linkage tests of these loci in the standard sequence are impossible, they would not be considered in the construction of the standard genetic map of this chromosome. However, an approximate location of these genes will be discussed later in connection with cytological map.

The Fourth Chromosome

Curly (Cy)

Curly, a dominant gene, was discovered by Miss Beers (D.I.S. No. 1 p. 41) in race B of Drosophila pseudoobscura. The wings may be curled either upward or downward. Flies homozygous for Curly are always inviable. In heterozygous condition, the mutant fly is fully viable and fertile. Curly can be easily distinguished from normal. The gene has been successfully transferred from race B to race A. Since this was the first gene known in this chromosome, it has been used very extensively for tests of various kinds.

jaunty (j)

Jaunty (j) was reported by Crew and Lamy (1935). It is recessive, and the mutant flies show slight upturn of the tip of the wing. Occasionally, it overlaps normal. It was found by Crew and Lamy (1935) to give 9.8% of recombination with another fourth chromosome known as incomplete, which will be described in the next section. When jaunty was introduced into this laboratory, it was combined with incomplete. Consequently, the genetic analysis of the two genes went together. The author would, therefore, prefer to discuss them together after the description of the gene incomplete.

incomplete (in)

Incomplete, another fourth chromosome recessive gene found by Crew and Lamy (1935), was originally described as short-4. To avoid confusion of the name short-4 with the sex-linked short, the author prefers to use the name incomplete to replace short-4.

According to Crew and Lamy (1935) incomplete and jaunty belong to the same linkage group, which is independent of or-Sc-pr group, representative of third chromosome and also of Stubby-glass group, representative of the second chromosome. Since glass has been found located in the second chromosome, in j must either belong to the Curly group, i.e. the fourth chromosome, or the small dot-like fifth chromosome. In view of the fact that in and j gave about 10% of crossing over according to Crew and Lamy (1935), it seemed very likely that they are located in the fifth chromosome. However, a direct test was not practicable on account of the fact that Curly and jaunty interfere with each other in

combinations. Consequently, incomplete jaunty flies were mated to wild type, and in F_2 generations a few flies of each incomplete and jaunty were obtained, and from these separate stocks of incomplete and jaunty were raised. It took several generations to obtain pure cultures of each. When these flies were available, linkage test of incomplete and Curly was made. The fact that the mating $\frac{in}{cy}$ ♂♂ × ♀♀ gave only two classes of flies, Curly and incomplete, but no recombinations, directly proved that incomplete and jaunty do belong to the Curly linkage group. The result of pair matings in the reciprocal crosses is summarized in table 32.

Table 32. Nature of the cross: $\frac{in}{Cy}$ ♀ × in ♂

Culture No.	Non-crossovers		crossovers		total
	in	Cy	in	+	
1	38	33	35	32	138
2	30	45	38	41	154
3	36	50	46	44	176
	104	128	119	117	468

The reason why single pair mating system was adopted here is to eliminate possible admixture of jaunty flies with Curly. Incomplete flies were derived from the incomplete jaunty stock. Some of them might still be heterozygous for jaunty, even though the stock had been selected for several generations. If many incomplete flies had been used in back-crosses, it could have been possible that some flies turned out in the progeny might be homozygous jaunty which would overlap the expression of Curly. If a single pair mating is used, any admixture of jaunty flies to Curly flies would lead to a widely different numerical result from

the situation when no such admixture is present. Hence, a discrimination of different single pair matings could be made, and as a result, the crossing over percentage would be more accurate. Among six single pair matings, fly counts of three cultures were made. Since their results agree fairly well with each other, the crossing over frequency arrived at from a total of 468 flies may be considered established. It was found to be 50.04%, which indicates that the loci for genes incomplete and Curly are very far apart from each other.

It has been pointed out above that the frequency of crossing over between jaunty and incomplete was reported by Crew and Lamy (1935) to be 9.8%. However, basing upon a single culture of mating $\frac{in\ j}{+}$ ♀♀ × in j ♂♂, which gave 461 flies, (table 33), crossing over frequency between

Table 33. Nature of the cross: $\frac{in\ j}{+}$ ♀♀ × in j ♂♂

Non-crossovers		crossovers		total
in j	+	in	j	
177	199	26	59	461

incomplete and jaunty is calculated to be 18.44%, a value much too high in comparison with 9.8%. Nevertheless, other crosses gave similar values like the former as will be shown later.

So far the linkage relations between incomplete and Curly and between incomplete and jaunty have indicated that Curly is located very far from incomplete, and jaunty, which was originally suspected to be a recessive allellomorph of Curly, is about 10 to 18 units away from incomplete. Since Curly and jaunty interfere with each other, a three point cross could not be carried out to determine the sequence of these three genes. However, an experiment to find out the relative distance

between Curly and jaunty was performed by mating Curly to jaunty and back-crossing F_1 Curly females to jaunty males. The result of fly counts from three separate cultures is represented in table 34. Among 1121 flies,

Table 34. Nature of the cross: $\frac{Cy}{j}$ ♀♀ × j ♂♂

Culture No.	Non-crossovers Cy	Non-crossovers j	crossovers Cy j	crossovers +	total
1		284		79	363
2		296		74	370
3		306		82	388
		886		235	1121

235 were of wild type representing one crossover class. All other 886 flies exhibited different degrees of curled wing. Though it was possible to distinguish some Curly jaunty flies, which are characterized by extreme curliness, it was safer to calculate the percentage of crossing over by doubling the wild type flies, for some jaunty Curly flies were undoubtedly classed among the extreme Curly ones. The frequency of crossing over was found to be 41.9%. By comparing this value with 50.04% of crossing over between Curly and incomplete it is reasonable to assume that jaunty is located between incomplete and Curly, though it is much nearer to the former than to the latter.

Multiple allellomorphs of tangled (tg)

The first mutant of tangled, tangled^1 (tg^1) was discovered by Crew and Lamy, who described it as fused (fu) in D. I. S. No. 1, p. 40. Later, they (D. I. S. No. 3, p. 45) changed the name to tangled. It is recessive, and in the mutant flies the second and third longitudinal veins

of both wings come together at their distal ends, often with extra crossveins. The same also occurs with fourth and fifth longitudinal veins. In extreme cases, the wing is tilted up.

Soon after $\underline{tg}^1$ had been introduced into this laboratory, several allels of the same locus were found in separate stocks. Tangled^2 (tg^2) and tangled^3 (tg^3) were found by the author and tangled^4 (tg^4) was discovered by Prof. Sturtevant.

A single tangled^2 male, similar to tangled^1, except having fewer extra veins, was found in the Bare orange Curly stock. It was mated to wild type females, and in F_2 several $\underline{Ba}$ $\underline{or}$ $\underline{Cy}$ $\underline{tg}^2$ and $\underline{or}$ $\underline{tg}^2$ flies were obtained. From these flies, separate stocks of $\underline{Ba}$ $\underline{or}$ $\underline{Cy}$ $\underline{tg}^2$ and $\underline{or}$ $\underline{tg}^2$ were raised.

A single $\underline{tg}^3$ male fly obtained from Curly stock had only one extra crossvein connecting the distal ends of the second and third longitudinal veins. It was mated to wild type females, giving in F_1 all wild type flies and in F_2 8 $\underline{tg}^3$ (6 ♀, 2♂) and 435 wild type ones. The great excess of wild type flies over the mutant type may be accounted for by presence of some modifier in the wild type parent, for after several generations of selection $\underline{tg}^3$ do breed true.

After a stock of tangled^3 had been established, the following reciprocal matings were carried out: $tg^3 \times px$, $tg^3 \times tg^1$, $tg^3 \times tg^2$. The fact that mating with plexus gave only wild type flies and mating with either $\underline{tg}^1$ or $\underline{tg}^2$ gave exclusively tangled flies of different degrees of expression of the character, indicates that the slight tangled (tg^3) is a third allel found in this locus.

A fourth allel of tangled (tg^4) was obtained by Dr. Sturtevant in echinus stock. The mutant flies resemble very much either tg^1 or tg^2. When tg^4 flies were mated to px, all F_1 were normal. But when they were crossed to either of the other tangled allels, all offspring were tangled, showing that tg^4 is another allel in the locus of tangled.

Among the four allels of tangled, tangled^3 (tg^3) was most favorable for experimental purposes, because tg^3, having only one clear cut extra crossvein instead of many characteristic of tg^1, tg^2 and tg^4, can be easily distinguished in combinations with other wing mutants. Moreover, tangled^3 wings, unlike other allels of the same locus, never become tilted at the tip. Hence, for genetic analysis of the locus, tangled^3 flies have been almost exclusively used.

According to Crew and Lamy (1935) tangled^1 does not belong to either the or-Sc-pr linkage group or the Stubby-glass group. On the basis of probable independence of tg^1 from incomplete and jaunty, they proposed that it might belong to the fifth group, that is the fifth chromosome. In view of the difficulties of classification encountered in combinations of tg^1 with incomplete and jaunty, it would seem to the author that Crew and Lamy's suggestion as to independence of tangled from incomplete and jaunty is not justified. Curly tangled^3 flies were mated to wild type, and in F_1 Curly females and males were selected and reciprocally backcrossed to tangled^3 individuals. The mating of $\frac{tg^3\ Cy}{+}$ ♂♂ × tg^3 ♀♀ gave 68 tg^3 Cy and 62 wild type flies. Since no flies of recombination classes were found, tangled^3 is located in the same chromosome to which Curly belongs. Counts of a single culture of the reciprocal cross are presented in table 35.

Table 35. Nature of the cross: $\frac{tg^3\ Cy}{+}$ ♀♀ × tg^3 ♂♂

Non-crossovers		crossovers		total
tg^3 Cy	+	tg^3	Cy	
288	261	54	30	633

Among 633 flies, there were 84 crossovers amounting to 13.3%.

In order to determine on which side of Curly tangled lies, it was necessary to plan a three point cross. Since all four genes so far described in the fourth chromosome affect the wing character, it is difficult to combine them together without damaging the wing or causing difficulties in classification. The only possibility was to try the combination incomplete, jaunty and tangled^3. It took several generations to establish that stock, which at first had some flies whose wings could not be classified. This difficulty was finally overcome by selection and inbreeding for several generations. Incomplete jaunty tangled^3 flies were then mated to wild type. Lest the interactions of genes would still obscure the classification of different combinations of flies, a parallel two point cross of jaunty tangled^3 was also carried out. The results of the matings $\frac{+}{j\ tg^3}$ ♀♀ × j tg^3♂♂ and $\frac{in\ j\ tg^3}{+}$ ♀♀ × in j tg^3♂♂ are summarized in tables 36 and 37 respectively. Calculated from the data given in table 36,

Table 36. Nature of the cross: $\frac{+}{j\ tg^3}$ ♀♀ × j tg^3 ♂♂

Culture No.	Non-crossovers +	j-tg^3	crossovers j	tg^3	total
1	75	78	57	50	260
2	74	79	55	53	261
	149	157	112	103	521

Table 37. Nature of the cross: $\frac{in\ j\ tg^3}{+}$ ♀♀ × in j tg^3 ♂♂

Culture No.	Non-crossovers		single crossovers Reg.1		single crossovers Reg.2		double c.o. 1,2		total
	in-j-tg^3	+	in	j-tg^3	in-j	tg^3	in-tg^3	j	
1	50	97	39	13	55	30	1	5	290
2	40	75	20	8	49	40	4	8	244
	90	172	59	21	104	70	5	13	534

j and tg^3 show 41.2% of crossing over. The result given in table 37, showing in tg^3 and j as double crossovers, definitely establishes the fact that jaunty is located between incomplete and tangled^3. The calculated linkage value here is 18.3% between incomplete and jaunty and 36.0% between jaunty and tangled^3. The occurrence of 18.3% of crossing over between in and j is in perfect agreement with 18.4%, which was found on the basis of the data given previously in table 33.

Rough (Ro)

Rough is a dominant eye mutation found by Miss Groscurth in race A. Preliminary unpublished report showed that it is located very near to Curly. Not infrequently, Ro overlaps normal. Rough was mated to tg^3 Cy. In F_1 generation, Ro Cy females were selected and backcrossed to tg^3 males. The result of counts is shown in table 38. The absence of

Table 38. Nature of the cross: $\frac{Ro}{tg^3\ Cy}$ ♀♀ × tg^3♂♂

Culture No.	Non-crossover		single crossovers reg.1		single crossovers reg.2		double c.o. 1,2		tot.
	Ro	tg^3-Cy	tg^3-Ro	Cy	tg^3-Cy-Ro	+	Ro-Cy	tg^3	
1	75	93	6	8	1	3	0	0	186
2	82	67	5	11	1	2	0	0	168
	157	160	11	19	2	5	0	0	354

Ro Cy and tg^3 flies indicates that Ro is located to the right of Curly. Linkage value between tg^3 and Cy is found to be 9.5%, and that between Cy and Ro 2.0%.

Construction of the genetic maps

The previous section has presented the evidences upon which the sequence of mutant genes was based and has indicated the method by which the various regions were oriented with respect to the others.

All experiments designed to test the linkage relations between genes in different linkage groups of the standard strain are now summarized in tables 39, 40, and 41 for the second chromosome, tables 42, 43, and 44 for the third chromosome, and tables 45 and 46 for the fourth chromosome. In the first column from the left in each table, reference is made to particular number of table for the detail data of individual type of cross. Under the column headed "type of cross", types of two parents involved in the cross are shown, the one on the left being female and the other on the right male. The column headed "loci" shows not only the loci concerned, but also their sequence. In the fourth and following column classes entered under their headings indicating the type of crossing over they represent. The results are, of course, obtained from the backcrosses of the F_1 hybrids to multiple recessives.

Tables 47, 48 and 49 show the total data for each pair of loci in the second, third and fourth chromosome respectively. The resulting maps, shown in figure 42, together with cytological maps, are constructed by adding the linkage values of successive regions in each chromosome,

taking 1% of crossover as 1 unit of map distance. The complete length of each chromosome, therefore, represents the sum of the distances between successive genes. Since it is highly probable that the genes represented as lying at the two extremes in each map are not located at the very extreme points of the chromosome, and since there are several long gaps in each chromosome whose extents are based on direct crossover experiments on account of the lack of intermediate loci to provide for detectable double crossovers, the actual distances on each map are possibly, and even probably, longer than represented now.

In determining the map distances, the nearest known gene to the spindle fiber attachment in each chromosome is taken as zero point or ~~right~~ the left end. The evidences leading to the determination of genes in relation to spindle fiber end will be presented later. Suffice it to mention here, however, the nearest known gene locus to the spindle fiber is Smoky (Sm) in the second chromosome, orange (or) in the third chromosome, and incomplete (in) in the fourth chromosome. Hence, loci Smoky, orange and incomplete are taken as zero points.

Tables 47, 48 and 49 are built on the basis of the data furnished in tables 39 to 46. A glance over the tables which show the relative frequency of crossover between individual pair of loci reveals the logical sequence of the genes. There are, however, a few places, which show a discordant result. In table 47, the recorded percentage of crossover between Smoky and bithorax is slightly higher than that between Smoky and pink or between Smoky and Bare. This discrepancy, as has already been pointed out previously, may be attributed to the long distance between

Smoky and bithorax, or to the differential viability of the various cross-over classes in the four point cross (table 4), on which alone the linkage value between Smoky and bithorax was based. Probably the distance between Smoky and bithorax should be shorter than 43.6%. But when the coincidence is taken into consideration in this long gap, the assignment of 43.6% units for the map distance between Smoky and bithorax may not be considered overestimated.

Comparison of direct crossover values between crossveinless and Scute, 45.6%, crossveinless and Jagged, 38.0%, and crossveinless and orange, 42.5% reveals another discordant result. The occurrence of 45.6% as the direct crossover value between crossveinless and Scute is apparently too high. This was, however, based on a single type of cross.

As shown in table 49 the crossover value between tangled^3 and Curly, 11.5%, is slightly higher than that between tangled^3 and Rough, 10.5%. Since Rough is located very near to Curly and since the crossover frequency between tangled^3 and Rough was based on single type of cross, the above discrepancy may not be considered too serious.

The known lengths of the genetic maps is 92.9 units for the second chromosome, 68.4 units for the third, and 69.2 units for the fourth. In view of the presence of a very long gap in the second and fourth chromosomes (43.6 units between Smoky and bithorax in the second chromosome and 38.6 units between jaunty and tangled^3 in the fourth) the actual genetic map length will probably be greater in the second and fourth chromosome than the third chromosome.

Table 39. Two point linkage experiment with the second chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non-crossovers		single crossovers		total
Table 6	bi Ba	bi × Ba	145	135	21	7	308

Table 40. Three point linkage experiments with the second chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non-crossovers		single crossovers reg. 1		single crossovers reg. 2		double c.o. 1,2		total
Table 71	Sm p ps	Sm-p-ps or × +	59	134	50	92	45	81	39	39	542
Table 1	Sm Ba gl	gl × Sm-Ba	90	164	52	137	27	36	20	20	546
Table 8	bi p Ba	bi-p or × Ba or	206	226	20	19	5	7	4	4	481
Table 10	bi p ps	bi-p-ps or × or	138	223	10	9	89	165	7	4	645
Table 11	bi gl ps	+ × bi-gl-ps	119	21	21	44	10	24	5	6	250

Table 41. Four point linkage experiment with the second chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non c.o.		single c.o. reg. 1		single c.o. reg. 2		single c.o. reg. 3		double c.o. 1,2		double c.o. 1,3		double c.o. 2,3		triple c.o. 1,2,3		total
Table 7	Sm bi Ba gl	bi-gl × Sm-Ba	186	210	100	199	17	20	78	45	13	28	28	60	1	7	1	7	1000

Table 42. Two point linkage experiment with the third chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non-crossovers		single crossovers		total
Table 14	Sc cv	Sc × cv	175	181	158	141	655

Table 43. Three point linkage experiment with the third chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non-crossovers		single crossovers reg. 1		single crossovers reg. 2		double c.o. 1,2		total
Table 25	or Bl Sc	Bl-Sc × or	160	175	19	13	34	39	1	1	442
Table 21	or ab pr	or-ab-pr× +	146	251	29	9	94	182	18	7	736
Table 69	or Sc pr	or-Sc-pr× +	45	74	19	17	17	23	5	5	206

Table 44. Four point linkage experiments with the third chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non co.		single c.o. reg.1		single c.o. reg.2		single c.o. reg.3		double c.o. 1,2		double c.o. 1,3		double c.o. 2,3		triple co. 1,2,3		total
Table 24	or ab Ja pr	or-ab-pr × Ja	146	286	14	43	24	44	45	108	4	8	4	4	2	6	1	2	741
Table 21	or Ja Sc pr	or-Sc-pr × Ja	152	205	37	36	10	15	54	42	1	1	5	5	3	4	0	5	575
Table 20	or Ja pr cv	or-pr-cv × Ja	202	273	40	39	98	85	68	59	12	7	4	17	8	7	1	2	922

Table 45. Two point linkage experiments with the fourth chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non-crossovers		crossovers		total
Table 33	in j	in-j × +	177	199	25	59	461
Table 81	in j	+ × na-in-j	354	341	73	54	822
Table 32	in Cy	in × Cy	104	128	119	117	468
Table 36	j tg^3	+ × j-tg^3	149	157	112	103	521
Table 34	j Cy	Cy × j		886		235	1121
Table 35	tg^3Cy	tg^3Cy × +	288	261	54	30	633

Table 46. Three point linkage experiments with the fourth chromosome of Drosophila pseudoobscura

Reference	loci	type of cross	non c.o.		single crossovers reg. 1		single crossovers reg. 2		double c.o. 1,2		total
Table 37	in j tg^3	in-j-tg^3 × +	90	172	59	21	104	70	5	13	534
Table 38	tg^3Cy Ro	Ro × tg^3Cy	157	160	11	19	2	5	0	0	354

Table 47. Total data for each pair of loci in the second chromosome of Drosophila pseudoobscura

Loci	total	crossovers	% of crossing over
Sm bi	1000	436	43.6%
Sm p	542	223	41.2%
Sm Ba	1546	661	42.8%
Sm gl	1546	719	46.5%
Sm ps	542	271	50.0%
bi p	1126	67	5.9%
bi Ba	1789	173	9.7%
bi gl	1250	365	29.2%
bi ps	895	372	41.6%
p Ba	481	20	4.2%
p ps	1187	469	39.6%
Ba gl	1546	330	21.2%
gl ps	250	45	18.0%

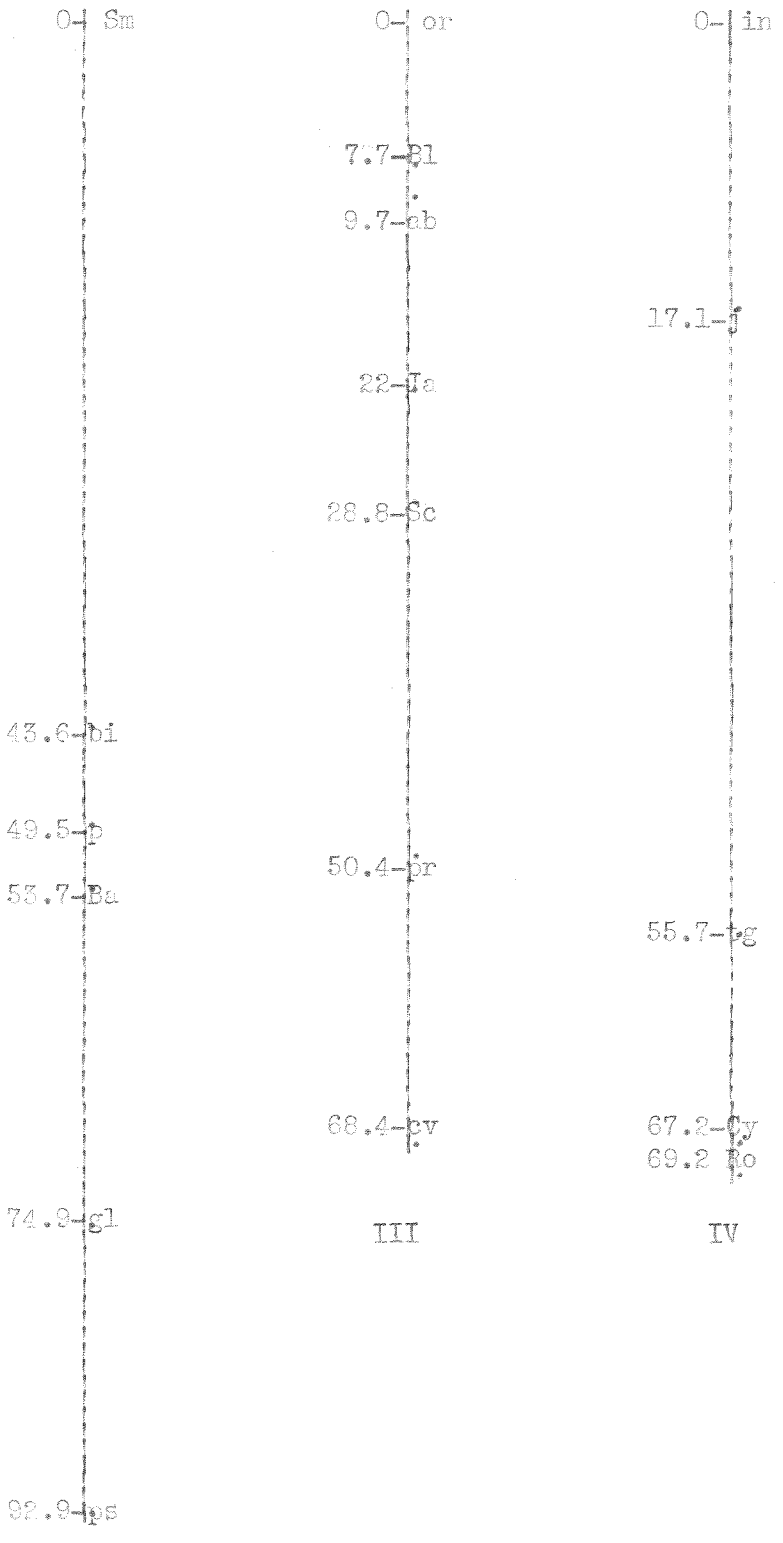
Table 48. Total data for each pair of loci in the third chromosome of Drosophila pseudoobscura

Loci	total	crossover	% of crossing over
or Bl	442	34	7.7%
or ab	1477	143	9.7%
or Ja	2235	353	15.8%
or Sc	1223	267	21.8%
or pr	3180	1168	36.7%
or cv	922	392	42.5%
Bl Sc	442	75	17.0%
ab Ja	741	91	12.3%
ab pr	1477	542	36.7%
Ja Sc	575	39	6.8%
Ja pr	1497	353	23.6%
Ja cv	922	350	38.0%
Sc pr	781	169	21.6%
Sc cv	655	299	45.6%
pr cv	922	166	18.0%

Table 49. Total data for each pair of loci in the fourth chromosome of Drosophila pseudoobscura

Loci	total	crossovers	% of crossing over
in j	1817	310	17.1%
in tg^3	534	254	47.6%
in Cy	468	236	50.1%
j tg^3	1055	407	38.6%
j Cy	1121	470	41.9%
tg^3 Cy	987	114	11.5%
tg^3 Ro	354	37	10.5%
Cy Ro	354	7	2.0%

Text figure.— Genetic maps of three autosomes of Drosophila pseudoobscura



CYTOLOGICAL MAPS

Introduction

Genetic maps of chromosomes are built on the basis of data concerning the frequency of recombination of linked genes. Cytological maps show the approximate location of genes within the microscopically visible chromosomes. The material for their construction is supplied by observations on sections of chromosomes transferred from one place to another in translocations, absent in deficiencies, or present as extra piece in duplications.

The cytologically visible alterations of the chromosomes produced by translocations were first discovered by Stern (1926, 1929) in X-Y translocations. Muller and Painter (1929), Painter and Muller (1929), Dobzhansky (1929a, 1930b) and many others in Drosophila melanogaster, McClintock (1930, 1931) and others on Zea mays, and Nabours and Robertson (1933) on Apotettix eurycephalus supplied further data. The points at which the chromosomes are broken and reattached in various chromosome rearrangements can be determined genetically by essentially the same methods which are used for the localization of genes. Since the location of breakages and reattachments may also be seen cytologically, their position can be determined with respect to the ends of the chromosomes, the spindle attachments and other characteristic regions. If the breakages lie genetically close to the loci of known genes, one may reasonably assume that the latter lie in the chromosomes in a more or less close proximity to the observed breakage points. The correlation between the genetic and cytological maps of a given chromosome can be in such a manner established.

Cytological maps constructed on the basis of studies on metaphase chromosomes were published in Drosophila melanogaster by Painter and Muller (Painter and Muller 1929, Painter 1931, Muller and Painter 1932) and by Dobzhansky (1929a b, 1930a, b, 1931a, 1932b, c, d).

Since Heitz and Bauer (1933) have shown that the enormously enlarged chromosomes present in the nuclei of dipteran salivary gland cells are normal chromosomes rich in constant detail, and since Painter (1933, 1934a, b) has demonstrated that the series of observable structures along the length of such chromosomes can be correlated with the series of gene loci on the linkage maps, it has become imperative to make use of this new method for constructing cytological maps. In Drosophila melanogaster, salivary chromosome maps have already been worked out by Painter (1934a, 1935) for the X-chromosome and the third chromosome.

The methods for constructing cytological maps on the basis of the salivary chromosomes are essentially the same as in the case of the metaphase chromosome maps, but in the former the location of breaks and reattachment of various chromosome rearrangements can be determined much more accurately. Moreover, very small deficiencies, duplications and inversions which could not be detected in metaphase chromosomes, can now be studied in the salivary gland chromosomes; and consequently, they constitute a more valuable source of materials than translocations for an accurate localization of genes in a chromosome. Machensen (1935) has located genes in the X-chromosome of Drosophila melanogaster by studying deficiencies almost entirely.

One of the essential requirements for constructing cytological maps is to obtain as many translocations as possible, to furnish breaks

properly distributed along the entire length of a chromosome. In Drosophila pseudoobscura no translocations have been so far reported. It is, therefore, necessary first of all to induce a number of translocations with the aid of X-ray.

Origin of Translocations

The first translocation discovered by Bridges (Bridges 1923, Morgan and Bridges 1923) in Drosophila melanogaster and certain translocations found since then (Stern 1926, 1929, Sturtevant and Dobzhansky 1930) in the same species, arose spontaneously. The induction of translocation by X-rays was discovered by Muller (1928a, b) and Muller and Altenburg (1928, 1930). Their results were soon corroborated by findings of Weinstein (1928), Serebrovsky and his collaborators (1928), and others. The discovery of Muller has made it practicable to attempt to get will translocations involving any combination of chromosomes.

The technique of finding induced translocations is based on the results of Bridges (1923) who has shown that translocations produce an apparent linkage between genes located in different chromosomes, which, therefore, are normally independent. The technique was described by Muller and Altenburg (1930) and by Dobzhansky (1929a, 1930a).

Since the basic object of the present investigation is to secure information concerning the cytological maps of all the autosomes in Drosophila pseudoobscura, the experiment was planned for obtaining translocations involving different autosomes and the Y-chromosome.

Wild type males were treated with X-rays. The age of the flies at the moment of the treatment was not more than two days after the emergence from the puparia. About fifty flies were placed in a small

cellulose capsule, and several capsules were tied together, and so exposed to X-rays that they were 15.5 cm from target of the tube. The tube was operated at 32 kilovolts and 9 milliamperes. Different batches of flies were rayed under above conditions for three different lengths of time, 32 min., 48 min., and 64 min., corresponding to 4000, 6000 and 8000 r-units respectively.

The treated males were crossed to untreated females having the second chromosome heterozygous for the dominant gene Bare, third chromosome homozygous for the recessive orange, and fourth chromosome heterozygous for the dominant Curly. 43 cultures were made, each consisting of 3 treated males and 5 treated females. In the F_1 generation Bare Curly males were selected and crossed individually to orange females.

Bare Curly males which appeared in the progeny of treated wild type males and untreated Ba or Cy females received an untreated x-chromosome, untreated second chromosome carrying Ba, untreated third chromosome carrying or, and an untreated fourth chromosome carrying Cy from their mother on one hand, and received treated y-chromosome, second, third and fourth chromosomes carrying normal allelomorphs of Ba, or and Cy respectively. When such males are crossed in single pair cultures to untreated females homozygous for or, the progeny normally consists of 16 classes of offspring representing all the combinations of four "marking" characters mentioned. These classes are:

Females: +, Ba, or, Cy, Ba or, Ba Cy, or Cy, Ba or Cy

Males: +, Ba, or, Cy, Ba or, Ba Cy, or Cy, Ba or Cy

However, if a parental wild type 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 such a male carries a translocation involving the treated second and third chromosome, no Ba, or, BaCy, or Cy individuals of either sex are expected in his progeny. Likewise, if such a male carries a translocation involving the second and y-chromosome, no Ba males and no non-Ba females are expected in his progeny. (Ba males and non-Ba females may occur, but either very few or abnormal, representing duplication and deficiency carrying classes).

Of 180 cultures, 30 cultures gave no offspring, presumably due to the sterility of the male parent, which carried one set of treated chromosomes. 128 cultures produced normal progenies, that is, in the overwhelming majority of these cultures all 16 expected classes of offspring were present in approximately equal numbers. The results obtained in the remaining twenty-two cultures are presented in table 50. These results are, indeed, so strikingly different from the results obtained in all the normal cultures, that no doubt is possible in classifying a given culture as belonging to the normal or the translocation class.

Judged from the missing classes of offspring, 22 translocations can be ascribed to several different combinations, four of them (40, 110, 179, 58) represent translocations involving Y- and II chromosome, two of them (129, 80) involved Y- and III chromosome, and five of them (6, 27, 77, 54, 127) Y- and IV chromosome. Cultures No. 19, 30 and 74 represent translocations involving II and II chromosomes. Cultures No. 10, 160 and 109 represent translocations involving III and IV chromosomes. In culture 82, twelve of the expected sixteen classes are missing, suggesting that a translocation involving II, III and IV chromosomes has arisen.

Table 50. Cultures showing linkage of genes located in different chromosomes

Culture No.	Translocation involving chromosomes	Strain	wild type ♀	wild type ♂	Ba ♀	Ba ♂	or ♀	or ♂	Cy ♀	Cy ♂	Ba or ♀	Ba or ♂	Ba Cy ♀	Ba Cy ♂	or Cy ♀	or Cy ♂	Ba or Cy ♀	Ba or Cy ♂
40	Y-II	A		17	17			16		15	20		15			16	12	
110	Y-II	B		15	15			14		15	13		11			15	13	
179	Y-II	C		13	14			12		12	15	1	16			14	12	
58	Y-II	D		19	15			15		14	17		16			15	14	
129	Y-III	A		16		15	15	4		18	17			13	14	2	16	
80	Y-III	B		14		12	17			14	14			11	14		14	
6	Y-IV	A		15		14		16	16			15	10	1	18		12	1
27	Y-IV	B		25		24		24	26	1		21	15	1	28		16	
77	Y-IV	C		16		15	1	16	20			19	14	1	17	2	13	1
54	Y-IV	D	1	21	2	24	3	19	20		1	25	25	1	19	1	18	1
127	Y-IV	E		18		13		17	19	2		11	18		22	1	14	
19	II-III	A	13	18		1			15	20	12	18				1	15	13
30	II-III	B	16	15			1		17	16	18	15					15	16
74	II-III	C	23	25					28	26	22	24			1	3	26	25
10	III-IV	A	13	12	19	12			1					2	11	15	13	16
160	III-IV	B	38	33	35	33	2			1				1	33	32	28	11
109	III-IV	C	24	21	19	17	3		1		1			1	20	17	14	15
79	Y-III-IV	A	5	15	17	17									13	1	12	5
38	Y-III-IV	B	2	17	2	21	1		1	1			1	2	15	4	17	3
82	II-III-IV	A	28	25			2								5	4	19	16
8	Y-II-III-IV	A		19	1												21	1
174	Y-II-III-IV-V	A	4	15		1			5	2	1	1			5	1	17	8

In culture No. 8, only wild type males and Ba or Cy females are present, indicating the presence of a translocation involving four chromosomes, Y-, II, III and IV. Cultures No. 79, 38 and 174, showing in each case more than eight classes either entirely absent or represented by few flies, were later proven to carry translocations involving Y-, III and IV chromosomes in the first two and involving Y-, II, III, IV and V chromosome in the last. As shown in table 50, there are present in most of these cultures a few flies of the unexpected classes. They may or may not be abnormal in appearance, either representing non-disjunctions or heteroploids. They could not be determined with certainty without a genetic and cytological analysis, which will be described below.

Each of the twenty-two 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 an appropriate breeding. These strains are designated by the characters of the alphabet as shown in table 50.

Although no particular attention was paid to the effect of different dosages of X-rays with which flies were treated on the productivity of translocations and fertility of tested individuals, a comparison of rather limited data, though statistically insignificant, shown in table 51 reveals, however, that the frequency of translocations seems to increase with the intensity of radiation. This is in agreement with the result by Oliver (1931, 1932) found in Drosophila melangaster.

Table 51: Comparison of different dosage of X-ray treatment and number of translocations

Radiation dosage in r-units	No. of F_1 individuals tested	No. of fertile cultures	No. of sterile cultures	No. of translocations
4000	13♂	11♂	20	13
6000	34	31	3	7
8000	14	7	7	2

Genetic and Cytological Analysis of Translocations

In analyzing the breakage loci in the translocations, genetic and cytological methods were used simultaneously. Since the salivary gland chromosomes in individuals heterozygous for translocations usually give a vivid presentation of the nature and extent of the chromosome rearrangements as manifested by geometrical configurations, it seemed in many cases desirable to make the cytological analysis in precedence of the genetic tests.

Translocations that involve two chromosomes

Y-chromosome translocations

Translocations involving Y-chromosome and one autosome constitute half of the total number of translocations obtained. Since under ordinary conditions crossing over is entirely absent in male flies, the determination of the location of the breakage points in translocations involving the Y by means of linkage tests is impracticable. However some of these translocations produce viable duplications or deficiencies, thus rendering it possible to deal with the problem by another method, described below.

Y-II translocations

As shown in table 50, four cases of translocations involving the Y- and the second chromosomes were obtained. Translocation males heterozygous for orange were mated to pink orange females. In the next generation the wild type translocation males were again selected and back-crossed to pink orange females. The result of independent crosses of the four translocations is summarized in table 52. In each case, the over-

Table 52. Ty-II $\frac{p\ or}{or}$ ♂♂ × p or ♀♀

	+		p or	
	♀	♂	♀	♂
Y-IIA	6	122	79	0
Y-IIB	5	122	116	0
Y-IIC	24	125	119	50
Y-IID	2	101	99	0

whelming majority of flies fall into two classes, orange males and pink orange females, representing heterozygous translocation and normals respectively. Presence of 50 p or males in Y-II C signifies the survival of flies heterozygous for a duplication. In each case of the four translocations there were a few wild type females, which may either represent non-disjunctions or deficiencies. Since in Y-II A, Y-II B, and Y-II D even duplication flies are not viable (as shown by the absence of $\frac{p\ or}{}$ males), it is more probable that wild females were non-disjunctions of XXY constitution.

With a view to find what recessive genes in the second chromosome are covered by the duplicating fragment in Y-II C, and at the same

time to test further possibility of obtaining viable duplications in other three translocations, two series of experiments were carried out. In one series orange translocation males heterozygous for genes p and ps were mated to p ps or females, and in the other series such males heterozygous for bi gl were mated to bi gl females. In an attempt to obtain viable duplications in cases of Y-II A, Y-II B, Y-II D, these cultures were raised in a cold room at 15° to 18° C. For each translocation complete ~~fly~~ counts in two cultures were made. The results of the two separate series of experiments are summarized in tables 53 and 54. It is obvious from the

Table 53. $\frac{\text{Y-II}}{\text{p ps or}}$ ♂♂ × p ps or ♀♀

	or ♀	or ♂	p ps or ♀	p ps or ♂	p or ♀	p or ♂
Y-II A	0	188	166	0	0	0
Y-II B	0	143	145	0	0	0
Y-II C	0	131	109	0	0	70
Y-II C	0	84	90	0	0	0

Table 54. $\frac{\text{Ty-II}}{\text{bi gl}}$ ♂♂ × bi gl ♀♀

	+ ♀	+ ♂	bi gl ♀	bi gl ♂
Y-II A	0	142	180	0
Y-II B	0	103	122	0
Y-II C	1	169	158	94
TY-II D	0	12	13	0

figures given in these tables that in both series of experiments duplication males are only produced in Y-II C translocation. The fact that the

cross $\frac{\text{Y-II C}}{\text{p ps}}$ $\frac{\text{or}}{\text{or}}$ ♀♀ and p ps or ♂♂ produced p or duplication flies indicates that the wild allel of the gene ps is suppressed by the duplicating fragment. On the other hand, in the cross $\frac{\text{Y-II C}}{\text{bi gl}}$ ♀♀ x bi gl ♂♂ duplication males were bi gl type, showing that wild allels of both bi and gl are not contained in the duplicating fragment. Hence, the breakage point on the second chromosome in Y-II C translocation lies between gl and ps, the two, as shown in the genetic map, being 18.0 units apart. The duplication males of Y-II C are slightly abnormal in appearance. When duplication p or males were mated to p ps or females, only one out of three such sultures produced 80 flies, composed of 40 or males, 21 p ps or females and 19 p or males. It seems probable that duplication males are only partially fertile.

A comparison of the figures given in tables 52, 53 and 54 reveals another interesting fact, that is in the crosses shown in tables 53 and 54 practically no non-disjunction females were present, but in the crosses shown in table 53, non-disjunction females occur rather frequently. These differences may possibly be due to the effect of the cold temperature which prevents non-disjunctions. The possibility that the production of non-disjunctions in the crosses shown in table 52, which were made only two generations after X-ray treatment, may be due to an after effect of the X-ray treatment, is also not excluded, though such an effect has not been reported in the genetic literature.

In the salivary gland chromosomes, breaks in the second chromosome in Y-II A, B and D have been observed to lie close to each other, but distinctly not at same point.

Fig. 1 represents the Y-II A translocation. It shows that a long piece of the second chromosome, including sections 47 to 62, is transferred to Y-chromosome, which, consisting of heterochromatin material, is embedded in chromocenter. The breakage point in the second chromosome seems to lie at the boundary between sections 48 and 49 (figs. 1 and 42). The translocated piece is always found paired with its homologue, probably because of its considerable length. There may, however, as shown in fig. 1, be small sections in which the pairing of homologous parts fails.

Figs. 2 and 3 illustrate the translocation Y-II B. These two figures were obtained from two cells of ~~two different~~ a single larvae, and both show the breakage point in the second chromosome in section 49. In fig. 3, it may be noticed that the broken end of the translocated strand of the second chromosome is connected by a fine achromatic strand, possibly representing the Y-chromosome, to the chromocenter. In fig. 2, however, such a connection seems to have been lost. As a matter of fact, several heavy bands of sections 49 and 50 are not shown in the homologous translocated piece (fig. 2). These might have been included in the granules united to the end as a result of mechanical injury in the process of smearing.

The cytological nature of Y-II D translocation is shown in figures 4 and 5. Fig. 4 shows one of the strands broken. Since there appears no sign of attachment of the broken end to the chromocenter, it can not be considered as a conclusive evidence that a piece of the second chromosome is transposed on the Y-chromosome. Fig. 5 was obtained from a cell of another hybrid larva. This shows that one strand of the second

chromosome is broken in section 48, and both broken ends are attached to the chromocenter. The phenomenon of attachment of both broken ends to the chromocenter suggests the reciprocal nature of this translocation. From the middle of section 48 on to the free end in the second chromosome is exchanged with a piece of Y-chromosome. The exact amount of Y involved in the exchange could not be possibly worked out in the salivary chromosomes. Considering the hypothetical mode of origin of reciprocal translocations, it may be presumed that the piece of Y chromosome transferred to the second includes also the free end. Indeed, it is highly probable, from the view point of translocation including the end piece, that Y-II A and Y-II B may also include the exchange of a piece of Y to second chromosome.

Figures 6, 7 and 8 show the breakage point in the second chromosome in Y-II C translocation. It is obvious from these figures that II chromosome is broken at about the middle of section 60. Fig. 8 drawn from the same cell in which 7 was observed, shows that the broken off piece of the second chromosome is attached to the chromocenter, which is composed of heterochromatin material. To avoid possible confusion of the heterochromatin mass with the dot-like fifth chromosomes, the latter together with the basal end of the third chromosome is also shown in fig. 8.

Genetically, as has already been shown previously, the duplicating fragment of Y-II C includes the wild allel of gene ps. Here in the salivary gland chromosomes the transposed piece consists of section 62, 61 and a part of 60. Hence the gene locus for ps must be localized within this small region of the second chromosome.

Considering the great difference in the length of the translocated piece of the second chromosome between Y-II C on one hand and of

Y-II A, Y-II B and Y-II D on the other, it is not surprising to find that the duplication flies are viable in the former and inviable in the latter.

The genetic break of Y-II A, B and D could be detected by linkage test in those non-disjunction females, which might be obtained if a large number of flies be examined. Since the break loci in these three cases lie close to each other, and since no known genes are present in this region, as will be shown later, it was considered not worthwhile to take up that laborious test. Incidentally, it may be mentioned that all these three breaks lie in the big gap between Smoky and bithorax.

Y-III translocations

Among 22 translocations only two involve the transfer of a section of the third chromosome to the Y-chromosome. Translocation males heterozygous for the gene orange, as originally obtained, have been constantly maintained by selecting wild type males to mate with orange sibs. Among the progeny of such matings, orange males are invariably present in both Y-III A and Y-III B. Table 55 shows the result

Table 55. Ty-III ♂♂ × or ♀♀

	+ or		or	
	♀	♂	♀	♂
Y-IIIA	0	223	275	136
Y-IIIB	0	314	327	34

of fly counts of two separate cultures for each translocation. In both cases, wild type males are present in approximately equal numbers with the orange females, indicating the full viability of translocation individuals.

The complete absence of wild type females indicates the complete non-viability of deficiencies. As to the number of duplications represented by orange males, it varies in the two cases. From comparing the proportion of or males with respect to wild type males, it is apparent that duplications are viable in more than 1/2 in Y-III A and only about 1/10 in Y-III B. Phenotypically, Y-III A duplication males are indistinguishable from normals, whereas Y-III B duplications are abnormal in appearance, being usually characterized by somewhat shorter abdomen and the presence of an extra posterior scutellar bristle. When duplication males were mated to normal females, in both cases no progeny was produced showing that duplications are completely sterile.

As inferred from the result shown in table 55, the duplicating fragment in both Y-IIIA and Y-III B does not suppress the gene orange. When translocation males heterozygous for or Sc pr were mated to or pr females, the two translocations behaved differently. Table 56 shows the summary of fly counts in 4 separate cultures for each case.

Table 56. $\frac{\text{Ty-III}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀

	+ ♀	+ ♂	or Sc pr ♀	or Sc pr ♂	or Sc ♀	or Sc ♂
Y-III A	0	273	293	152	0	0
Y-III B	0	464	400	0	0	108

The duplication males of Y-III A were or Sc pr, but those of Y-III B show only orange and Scute. The results may be interpreted as follows: none of the wild allels of genes or Sc and pr is contained in the duplicating fragment of Y-III A, and with Y-III B wild allel of the gene purple

is unquestionably included in the duplicating fragment. Since Scute is dominant over wild type, it is difficult to ascertain whether or not its locus is included in the fragment. Careful examination of Y-III B or duplications, however, enables one to find less bristles missing in these males than in those not carrying the duplications. In few or duplication males almost the entire complement of bristles were found present. These observations lead one to assume that these flies carry two wild allels of Scute and one Scute gene. Consequently, the duplicating fragment in Y-III B probably includes the locus of the gene Scute.

Since tests of other genes in the third chromosome were made after the cytological analysis of these translocations, it seems best to present the salivary chromosome results first.

Fig. 9 represents the heterozygous Y-III A translocation. The third chromosome is broken near the free end, just a little below section 80. The small piece, though paired with its homologue, is attached to the chromocenter by fine achromatic strands, which may represent Y-chromosome. The other broken end is attached to a small mass of heterochromatin material, suggesting that a part of the Y is translocated to this place.

Figs. 10 and 11 show heterozygous Y-III B translocation. They were drawn from cells of different larvae. In both figures it can be seen that the third chromosome is broken in section 73. Fig. 10 shows the attachment of the other broken end to the chromocenter. These two figures, when combined, show that this translocation involves the exchange of a piece of the third chromosome for a piece of the Y-chromosome.

Figures showing a duplication of the translocated fragment have also been observed in both Y-III A and Y-III B. They represent two normal homologous third chromosomes completely paired, and a duplicating fragment united to chromocenter, which may or may not pair with its homologue.

As has been pointed out above, the duplicating fragment in Y-III A translocation consists of only two sections and does not cover any gene from or to pr. The fact that genes Sc and pr are suppressed by Y-III B duplicating fragment indicates that or is located near to spindle fiber region. Since the genetic map of the third chromosome shows that the locus crossveinless is 18 units to the right of purple, it was found necessary to test this against Y-III A translocation. The result of the test, shown in table 57, gives no cv males, but many more

Table 57. Y-III A ♂♂ × cv ♀♀

Culture No.	+ ♀	+ ♂	cv ♀	cv ♂
1	0	58	36	0
2	0	48	48	0
3	0	31	19	0

wild type males than cv females, indicating that the locus for crossveinless is not contained in the short duplicating fragment of Y-III A.

To the left of the locus Scute there are two dominant genes, Jagged and Blade. They were tested against Y-III B duplication males. Since Y-III B duplication males can easily be distinguished from normal males, the expression of Jagged and Blade can be compared in the two kinds

of males to see whether one or two wild allels of the dominant mutation is present. In the culture of the cross, $\frac{\text{Y-III B}}{\text{or pr}}$ ♀♀ × Ja ♂♂ (table 58),

Table 58. $\frac{\text{Ty-III B}}{\text{or pr}}$ ♂♂ × Ja ♀♀

Culture No.	Ja ♀	Ja ♂	+ ♀	+ ♂
1	23	34 (5^x)	26	41 (5^x)
2	51	36(10^x)	42	38 (3^x)

x - duplications

15 Jagged duplication males were obtained. The wings of these flies showed even more extreme Jagged than those in translocation males, indicating with high probability that the wild allel of Jagged is not covered by the duplicating fragment. In a single culture of the cross, $\frac{\text{Y-III B}}{\text{or pr}}$ ♀♀ × or Bl ♂♂ (table 59), 10 or Bl duplication males were

Table 59. $\frac{\text{Y-III B}}{\text{or pr}}$ ♂♂ × or Bl ♀♀

+ ♀	+ ♂	Bl ♀	Bl ♂	or ♀	or ♂	or-Bl ♀	or-Bl ♂
0	24	0	35	22	20	27	10

produced. They showed the character of Blade in a more extreme form than the Blade translocation males. These results suggest that the Y-III B transferred piece of the third chromosome does not cover any known gene locus to the left of Scute.

Genes narrow and plexus, as described in the previous section, are found in sequence with 'arrowhead inversion', the limits of which have been found in corroboration with Prof. Dobzhansky (unpublished) to include from the middle of section 70 to the middle of section 77

(cf. fig. 42). Whether the loci na and px lie within the inversion or outside of it remains to be seen. The result of tests of the two Y-III translocations with respect to these genes is summarized in tables 60 and 61. The fact that in both cases the duplicating males show narrow and plexus indicates that both of these genes are not located in sections 73 to 81.

Table 60. $\frac{\text{Y-III}}{\text{na}}$ ♂♂ × na ♀♀

	+		na	
	♀	♂	♀	♂
Y-III A	1	74	51	69
Y-III B	0	47	48	13

Table 61. $\frac{\text{Y-III}}{\text{px}}$ ♂♂ × px ♀♀

	+		px	
	♀	♂	♀	♂
Y-III A	0	30	56	28
Y-III B	0	129	137	58

Y-IV translocations

Five cases of Y-IV translocations were obtained (table 50). Translocation males were in each case mated to Cy females. In the next generation, Cy males were selected and backcrossed to normal females. The result of separate crosses of the five translocations is summarized in table 62. In each case, wild type translocation males are as many

Table 62. $\frac{\text{Y-IV}}{\text{Cy}}$ ♂♂ × + ♀♀

	+		Cy	
	♀	♂	♀	♂
Y-IV A	8	198	191	34
Y-IV B	1	121	136	36
Y-IV C	2	112	141	73
Y-IV D	0	103	191	43
Y-IV E	0	108	108	68

as normal females, indicating the full viability of heterozygous translocations. Curly males may represent either duplications or XO non-disjunctions. Wild type females may represent either deficiencies or XXY non-disjunctions. Each of the Y-IV translocations was tested for the constitution of its duplicating fragment. The results of the crosses Y-IV/in j ♂♂ × in j ♀♀ and Y-IV/tg^3♂♂ × tg^3 ♀♀ are given in tables 63 and 64 respectively. For each kind of mating, complete counts in 2

Table 63. $\frac{\text{Y-IV}}{\text{in j}}$ ♂♂ × in j ♀♀

	+		in j		in		j	
	♀	♂	♀	♂	♀	♂	♀	♂
Y-IV A	1	167	132	17				
Y-IV B	3	151	190	15				
Y-IV C	1	193	170	70				
Y-IV D	0	144	114	0	3	18	6	
Y-IV E	4	122	113	3				

Table 64. $\frac{\text{Y-IV}}{tg^3}$ ♂♂ × tg^3 ♀♀

	+		tg^3	
	♀	♂	♀	♂
Y-IV A	3	149(13^X)	172	1
Y-IV B	17	247(14^X)	231	14
Y-IV C	3	185(28^X)	169	2
Y-IV D	14	182	253	38
Y-IV E	3	155 (2^X)	165(2^X)	43

x - duplications

cultures of each translocation were made. As shown in table 63, duplication males of Y-IV A, B, C and E were in j, indicating that the

respective duplicating fragments do not cover the gene loci in and j. In Y-IV D, however, duplications showed incomplete but not jaunty, suggesting that the wild allel of jaunty is included in the duplicating fragment. Incomplete jaunty males in the cases Y-IV A, B, C, and E are partly normal and partly abnormal in phenotype and the latter can be distinguished from the former by the presence of minute bristles and short abdomen. Incomplete males obtained in the cross with TY-IV D (table 63) were normal in appearance, but jaunty females were somewhat abnormal, representing deficiencies. Three incomplete females, which were normal in appearance, may very likely represent XXY non-disjunctions. Indeed, a few wild type females were present in each of the four other translocations. Since duplication males are phenotypically abnormal, these females may also very likely be non-disjunctions having the constitution of XXY.

When Y-IV translocation males heterozygous for tg^3 were mated to tg^3 females (table 64), a certain proportion of wild type males in Y-IV A, B, C, and E were abnormal in appearance, definitely representing duplications. This fact suggests that the locus for tg^3 is included in the duplicating fragment in all of the four cases. In Y-IV D, tg^3 males apparently represent duplications. Since abnormal duplication males are present in the midst of wild types, the phenotypically normal tg^3 males may very probably represent non-disjunctions of XO type. The obvious differentiation of duplication males and XO males can now explain why some Cy males and in j males as shown in tables 62 and 63 were partly normal and partly abnormal.

Once more Y-IV translocations were tested for the constitution of duplicating fragments. Translocation males heterozygous for

in j tg^3 were mated to in j tg^3 females. (Table 65) The fact that in

Table 65. $\frac{\text{Y-IV}}{\text{in j tg}^3}$ ♂♂ × in j tg^3 ♀♀

	+		in j tg^3		in j		in		j	
	♂	♂	♀	♂	♀	♂	♀	♂	♀	♂
Y-IV A	0	161	187	0	0	4				
Y-IV B	2	108	139	1	0	4				
Y-IV C	2	100	80	17	0	7				
Y-IV D	0	104	160	6	0	0		25		1
Y-IV E	1	109	144	26	0	2				

j males were abnormal and in j tg^3 males were normal, representing duplications and XO types respectively, definitely brings confirmation to the explanation given above for the results shown in tables 62, 63 and 64. Duplications are viable in rather small proportion in all Y-IV translocations.

With the exception of duplication males in Y-IV D, which were proven to be fertile, tests of limited number of duplication males in other four translocations show complete sterility of these flies.

It may be recalled from previous section that the gene cross-veinless originally reported by Crew and Lamy in the fourth chromosome is really located in the third chromosome. To insure the above finding, and as a matter of fact due to the misleading information in the Crew and Lamy's report, Y-IV translocations were tested for gene cv. The results as summarized in table 66, showing equal distribution of normal and cv flies in both sexes, thus leave no doubt that cv can not possibly be located in the normal fourth chromosome.

Table 66. $\frac{\text{Y-IV}}{\text{cv}}$ ♂♂ × cv ♀♀

	+		cv	
	♀	♂	♂	♂
Y-IV A	53	39	38	34
Y-IV B	23	32	25	38
Y-IV C	27	30	24	24
Y-IV D	27	19	14	23
Y-IV E	52	42	41	34

Fig. 12 represents the salivary gland chromosome configuration in the larva of Y-IV A translocation. Section 91 is broken. The piece including the free end is attached to the chromocenter.

Fig. 13 represents Y-IV B translocation showing the breakage point in section 92.

Fig. 14 and 15 show Y-IV C translocations, the break of which apparently lies between sections 94 and 95. In fig. 14, there is shown a wide, faint, yet traceable space like connection between the break in the fourth chromosome and the chromocenter. This space may be the Y-chromosome.

Figures 16, 17 and 18 represent three different drawings showing Y-IV translocations. In all cases, the breakage points lie in section 92. The attachment of the translocated piece to the chromocenter is shown in fig. 17. The sharp break in the fourth chromosome can be best identified in fig. 18.

A comparison of the distribution of the breakage points of the above four translocations (cf fig. 42) shows that they lie close to each other. As a matter of fact, Y-IV B and Y-IV E might have been broken at same locus. This has, however, not been established. The

break of Y-IV C lies closer to the free end than the other three. To correlate cytological observations with the genetic data given previously, tg^3 being included in Y-IV C duplicating fragment can not possibly be localized further left than section 95. Since in j are not covered in either of the four translocations, they must be located anywhere left of section 91. Hence, in and tg^3 which are located at opposite ends of the genetic map of the fourth chromosome are now proven to lie near to spindle attachment and the free end respectively.

The salivary gland chromosome picture of translocation Y-IV D, as shown in figs. 19 and 20, represents a different type of chromosome rearrangement from the other Y-IV translocations. Both figures show that fourth chromosome is broken twice, one between sections 85 and 86 and the other between 88 and 89. Fig. 20 clearly demonstrates that both ends are united to the chromocenter. This phenomenon can be explained by assuming that a piece of the fourth chromosome, including sections 86, 87 and 88, is deleted from the fourth chromosome and inserted into the Y-chromosome. Among altogether 11 cases of Y translocations, this is the only one which shows that the piece transferred to the Y does not involve the free end.

Genetically, the locus jaunty is included in the deleted piece of the fourth chromosome, which is inserted into the Y. Since the genetic position of the gene incomplete is to the left of jaunty, it may lie somewhere from section 82 to 85 of the fourth chromosome.

The relation of the length of the duplicating fragments to the relative frequency of viable duplication flies is no less striking. As one may notice in tables 64 and 65, the frequency of viable

duplication males is highest in Y-IV D, next is Y-IV C, and lowest in Y-IV A, B, and E. Salivarily, the length of the duplicating fragment is shortest in Y-IV D, next in Y-IV C, and longest in Y-IV A, B and E, the last three being approximately equal in length. Apparently, the frequency of viable duplications is inversely proportional to the length of the duplicating fragment. Indeed, the results obtained in Y-II and Y-III translocations follow essentially the same principle.

Reciprocal Translocations

Chromosome rearrangements that involve the exchanges of sections between non-homologous chromosomes are known as reciprocal translocations. Both chromosomes are, therefore, simultaneously donors and recipients. In simple or non-reciprocal translocations a section of one chromosome (the donor) is attached to another chromosome (the recipient), but no section of the recipient is transferred to the donnor. Among three II-III translocations, two have been shown to be non-reciprocal and one reciprocal. But among three III-IV translocations, two are reciprocal and one non-reciprocal.

In the crosses of flies heterozygous for reciprocal translocations with 'normal', usually only two kinds of individuals are produced, 'normal' and heterozygous translocation. The inviability of heteroploid individuals can be easily accounted for by the fact that gametes which carry duplications for a section of one chromosome are also deficient for a section of another chromosome. Such gametes are expected to be inviable in a large majority of cases. Consequently, for reciprocal translocations, the break loci can be determined only by means of linkage tests.

T II-III C

T II-III C is a reciprocal translocation between the second and third chromosomes. When translocation males heterozygous for orange were crossed to p or females, the offspring (table 67) shows approximately

Table 67. T II-III C / or ♂♂ × p or ♀♀

+		or	
♀	♂	♀	♂
127	123	127	100

equal numbers of wild type males and females and or males and females, indicating that heterozygous translocation individuals of II-III C are completely viable.

When translocation males heterozygous for p or were mated to p or females, only two types of fly were produced, wild type and p or. The absence of recombination classes of individuals indicates the complete inviability of heteroploids. The breaking point in the third chromosome was tested by the cross, II-III C / or Sc pr ♀♀ × or pr ♂♂, the result of which is shown in table 68. Counts of five separate cultures

Table 68. T II-III C / or Sc pr ♀♀ × or pr ♂♂

Culture	Non-crossovers		single crossovers				double c.o		
			Reg.1		Reg.2		1,2		
No.	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	total
1	139	185	4	1	4	5	0	1	339
2	83	110	4	3	2	2	0	0	204
3	128	122	1	6	6	3	0	0	266
4	103	135	3	3	6	4	1	0	255
5	114	127	0	0	1	6	0	0	248
	567	679	12	13	19	20	1	1	1312

yield a total of 1312 flies, from which crossover value between or and Sc and that between Sc and pr are calculated to be 2.06% and 3.12% respectively. When these values are compared with the corresponding values, 22.8% and 24.7%, from the control (the counts of the latter are shown in table 69), it is apparent that the frequency of crossing

Table 69. $\frac{\text{or Sc pr}}{+}$ ♀♀ × or pr ♂♂

Non-crossovers		single crossovers				double c.o.		
		Reg.1		Reg.2		1,2		
or-Sc-pr	+	or	Sc-pr	or-Sc	pr	or-pr	Sc	total
45	75	19	17	17	23	5	5	206

over is greatly reduced in both regions. Since the amount of reduction appears to be proportionally greater in the first region than in the second region, the break in the third chromosome probably lies to the right of the locus Scute.

Having learned from above that crossing over in the whole third chromosome is considerably reduced by the presence of II-III C translocation, the author, using the gene orange as the marker of the break in the third chromosome, made the cross of translocation females heterozygous for Sm p ps in the second chromosome and or in the third chromosome to the multiple recessive males. The result of the mating is shown in table 70. A comparison of the calculated crossover values from this experiment, being 46.1% between Sm and p, 30% between p and or and 13.9% between or and ps, and the corresponding values calculated from the data of the control as shown in table 71 (41.1% between Sm and p and 37.6% between p and ps, or being located in a separate chromosome assorts independently with any combination of second chromosome genes)

Table 70. $\frac{T\ II\text{-}III\ C}{Sm\ p\ or\ ps}$ ♀♀ × p or ps♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers								triple crossovers		total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3				1,2,3		
	+	Sm-p-or-ps	Sm	p-or-ps	Sm-p	or-ps	Sm-p-or	ps	Sm-or-ps	p	Sm-ps	p-or	Sm-p-ps	or			Sm-or	p-ps	
1	17	22	19	16	2	8	5	0	7	4	3	3	0	1			2	1	110
2	21	9	8	16	4	17	0	0	4	5	1	1	0	1			2	0	89
3	14	19	8	18	4	11	0	7	3	5	3	2	3	0			2	5	104
	52	50	35	50	10	36	5	7	14	14	7	6	3	2			6	6	303

Table 71. $\frac{\text{or Sm p ps}}{+}$ ♀♀ × p ps ♂♂

Cult. No.	Non-crossovers				single crossovers								double c. o.				total
					Reg. 1				Reg. 2				Reg. 1,2				
	Sm p ps		+		p ps		Sm		Sm p		ps		p		Sm ps		
	or	+	or	+	or	+	or	+	or	+	or	+	or	+	or	+	
1	21	17	50	46	13	19	24	35	13	18	36	22	16	13	14	15	372
2	11	10	18	20	8	10	13	23	9	5	12	11	8	2	2	8	170
	32	27	68	66	21	29	37	58	22	23	48	33	24	15	16	23	542

shows that the region between p and ps is somewhat reduced and the region between Sm and p remains unaffected. The fact that or recombines with p in 30.2% and with ps in only 7.9% indicates that the break lies much closer to ps than to p

Genetically between p and ps lies an intermediate gene, gl. A cross $\frac{\text{II-III C}}{\text{na Sm Ba gl}}$ ♀♀ × na gl ♂♂ was made with the aim of determining the break of the translocation in relation to gl, narrow being associated with an inversion in the third chromosome is used as the marker for the third chromosome break. As calculated from table 72, linkage values between Sm and Ba, between Ba and gl, and between gl and na are 44.2%, 30.2% and 7.9% respectively. The normal frequency of crossing over (table 1) is 42.0% between Sm and Ba and 18.9% between Ba and gl. It seems strange that in the region Ba-gl the experimental value is even higher than that in the control. Nevertheless, judged from na-gl crossover value, it is obvious that the break must lie to the right of gl. Since the break in the second chromosome lies to the left of ps, it may be safely concluded then that the breakage point should lie somewhere between gl and ps, the two being 18 units apart.

Table 72. $\frac{\text{T II-III C}}{\text{Sm Ba } g_1 \text{ na}}$ ♀♀ × gl na ♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers								triple crossovers		total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3				1,2,3		
	+	Sm-Ba-gl-na	Sm	Ba-gl-na	Sm-Ba	gl-na	Sm-Ba-gl	na	Sm-gl-na	Ba	Sm-na	Ba-gl	Sm-Ba-na	gl			Sm-gl	Ba-na	
1	23	11	16	22	5	11	2	2	5	4	2	2	1	1			1	0	108
2	8	7	8	7	3	2	0	2	0	1	0	0	0	0			0	0	38
3	9	6	7	15	19	5	1	1	2	4	0	1	1	0			0	0	71
4	12	5	7	7	3	6	1	1	4	2	1	0	0	1			0	0	50
	52	29	38	51	30	24	4	6	11	11	3	3	2	2			1	0	267

Test for viability of II-III C homozygous translocation was made by intercrossing translocation males and females heterozygous for Sm or Sc. If homozygous translocation individual were viable, the above mating should produce an appreciable number of wild type individuals. The fact that no such flies appeared in the test cross indicates definitely that homozygous II-III C translocation individuals are not viable. Even if a few such flies do turn out as it would be shown later in II-III A and B, they could very probably be flies resulting from double crossovers.

The salivary chromosome configuration of heterozygous translocation II-III C is shown in fig. 21. Second chromosome is broken about the middle of section 59, and third chromosome is broken between sections 69 and 70. The short broken off piece from the second is attached to the basal part of the third, which in exchange gives the excised long section to the second.

From the study of translocation Y-II C, ps is located in sections 60 to 62. Since the frequency of crossing over between the break and ps in II-III C is 13.9%, the locus, ps, presumably lies very close to the terminal end of the second chromosome. The locus of gl, apparently must lie to the ~~right~~ left of section 59.

As it has already been shown in Y-III B translocation, the locus Scute lies to the ~~left~~ right of section 72. The break of II-III C, now located between sections 69 and 70 therefore, lies a few sections to the ~~left~~ right of Scute. This may also account for the genetic result given above that crossover frequency is more reduced in the region between or and Sc than that between Sc and pr.

T III-IV B, C

Of three translocations that involve third and fourth chromosomes, two are reciprocal. These are III-IV B and III-IV C. Heterozygous translocation males carrying the gene orange in the non-treated chromosome were mated to or Cy females. The result of counts in the two cases, as shown in table 73, gives in each case even more

Table 73. $\frac{\text{T III-IV}}{\text{or}}$ ♂♂ × or Cy ♀♀

	+		Cy		or Cy		or	
	♀	♂	♀	♂	♀	♂	♀	♂
III-IV B	46	46	41	50	32	31	27	42
III-IV C	22	27	37	26	26	27	22	22

translocation flies than 'normals', indicating that heterozygous translocations are completely viable.

Judged from the results of crosses $\frac{\text{T III-IV}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂ and $\frac{\text{T III-IV}}{\text{na in j}}$ ♀♀ × na in j ♂♂, which are summarized in tables 74 and 75

Table 74. $\frac{\text{T III-IV}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀

	+	or Sc pr
III-IV B	403	359
III-IV C	429	324

Table 75. $\frac{\text{T III-IV}}{\text{na in j}}$ ♂♂ × na in j ♀♀

	+	na in j
III-IV B	75	55
III-IV C	110	58

respectively, heteroploid flies do not exist in either of the two translocations.

Genetic test for the break in the third chromosome was attempted by making the mating $\frac{\text{T III-IV}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂, the results of which are presented in table 76 for III-IV B and table 77 for III-IV C.

Table 76. $\frac{\text{T III-IV B}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		single crossovers Reg. 1		single crossovers Reg. 2		double c.o. 1, 2		tot.
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	109	85	1	1	14	13	0	0	223
2	58	68	0	0	11	17	0	0	154
3	110	108	0	0	19	18	0	0	255
4	55	60	0	0	6	9	0	0	130
5	65	58	1	0	17	14	0	0	155
	397	379	2	1	67	71	0	0	917

Table 77. $\frac{\text{T III-IV C}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		single crossovers Reg.1		single crossovers Reg.2		double c.o. 1,2		tot.
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	65	46	0	0	15	14	0	0	140
2	95	104	0	1	24	21	0	0	245
3	72	58	2	3	18	22	1	0	176
4	83	54	0	0	27	21	0	3	188
	315	262	2	4	84	78	1	3	749

The calculated crossover values in the two cases as well as those in the control (cf. table 69) are given in table 78.

Table 78. Comparison of crossover value in the third chromosome

	Reg.1, or/Sc	Reg.2, Sc/pr
T III-IV B	0.3	15.5%
T-III-IV C	1.33	22.2%
Control	22.8	24.7%

A comparison of these values shows a remarkable reduction in the frequency of crossovers in the region between or and Sc in both III-IV B and III-IV C, indicating that the break in the third chromosome lies in this region in either case. The fact that linkage between Sc and pr is greater in III-IV B, 15.1%, than in III-IV C, 22.2%, suggests the possibility that the break is farther away from the gene Scute in the latter than in the former.

Using na as a crossover reducer to mark the break in the third chromosome, $\frac{\text{T III-IV}}{\text{na in j}}$ ♀♀ were mated in na in j ♂♂. The results of separate crosses for the two translocations are summarized in tables 79

Table 79. $\frac{\text{T III-IV B}}{\text{na in j}}$ ♀♀ × na in j ♂♂

Cult. No.	+	na-in-j	na	in-j	na-in	j	na-j	in	tot.
1	90	54	36	44	3	8	5	6	246
2	99	46	36	32	20	7	2	4	246
3	63	45	20	31	8	6	1	5	179
	252	145	92	107	31	21	8	15	671

and 80 respectively. The data from the control are shown in table 81. Frequency of crossing over between in and j is 11.2% in III-IV B and 2.4%

Table 80. $\frac{\text{T III-IV C}}{\text{na in j}}$ ♀♀ × na in j ♂♂

Cult. No.	Non-crossovers +	Non-crossovers na-in-j	Crossovers na-in	Crossovers j	tot.
1	93	56	3	3	155
2	100	89	1	0	190
	193	145	4	3	345

Table 81. $\frac{+}{\text{na in j}}$ ♀♀ × na in j♂♂

Cult. No.	Non-crossovers + +	Non-crossovers + na	Non-crossovers in j +	Non-crossovers in j na	Crossovers in +	Crossovers in na	Crossovers j +	Crossovers j na	total
1	75	47	60	47	16	9	11	6	271
2	85	48	54	73	16	10	13	8	307
3	57	42	51	56	14	8	9	7	244
	354		341		73		54		822

in III-IV C. A comparison of these values with the value obtained from the control, 15.5%, shows that the break in the fourth chromosome is far away from the spindle attachment in III-IV B and near to it in III-IV C.

To repeat the same kind of experiments as above, $\frac{\text{T III-IV}}{\text{na or in j}}$ ♀♀ were mated to na or in j ♂♂, and the results are shown in tables 82 and 83

Table 82. $\frac{\text{T III-IV B}}{\text{na or j in}}$ ♀♀ × na or j in ♂♂

Cult. No.	Non-crossovers +	Non-crossovers na-or-j-in	Single crossovers Reg. 1 na-or	Single crossovers Reg. 1 j-in	Single crossovers Reg. 2 na-or-j	Single crossovers Reg. 2 in	double c.o. 1,2 na-or-in	double c.o. 1,2 j	tot.
1	33	41	28	23	6	6	6	5	148
2	69	68	32	21	5	15	11	6	227
	102	109	60	44	11	21	17	11	375

Table 83. $\frac{\text{T III-IV C}}{\text{na or j in}}$ ♀♀ × na or j in ♂♂

Cult.No.	+	na-or-j-in	in	total
1	155	100	0	255
2	54	60	1	115
	209	160	1	370

for III-IV B and III-IV C respectively. That in the control is shown in table 84. There appeared in III-IV B six crossovers between na and

Table 84. $\frac{\text{na or in j}}{+}$ ♀♀ × na or in j ♂♂

Non-crossovers		Crossovers		
+	in-j	in	j	total
91	89	16	11	207

or. Since this number is negligibly small, they were left out of consideration in the calculation of linkage values. in and j are now found to give 6.0% crossing over in III-IV B, but the result as a whole seems consistent with that obtained from $\frac{\text{T III-IV B}}{\text{na in j}}$ ♀♀ × na in j ♂♂. Concerning the result of the mating $\frac{\text{III-IV C}}{\text{na or in j}}$ ♀♀ × na or in j♂♂, in which no orange and narrow crossover was obtained, there was only one fly representing the crossover between in and j. That the crossing over in the regions between in, j and the break is almost entirely suppressed indicates that the break in the fourth chromosome in III-IV C lies very close to the regions in and j, probably between them.

In addition to the above experiments, III-IV B and C were tested for the linkage of the genes near to the free end. For this purpose, $\frac{\text{T III-IV}}{\text{na or tg}^3\text{ Cy}}$ ♀♀ were mated to na or tg^3 ♂♂. The results, as

shown in tables 85 and 86 for III-IV B and III-IV C respectively, are

Table 85. $\frac{\text{T III-IV B}}{\text{na or tg}^3\text{ Cy}}$ ♀♀ × na or tg^3 Cy ♂♂

Cult. No.	Non-crossovers +	Non-crossovers na-or-tg^3-Cy	Single crossovers Reg. 1 na-or	Single crossovers Reg. 1 tg^3-Cy	Single crossovers Reg. 2 na-or-tg^3Cy	Single crossovers Reg. 2 Cy	Double c.o. 1,2 na-or-Cy	Double c.o. 1,2 tg^3	total
1	61	61	0	1	2	8	2	2	137
2	70	72	0	2	4	3	0	2	153
	131	133	0	3	6	11	2	4	290

Table 86. $\frac{\text{T III-IV C}}{\text{na or tg}^3\text{ Cy}}$ ♀♀ × na or tg^3 ♂♂

Cult. No.	Non-crossover +	Non-crossover na-or-tg^3Cy	Single crossovers Reg. 1 na-or	Single crossovers Reg. 1 tg^3-Cy	Single crossovers Reg. 2 na-or-tg^3	Single crossovers Reg. 2 Cy	Double C.O. 1,2 na-or-Cy	Double C.O. 1,2 tg^3	tot.
1	56	35	36	42	9	13	8	3	202
2	26	28	27	17	6	3	0	0	107
	82	63	63	59	15	16	8	3	309

in perfect agreement with those from other tests. Frequency of crossing over between the break and tg^3 is 43.9% in III-IV C and only 3.1% in III-IV B, indicating that the break lies much closer to the locus tg^3 in III-IV B than in III-IV C. Moreover, the linkage values between tg^3 and Cy are 9.6 in III-IV B and 13.6% in III-IV C, showing more reduction in the former than in the latter. The frequency of crossing over between tg^3 and Cy is 17.0%.in the control, as calculated from the data given in table 87.

Table 87. $\frac{\text{na or tg}^3\text{ Cy}}{+}$ ♀♀ × na or tg^3 ♂♂

Non-crossovers tg^3-Cy	Non-crossovers +	Crossovers tg^3	Crossovers Cy	total
96	94	17	22	229

Genetic tests for the viability of homozygous III-IV translocations were also made. The plan of the test was essentially the same as described for testing II-III translocations. T III-IV heterozygous for Cy or Sc pr were mated to each other. In III-IV B, about 1/4 of the progeny was wild type, which represents the homozygous translocation individuals. These wild type individuals are normal in appearance. Some of such males were mated to na or in j females. The salivary gland chromosome figures of the hybrid larvae show attachment of the third chromosome head to the base of the fourth, and of head of the fourth chromosome to the base of the third. Genetically, when the hybrid males were mated to na or in j females, only wild type and na or in j individuals were obtained, as shown in table 88, proving definitely that III-IV B

Table 88. T III-IV B / na or in j ♂♂ × na or in j ♂♂

Culture No.	+		na or in j	
	♀	♂	♀	♂
1	30	36	35	30
2	8	10	15	13

homozygous translocation individuals are viable. This has rendered it possible to keep III-IV B translocation in homozygous condition.

But in the case III-IV C, no wild type flies were obtained from the test cross, indicating that III-IV C homozygous translocation is not viable.

The typical salivary gland chromosome configuration, showing a reciprocal translocation, is shown in fig. 28 for III-IV B. The third chromosome is broken just below section 68, and the excised piece

is transferred to the broken end of the fourth, which lies between sections 94 and 95. The piece transferred from the fourth to the third includes 95 to the free end.

The figure (fig. 23) observed in III-IV C translocation, however, shows that breaks in both third and fourth chromosomes lie close to chromocenter. The third chromosome is broken in section 66, and the fourth in section 86. Comparisons of the cytological and the genetic data show a consistent agreement. That the break in the third chromosome is nearer to spindle attachment in III-IV C than in III-IV B explains well why the frequency of crossing over between Sc and pr is higher in the former than in the latter. Similar correlation also holds for the breaks in the fourth chromosome. The fact that the fourth chromosome is broken in section 86 in III-IV C, almost completely suppressing the crossover between in and j, gives strong support to the conclusion reached from the study of Y-IV translocation that the loci jaunty and incomplete lie to the right and left of section 86 respectively. That crossing over is considerably reduced between the break and tg^3 in III-IV B, in which the break lies closer to free end, furnishes another evidence that tg^3 and Cy are located in the opposite end of the chromosome from in and j. Finally, as indicated by the higher frequency of crossing over between the break and Cy than between the break and tg^3, the locus Cy lies to the right of tg^3.

Non-reciprocal translocations

In non-reciprocal or "simple" translocations, a section of one chromosome (the donor) is attached to another chromosome (the recipient), but the section of the recipient is transferred onto the

donor. Among twenty-two translocations, three translocations are apparently simple ones. In two of them, designated II-III A and II-III B, a section of third chromosome is excised, and intercalated into the second chromosome. In the third, III-IV A, a section excised from third chromosome has been intercalated into the fourth chromosome. The following is a description of the results of genetic and cytological analysis of these cases. Since III-III A and II-III B involve same two chromosomes, they are treated together.

T II-III A and B

When II-III A and II-III B translocation males heterozygous for or were mated to p or females, more wild type flies were produced than orange flies (table 89), indicating that the heterozygous translocations are at least as viable as the 'normals'. When $\frac{\text{II-III}}{\text{p or}}$ males were

Table 89. $\frac{\text{T II-III}}{\text{or}}$ ♂♂ × p or ♀♀

	+		or	
	♀	♂	♀	♂
T-II-III A	118	114	84	73
T II-III B	75	76	91	76

mated to p or females a few p and or flies were produced in each case, (table 90), suggesting that the duplications and deficiencies are viable.

Table 90. $\frac{\text{T II-III}}{\text{p or}}$ ♂♂ × p or ♀♀

	+	p-or	p	or
T II-III A	249	154	13	3
T II-III B	98	81	7	4

The exact nature of the duplicating fragments was not quite clear, until the result of four separate crosses of $\frac{\text{T II-III}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀ for each translocation (table 91) gave a considerable number of or and Sc pr individuals in each case. Sc pr flies are normal in appearance and

Table 91. $\frac{\text{T II-III}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀

	+	or-Sc-pr	or	Sc-pr
T II-III A	351	234	94	210
T II-III B	229	211	164	187

appear in almost equal number as 'normals' (or Sc pr). In both cases, or flies, having minute bristles and somewhat defective wings, are abnormal in appearance, and the number of such flies was not as large as of Sc pr individuals. The above facts raise a strong presumption that Sc pr flies are duplications and or flies the deficiencies. In both cases, apparently, the duplicating fragment suppresses the expression of the gene, orange.

The result of reciprocal crosses, $\frac{\text{II-III}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂ as summarized in tables 92 and 93, shows an unequal frequency of or and

Table 92. $\frac{\text{T II-III A}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		Single crossovers Reg. 1		Single crossovers Reg. 2		double c.o. 1, 2		tot.
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	79	56	53	63	37	26	18	13	345
2	45	22	11	42	15	9	13	2	159
3	64	25	28	27	22	17	15	4	202
4	97	34	37	69	25	24	15	3	304
	285	137	129	201	99	76	61	22	1010

Table 93. $\frac{\text{T II-III B}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		Single crossovers Reg. 1		Single crossovers Reg. 2		double c.o. 1, 2		tot.
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	43	30	21	41	18	17	9	4	183
2	36	29	14	33	23	15	14	4	168
3	43	37	38	34 b	23	15	9	11	210
4	34	45	24	27	13	16	10	7	176
5	52	33	36	42	27	7	16	11	224
6	53	35	25	41	21	12	7	3	197
	261	209	158	218	125	82	65	40	1158

Sc pr flies, the latter being almost twice as frequent as the former. Moreover, if calculated on the basis of figures given in these tables, the crossover values in the first region, i.e. between or and Sc, are 40.9% for II-III A and 41.5% for II-III B, and for the second region, i.e. between Sc and pr, 25.5% for II-III A and 27.0% for II-III B respectively. These values, especially in the first region, when compared with the control data (cf. table 69), show a great increase. This phenomenon may now be interpreted as due to the fact that there were a high number of duplication and deficiency flies involved. Since these translocations are now definitely known to give viable duplications, and since under such circumstances the crossover values could hardly be trusted for test of the break in the third chromosome, attention is now paid to find the extent of the duplicating fragments in the two cases by another method. For this reason, cytological analysis was taken up first.

Investigations of salivary gland chromosomes reveal in both II-III A and II-III B the intercalation of a short piece from third chromosome into the second chromosome. The deleted regions in the third chromosome are not the same in the two cases, but they overlap each other. But in the second chromosome, the locus at which inserted piece lies is different in II-III A and II-III B. Although the salivary glands were dissected from the larvae of the crosses between heterozygous translocation males and normal females, no good figures showing heterozygous translocations have ever been observed. All the satisfactory figures obtained were of duplication flies. Efforts were repeatedly made to obtain heterozygous translocations, but they were not successful. The cause for that is unknown. It might be possible that duplication larvae are more vigorous than the translocation ones, though this has not been definitely proven.

In any event, duplication figures showing the pairing of the inserted piece with its homologue have been observed, and they gave the clue for establishing what part of the third chromosome is inserted into the second chromosome. Such pairing was, however, rare in II-III A, the reason for which will be made clear later.

Figs. 24 and 25 show where in the third chromosome a section is deleted and where it is inserted in the second chromosome in II-III A and II-III B respectively. In fig. 24 a buckle is formed in the middle region of the second chromosome. This buckle represents the intercalated piece, and it can be homologized with the sections 64 and 65 of the third chromosome. Actual pairing of the parts have never been observed,

because of the fact that the transferred piece is small and inserted into a region in the second chromosome quite far away from the chromocenter, near which its homologue lies. Of a good many figures observed, fig. 24, though not showing actual pairing, is about the best that can be obtained.

Fig. 25 shows the duplication of II-III B. Unlike II-III A, intercalation in the second chromosome here occurs almost at the spindle attachment region. Partial pairing of the transposed piece and its homologue has been frequently observed. It is shown in this figure that the two strands of the second chromosome fail to pair in a part of its length, but pair very near to the chromocenter. One of the two strands is attached to the chromocenter, one band after the paired region, and the other is continued with the extra piece which goes to the chromocenter region also. The inserted piece in II-III B, also including the characteristic section 65, seems to differ from II-III A by containing a part of section 66 instead of section 64.

Figures 26 and 27 show the second chromosome of a fly heterozygous for the duplication of II-III A at a higher magnification than other drawings. One strand of the second chromosome is broken, and the section of the third chromosome is intercalated. The parts of II above and below the breakage sometimes pair nearly completely with the inserted duplication forming a characteristic buckle. The two figures show exactly the same parts of the second chromosome and the same inserted piece, though they were obtained from glands of two different larvae, and the chromosome in fig. 27 is much more distended. Sections No. 43 to 62 and 63 to 81 belong to the second and third chromosomes respectively.

Only sections 52 and 53 of the second chromosome are shown in these figures. The duplicating fragment of the third chromosome, which includes the sections 64 and 65 and small parts from the section 63 and 66, is intercalated in an inverted order into the section 52 of the second chromosome, between the two light bands "Q" and "R" indicated by arrows. Band P is a characteristic very heavy one and is found paired in both figures. Bands S are also paired in fig. 26 but not in fig. 27, in which, however, they stand out distinctly as two homologous bands in an unpaired condition. Between the two heavy bands there lie the two light ones "Q" and "R". Q can be seen in fig. 27 almost paired and R, though not found paired in either figure, could be clearly homologized in both cases. It has been seen paired in other figures. The two figures agree that the insertion point in the second chromosome is between Q and R, and no visible band in the second chromosome is lost. In other words, the second chromosome has received a section from the third chromosome but has not lost even a single light band in exchange. This evidence apparently fulfills the condition necessary for a demonstration of a simple translocation. The bearing of this finding on the problem of existence and origin of simple translocations will be discussed later.

Further investigation of salivary gland chromosomes in II-III B hybrid larvae has led to the discovery of another translocation, which was not detected genetically. Fig. 28 shows two things, namely, the partial pairing of the duplicating piece with its homologue near the chromocenter, and a translocation between the second and the fifth chromocenter near the free end. The two strands of the second chromosome are paired only in a little region at the middle. In the basal region, one

strand is apparently longer than the other, and the longer bears the inserted piece which partly pairs with its homologue in the third chromosome. It seems clear from the picture that the intercalated piece from the third consists of section 65 and a part of section 66. A careful analysis further shows that the inserted piece is not directly attached to the chromocenter but to a light band not far from it. Since this band has no homologue in the "paired" portion of the third chromosome, it may be presumed to be the last band of the second chromosome before the chromocenter. The same condition is also shown in fig. 25.

One of the unpaired strands of the second chromosome at the free end ends abruptly in section 56, and the other strand, including the free end, is partly paired with its homologue which is united to the fifth chromosome. The section excised from the second and transferred to the fifth chromosomes includes the region from the distal part of section 56 to the free end. It may be noticed in both figure 28 and 29 that the piece is not united to the very tip of the fifth chromosome. Instead, a very small piece involving only faint region is cut off from the tip. Where does this piece go can not be identified. It may either attach to the broken end of the second or simply get lost.

Both genetic and cytological data agree that the duplicating fragments in II-III A and II-III B overlap each other, suppressing the gene or, which can, therefore, be located in the region common to both of them, namely section 65. When Sc pr duplication flies were intercrossed, they proved not only completely fertile themselves, but also produced viable homozygous duplications, as indicated by proportion of

different classes of flies shown in table 94. But when flies deficient

Table 94. $\frac{+^{or}}{or\quad Sc\quad pr}$ ♀♀ × $\frac{+^{or}}{or\quad Sc\quad pr}$ ♂♂
$\frac{}{or\qquad\quad pr}$ $\frac{}{or\qquad\quad pr}$

	Sc-pr	pr	or-Sc-pr	or-pr
T II-III A	105	46	18	4
T II-III B	128	41	34	4

for or were intercrossed (table 95), they produced only or and or pr

Table 95. $\frac{+^{Sc}\quad +^{pr}}{or\qquad pr}$ ♀♀ × $\frac{+^{Sc}\quad +^{pr}}{or\qquad pr}$ ♂♂

	or	or-pr
T II-III A	164	59
T II-III B	138	42

offspring. The absence of wild type flies indicates the inviability of the homozygous deficiencies. Incidentally, it may be mentioned that homozygous translocations of both II-III A and II-III B were found to be inviable.

T II-III A and T II-III B duplications were also tested for the recessive gene abrupt, which genetically is about 9 units to the right from or. The results of the tests are summarized in table 96,

Table 96. $\frac{\text{T II-III}}{or\ ab\ pr}$ ♂♂ × or ab pr ♀♀

	+	or-ab-pr	or	ab-pr
T II-III A	105	33	92	8
T II-III B	32	24	48	8

and show that ab behaves like pr but not like or, indicating that the locus ab is not included in the duplicating fragment of either II-III A or of II-III B.

Since the gene or has been shown to lie in translocations II-III A and II-III B within the short inserted sections, which does not cross over with the third chromosome, or was used as the marker of the break in the third chromosome in tests for the locus of the break in the second. The results of the test crosses are given in tables 97 and 98 for II-III A and II-III B respectively. The calculated crossover value in II-III A is 30.7% between Sm and or, .52% between or and p, and 41.5% between p and ps, and that in II-III B is 3.1 between or and Sm, 40.3% between Sm and p, and 20.5% between p and ps. A comparison of these values in separate cases with those from the control given previously (cf. table 71) renders it possible to conclude that the break at the second chromosome is near to locus pink in II-III A and between spindle fiber and Sm in II-III B. The reduction of crossovers between p and ps in II-III B is due, as will be shown later, to the presence of a viable second chromosome duplicating fragment which suppresses the gene ps,

II-III A was further tested against the genes bi p and ps. The data given in table 99, showing no crossovers in the first and second

Table 99. $\frac{\text{T II-III A}}{\text{or bi p ps}}$ ♀♀ × or bi p ps ♂♂

Cult. No.	Non-crossovers +	Non-crossovers or-bi-p-ps	Single crossovers Reg.1	Single crossovers Reg.2	Single crossovers Reg.3 or-bi-p	Single crossovers Reg.3 ps	total
1	118	95	0	0	45	57	315
2	64	79	0	0	42	41	226
	182	174	0	0	87	98	541

Table 97. $\frac{\text{T II-III A}}{\text{Sm or p ps}}$ ♀♀ × or p ps♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers						triple crossovers		total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3		1,2,3		
	+	Sm-or-p-ps	Sm	or-p-ps	Sm-or	p-ps	Sm-or-p	ps	Sm-p-ps	or	Sm-ps	or-p	Sm-or-ps	p	Sm-p	or-ps	
1	36	13	5	13	0	0	8	23	0	0	1	11	0	0	0	0	110
2	9	4	3	7	0	0	3	8	0	0	3	7	0	0	0	0	44
3	40	14	4	12	0	0	12	16	0	1	6	11	0	0	0	0	116
4	30	18	6	12	0	0	8	27	0	0	8	8	0	0	0	1	118
	115	49	18	44	0	0	31	74	0	1	18	37	0	0	0	1	388

Table 98. $\frac{\text{T II-III B}}{\text{or Sm p ps}}$ ♀♀ × or p ps ♂♂

cult. No.	Non-crossovers		single crossovers						double crossovers								triple crossovers		total
			reg. 1		reg. 2		reg. 3		1,2		1,3		2,3				1,2,3		
	+	or-Sm-p-ps	or	Sm-p-ps	or-Sm	p-ps	or-Sm-p	ps	or-p-ps	Sm	or-ps	Sm-p	or-Sm-ps	p			or-p	Sm-ps	
1	27	23	0	0	22	21	4	14	2	0	1	0	2	1			2	0	119
2	13	2	1	0	0	5	1	4	0	0	0	0	1	1			0	0	28
3	3	3	0	0	2	4	0	0	0	0	0	0	1	1			0	0	14
	43	28	1	0	24	30	5	18	2	0	1	0	4	3			2	0	161

region but 42.0% between p and ps, suggest that the break in the second chromosome lies probably to the left of the locus bithorax.

Finally, the piece excised from the second and transferred to the fifth chromosome was tested for recessive genes. The results of the crosses shown in tables 100, 101 and 102 suffice to show that the duplicating fragment, cytologically including sections from 56 to 62, covers

Table 100. $\frac{\text{T II-III B}}{\text{p ps or}}$ ♂♂ × p ps or ♀♀

+	p-ps-or	p-or
34	40	9

Table 101. $\frac{\text{T II-III B}}{\text{p or ps bi}}$ ♂♂ × p or ps bi ♀♀

+	p-or-ps-bi	p-or-bi
57	30	16

Table 102. $\frac{\text{T II-III B}}{\text{na bi gl}}$ ♂♂ × na bi gl ♀♀

+	bi-gl	bi
30	42	10

the loci ps and gl. The duplication flies are abnormal in appearance, characterized by rough eyes and shorter wings. When mated, they were found to be sterile.

Since Y-II C duplicating fragment suppresses only the locus ps, gl can be located somewhere from the middle of section 56 to the middle of section 59. Judged from the amount of crossing over that takes place between the break and gl in II-III C, the locus gl probably lies close to the break of II-III B.

T III-IV A

Translocation III-IV A is the only observed case of a non-reciprocal translocation between the third and fourth chromosome. When $\frac{\text{III-IV A}}{\text{or}}$ males were mated to or Cy females, the distribution of offspring, as shown in table 103, indicates the complete viability of

Table 103. $\frac{\text{T III-IV A}}{\text{or +}}$ ♂♂ × $\frac{\text{or Cy}}{\text{or +}}$ ♀♀

+		Cy		or Cy		or	
♀	♂	♀	♂	♀	♂	♀	♂
37	41	31	49	21	30	20	41

translocation individuals of both sexes. However, a single mating, $\frac{\text{III-IV A}}{\text{or Cy}}$ ♂♂ × or ♀♀ produced 5 abnormal Curly flies among the total of 346 (table 104), indicating the presence of duplication individuals. When

Table 104. $\frac{\text{T III-IV A}}{\text{or Cy}}$ ♂♂ × or ♀♀

+	or-Cy	or	Cy
185	156	0	5

$\frac{\text{III-IV A}}{\text{or Sc pr}}$ males were mated or pr females, the result shown in table 105,

Table 105. $\frac{\text{T III-IV A}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀

+	or-Sc-pr	pr-Sc	Sc	or-Sc
381	322	46	15	6

representing fly counts in four independent cultures, gave 46 pr Sc abnormal flies, which may be presumed to represent duplications. There were also present 15 Sc and 6 or Sc normal flies, the cause for their appearance

being unknown. The reciprocal cross as shown in table 106 produced 13

Table 106. $\frac{\text{T III-IV A}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		Single crossovers Reg.1		Single crossovers Reg.2		double c.o. 1, 2		total
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	104	83	0	5	3	5	0	1	201
2	126	97	0	1	1	3	0	0	228
3	103	106	0	7	3	3	0	0	222
	333	286	0	13	7	11	0	1	651

abnormal Sc pr flies and no or flies, indicating that crossing over is completely suppressed in this region. For the second region, namely between Sc and pr, crossing over amounts only to 2.9%, showing considerable reduction as compared with the control. Sc pr flies were repeatedly tested and found to be completely sterile. Homozygous translocation individuals were found to be inviable.

The genetic tests of the fourth chromosomes involved in the translocation show (tables 107 and 108) that the genes in and j are

Table 107. $\frac{\text{T III-IV A}}{\text{na or j}}$ ♂♂ × na in j ♀♀

+		na in j	
♀	♂	♀	♂
24	51	20	35

Table 108. $\frac{\text{T III-IV A}}{\text{na or in j}}$ ♀♀ × na or in j♂♂

Non-crossovers	
+	na-or-in-j
100	91

not included in the duplicating fragment, and that the locus of the breakage is so close to in and j that no crossovers are produced.

Fig. 30 shows the heterozygous III-IV A translocation. A piece of the third chromosome from section 64 to section 70 is deleted and intercalated into section 86 of the fourth chromosome. The fact that this transferred piece of the third includes the region common with the duplication in II-III A and II-III B, gives further support to the idea that the locus for or is localized in section 65. The considerable length of the deleted piece may account for the sterility of duplications and inviability of deficiencies.

Fig. 31 shows the combination of III-IV A duplication and 'narrow' inversion. This was observed in larva of the cross, $\frac{\text{III-IV A}}{\text{na or in j}}$ ♂♂ × na or in j ♀♀. It may be noticed in the figure that the part of the third chromosome from section 64 to section 67 is represented in triplicate. Beyond section 67, the chromosome is bifurcated, a single strand above, representing the inverted strand, and a strand of normal sequence below. The 'narrow' inversion starts at middle of section 70, but the III-IV A duplication runs a little over that point, thus the two overlapping each other in a very small region. Since III-IV A duplication does not suppress na, and neither does Y-III B duplication, the gene narrow must be located somewhere in sections 71 or 72, within the inversion.

Having learned that III-IV A duplication is considerably longer than that of either II-III A or II-III B on the right side, the author made the following matings to find the genetic length of the duplication. In the cross of $\frac{\text{III-IV A}}{\text{or ab pr}}$ males to or ab pr females

(table 109), three pr duplication flies were not abrupt, indicating that

Table 109. $\frac{\text{T III-IV A}}{\text{or ab pr}}$ ♂♂ × or ab pr ♀♀

+	or-ab-pr	pr
70	22	3

the locus ab is suppressed by the duplicating fragment, carrying its wild allel. In the cross of $\frac{\text{III-IV A}}{\text{na or in j}}$ males × $\frac{\text{or Sc pr}}{\text{Bl}}$ females (table 110), 8 Bl duplication flies appeared. However, these Bl flies, showing

Table 110. $\frac{\text{T III-IV A}}{\text{na or j in}}$ ♂♂ × $\frac{\text{or Sc pr}}{\text{Bl}}$ ♀♀

Culture No.	+	Bl	or	or-Bl
1	31	59 (4^x)	32	3
2	54	74 (4^x)	37	1

x - duplications

less extreme "bladeness" than normal heterozygous Blade flies, may be accounted for by supposing that they carry two wild allels and one Bl gene. This fact suggests that the duplication covers the locus Bl. Finally, in the cross $\frac{\text{III-IV A}}{\text{na or in j}}$ males to or Sc Ja females (table 111),

Table 111. $\frac{\text{T III-IV A}}{\text{na or in j}}$ ♂♂ × or Sc Ja

+		or	
Ja	Sc	Ja	Sc
22 (3^x)	16	26	31

x - duplications

three observed Ja duplications showed more extreme "jaggedness" than heterozygous Ja, indicating that the locus of the gene Ja is not included in the duplicating fragment. Since Ja is not suppressed in Y-III B duplication either, it seems very probable that it lies in section 71 or section 72.

Translocations that involve more than two chromosomes

Y-III-IV A, B

Cultures No. 79 and 38 (Table 50) show linkage of the genes located in third and fourth chromosomes, indicating that translocations involving these chromosomes are present. However, one may notice that in the wild type and Bare classes the males are more frequent than females, and in the or Cy and Ba or Cy classes females are more frequent than males. This led to the suspicion that not III-IV but Y-III-IV translocations a are involved, and that the few wild type and Ba females and or Cy and Ba or Cy males are deficiencies and duplications respectively. To prove that, wild type males from these cultures were crossed to or Cy females. The results are shown in table 112. Again the majority of wild type and

Table 112. $\frac{\text{Y-III-IV}}{\text{or}}$ ♂♂ × or Cy ♀♀

	+		Cy		or		or-Cy	
	♀	♂	♀	♂	♀	♂	♀	♂
Y-III-IV A	0	19	2	11	28	11	16	6
Y-III-IV B	1	9	1	6	3	1	3	0

Cy classes are males, and or Cy classes are mostly females. An experiment presented in table 113 shows the same result.

Table 113. $\frac{\text{Y-III-IV}}{\text{or Cy}}$ ♂♂ × or ♀♀

	+		or-Cy		or		Cy	
	♀	♂	♀	♂	♀	♂	♀	♂
Y-III-IV A	31	222	238	173	0	0	0	1
Y-III-IV B	5	82	73	38	0	1	0	0

On the supposition that the deficiencies and duplications may cover some of the third chromosome genes, the males and females heterozygous for T-Y-III-IV A and B and for or Sc pr were back-crossed to or pr females and males respectively (tables 114, 115, 116). The

Table 114. $\frac{\text{Y-III-IV}}{\text{or Sc pr}}$ ♂♂ × or pr ♀♀

	+		or Sc pr	
	♀	♂	♀	♂
Y-III-IV A	289		379	
Y-III-IV B	51	307	277	219

Table 115. $\frac{\text{Y-III-IV A}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		Single crossovers Reg.1		Single crossovers Reg.2		Double c.o. 1, 2		total
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	28	38	20	13	26	5	3	5	138
2	6	10	5	2	3	5	0	0	31
3	23	25	8	3	14	6	1	3	83
4	17	25	17	9	9	8	1	3	89
5	36	39	19	13	24	11	1	3	146
	110	137	69	40	76	35	6	14	487

Table 116. $\frac{\text{Y-III-IV B}}{\text{or Sc pr}}$ ♀♀ × or pr ♂♂

Cult. No.	Non-crossovers		Single crossovers Reg.1		Single crossovers Reg.2		Double c.o. 1, 2		tot.
	+	or-Sc-pr	or	Sc-pr	or-Sc	pr	Sc	or-pr	
1	40	79	12	1	12	7	0	0	151
2	10	24	1	0	3	0	0	0	38
3	9	22	5	0	13	0	0	0	49
4	4	7	0	0	7	0	0	0	18
	63	132	18	1	35	7	0	0	256

results show that none of the third chromosome gene tested is involved in the duplications or deficiencies. Moreover, the frequency of crossing over in the third chromosome is normal in Y-III-IV A, while in Y-III-IV B it is reduced (7.9% between or and Sc and 16.4% between Sc and pr). Similar tests were arranged for the fourth chromosome (table 117); again neither of the genes tested (incomplete and jaunty) showed

Table 117. $\frac{\text{Y-III-IV}}{\text{na in j}}$ ♂♂ × na in j ♀♀

	+		na-in-j	
	♀	♂	♀	♂
Y-III-IV A	31	86	90	29
Y-III-IV B	25	44	127	30

behavior indicating that these loci are not included in the duplications and deficiencies. Finally, the test with the fourth chromosome gene tangled^3 (tables 118, 119, 120) was successful, since in both translocations it is suppressed in duplication individuals, and manifests itself in deficiencies.

Table 118. $\frac{\text{Y-III-IV A}}{\text{na tg}^3\text{ Cy}}$ ♂♂ × na tg^3 ♀♀

+		na-tg^3-Cy		na-Cy	
♀	♂	♀	♂	♀	♂
9	30	38	2	6	12

Table 119. $\frac{\text{Y-III-IV B}}{\text{na in j}}$ ♂♂ × na tg^3 Cy♀♀

+		na		tg^3	
♀	♂	♀	♂	♀	♂
0	19	20	2	6	0

Table 120. $\frac{\text{Y-III-IV B}}{\text{na in j}}$ ♂♂ × p or tg^3 ♀♀

+		tg^3	
♀	♂	♀	♂
44	81	8	0

Further study of these two translocations was carried through with the aid of the cytological method. The salivary gland chromosome configuration for Y-III-IV A is shown in fig. 32. It shows that the part of the fourth chromosome including sections from 97 on to the free end is attached to the chromocenter. The part of the chromocenter to which this section of the fourth chromosome is attached is associated with the spindle attachment of the third chromosome. In the light of the genetic data given above, the probable feature of this translocation may be that each arm of V-shaped Y-chromosome receives a transferred piece, one from the fourth chromosome including from sections 97 on to the free end, and the other from the third chromosome including a part of its heterochromatic region.

Fig. 33 represents the heterozygous Y-III-IV B translocation. The third chromosome is broken; and the basal part is attached to the chromocenter. The major part of the third from section 64 to the free end is attached at the junction of sections 93 and 94 in the fourth chromosome. The piece of the fourth chromosome involving section 94-99 does not unite with the broken end of the third, but is attached independently to the chromocenter. In another larva, a duplication for the broken off section of the fourth chromosome is found as shown in fig. 34. The duplicating fragment is attached to the chromocenter. The simple interpretation of the situation in this translocation, therefore, is that a long piece of the third chromosome is transferred to

the fourth, a section of which in turn is attached to the Y. Probably Y has also lost a section which is united with the broken end of the third chromosome near the chromocenter region, but this has not been established directly. Anyway, the cytological analysis seems to fit the interpretation of the genetic results given above, namely that the females with tilted wings and minute bristles represent deficiencies. Indeed, when $\frac{\text{Y-III-IV B}}{\text{na or in j}}$ males were mated to p or tg^3 females, or $\frac{\text{Y-III-IV B}}{\text{na in j}}$ males were mated to na tg^3 Cy females (tables 119 and 120), some tg^3 females with tilted wing and minute bristles were produced. Duplications and deficiencies in both Y-III-IV A and Y-III-IV B have been proven to be fertile.

T II-III-IV

This is a translocation which involves three long autosomes. As shown by the results of the crosses given in tables 121 and 122, these translocations produce viable duplication for or. They have

Table 121. $\frac{\text{T II-III-IV A}}{\text{Ba or Cy}}$ ♂♂ × or ♀♀

+		Ba-or-Cy		Ba-Cy	
♀	♂	♀	♂	♀	♂
34	24	60	43	10	8

Table 122. $\frac{\text{T II-III-IV A}}{\text{p or } tg^3}$ ♂♂ × p or tg^3 ♀♀

Culture No.	+	p-or-tg^3	p-tg^3
1	136	109	41
2	32	34	6

minute bristles and rough eyes. When tested, they were proved sterile.

In the salivary gland chromosomes of this heterozygous translocation, a complicated configuration has been observed. Fig. 35 shows all that can be reported for this translocation. A long piece of the second chromosome from section 51 on is translocated to section 93 of the fourth chromosome. A long piece of the third chromosome from section 66 on to the free end is transferred to section 44 of the second chromosome. The basal portion of the third chromosome, though not clear in the figure, probably receives sections 47 and 48, which are paired with their homologues, from the second chromosome and free end from the fourth chromosome. As shown by the loop region of the fourth chromosome, a part of it can be homologized with sections 92 and 93, indicating an intra-chromosomal translocation in the fourth chromosome. Since this translocation is too complicated and has proven not of much use for constructing cytological maps, it was not attempted to work out details of various breaks. However, the duplication Ba Cy and p tg^3 flies shown in table 121 and 122 probably represent the duplication of the whole rebuilt chromosome, consisting of the third chromosome basal end (including the locus or), sections 47 and 48 of the second chromosome, and the free end of the fourth chromosome.

T Y-II-III-IV A

As shown in table 50, strain No. 8 gave almost exclusively wild type males and Ba or Cy females, indicating a translocation involving Y, second, third and fourth chromosomes. Further results of tests (table 123) suggested that duplications or deficiencies are not viable.

Table 123. $\frac{T\ Y\text{-}II\text{-}III\text{-}IV}{p\ or\ tg^3}$ ♂♂ × p or tg^3 ♀♀

+		p-or-tg^3	
♀	♂	♀	♂
0	130	140	0

Heterozygous translocations seem, however, to be completely viable.

Salivary chromosomes of this translocation have been studied and found to involve very complicated rearrangements. The details have not been worked out. An outline sketch of one figure is shown in fig. 36. It can be interpreted so that the second chromosome is broken into three pieces, the short free end is transferred to the fourth chromosome, a long middle section is intercalated into the third chromosome, and the basal portion seems to receive a long inverted segment from the fourth chromosome. The very tip of the fourth chromosome is removed, and probably united to Y. The purpose of presenting this sketch is merely to show that individuals heterozygous for such an enormous amount of chromosome rearrangement can still be completely viable and fertile.

T-II-III-IV-V A

In view of the irregular distribution of different classes of offspring obtained from the strain No. 174 as shown in table 50, salivary chromosome configuration was immediately examined. Fig. 37 is one of the two unusually clear pictures which were found for this heterozygous translocation. A very long second chromosome strand is transferred to about section 67 of the third chromosome. The section of the third, from section 67 on to the free end ~~containing~~ with the region from section 68 to 77, in inverted order, is transferred ~~in~~

~~inverted order~~ to section 96 of the fourth chromosome. A short fourth chromosome piece is transferred to the fifth chromosome. Since the genetic data (table 124) indicate that Y-chromosome is involved in this translocation, it seems reasonable to assume that a piece of Y-chromosome is transferred to the broken basal region of the second chromosome.

Table 124. $\frac{TY\text{-}II\text{-}III\text{-}IV\text{-}V\ A}{p\ or\ tg^3}$ ♂♂ × p or tg^3 ♀♀

+		p-or-tg^3		p-or		tg^3	
♀	♂	♀	♂	♀	♂	♀	♂
2	65	80	13	1	31	5	21

The results presented in table 124 show a number of different exceptional classes of flies. In the light of the cytological observations, the interpretation of the respective classes of individuals might be as follows: wild type females may represent non-disjunction (XXY), p or tg^3 males may represent the other non-disjunctional class (XO), p or males may be XO bearing a duplication for the transferred part of the fourth chromosome, which suppresses tg^3. tg^3 males may represent deficiencies for the part of the fourth. p or females may represent XXY with a deficiency for the section of the fourth. The above interpretation may be entirely wrong. Nevertheless, it cannot be doubted that the locus of the wild allel of tg^3 is located in the broken off part of the fourth chromosome which is transferred to the fifth.

Interracial Inversions

In two previous papers (Tan 1935a, b) the author reported that the two races of Drosophila pseudoobscura differ in four long inverted sections, one each in the left limb of X-chromosome, right limb

of X-chromosome, second chromosome and third chromosome, and in two constantly unpaired regions in the left limb of X-chromosome. Since the publication of the above, the two very short unpaired regions in the left limb of X-chromosome, which were once suspected to be inversions too, have actually been observed paired in the hybrids of the two races by Dobzhansky and Tan (1936).

Figures 38 and 39 show interracial inversions of the second chromosome. The lower limit of the inverted section lies in the intersection between section 51 and section 52 (fig. 38) and the upper limit is within section 56 (fig. 39). That gene Ba can not be transferred from race A to race B (unpublished data of Sturtevant and Dobzhansky) can be accounted for by the fact that the locus Ba lies within the inverted section, as has been indirectly demonstrated in II-III A translocation.

Figures 40 and 41 illustrate the interracial inversion in the third chromosome. The upper limit is shown (fig. 40) to lie within section 78 and the lower limit near the intersection between section 70 and section 71 (fig. 41). The study of Y-III A and Y-III B translocations has demonstrated that the loci Sc and pr lie in a region from section 73 to 78, which is almost entirely included in the interracial inversion. This may explain very well the fact that genes Sc and pr can not be easily transferred from race A to race B. On the other hand, gene or, which has been located in section 65, can be transferred from one race to the other very easily.

Construction of Cytological Maps

The previous section has presented the evidences upon which various genes are located in the salivary chromosomes. Fig. 42 shows the composite pictures of the second, third and fourth chromosomes of Drosophila pseudoobscura, and the various breaks observed in the translocations studied are indicated by the lines above. Below each chromosome are shown the corresponding genetic maps which represent the spatial relations of the genes in terms of the crossover units.

In the second chromosome Sm, giving ~~almost no~~ very little crossing over with the break of T II-III B, probably lies ~~very~~ close to the spindle attachment. Judged from the fact that the break of T II-III A does not reduce the frequency of crossing over in the region between pink and bithorax on one hand and pauciseta on the other, the gene loci for both bi and p may be localized to the right of the break of T II-III A. Gene gl, being suppressed by the duplicating fragment of T II-III(V) B and not by that of Y-II C, must be located somewhere between sections 57 and 60. The gene ps, being covered by no other duplicating fragment than Y-II C, lies, consequently, closest to the free end of the second chromosome.

In the third chromosome the gene or has been localized within a narrow region of section 65, which is in common for duplications II-III A, II-III B and III-IV A. Genes Bl and ab, being suppressed by III-IV A duplication but not by that of either II-III A or II-III B, are located somewhere between sections 66 and 70. Since Ja is not covered by either Y-III B or III-IV A duplicating fragments, it must lie in sections 71 or 72. Sc and pr, which are included in the duplicating

fragment of Y-III B, but not that of Y-III A, may be located somewhere from sections 73 to 78. The known locus nearest to the free end of the third chromosome is cv, which is included in Y-III A duplicating fragment.

Comparatively few genes are known in the fourth chromosome. Near the spindle attachment is the gene in. The gene, j, being covered by Y-IV D duplication, is located in sections 86 to 88. Since the locus tg^3 is covered by the duplicating fragments of Y-III-IV A and Y-II-III-IV-V A, it must lie to the right of section 96. The loci for genes Cy and Ro, known to lie to the right of tg^3, may be located very near to the free end of the fourth chromosome.

DISCUSSION

A comparison of genetic and cytological maps of the autosomes of Drosophila pseudoobscura, shown in fig. 42, reveals several interesting facts which seem to agree fairly well with the generalizations resulting from study of the metaphase chromosome maps of Drosophila melanogaster by Painter and Muller (Painter and Muller 1929, Painter 1931, Muller and Painter 1932) and by Dobzhansky (1929a,b, 1930a,b, 1931a, 1932b,c,d), and of the salivary chromosome maps of the X and third chromosome of the same species recently established by Painter (1934a, 1935). In both melanogaster and pseudoobscura, the linear seriation of genes has been found identical in the genetic and the cytological maps. This corroborates the hypothesis of the linear arrangement of genes within the chromosomes which was advocated by Morgan and by Sturtevant, and which has been one of the main working hypotheses in genetics.

It has been found in Drosophila melanogaster, in both metaphase and salivary chromosome maps, that the relative distance between genes in the genetic and cytological maps appear to be widely different. The genes lying near the spindle attachment are relatively much farther apart on the cytological than on the genetic map. For genes lying in the middle of chromosome the reverse is observed. Such discrepancy is due to the variable frequency of crossing over per unit length in the different parts of the chromosomes.

As shown in fig. 42, the positions of the loci in the three autosomes of Drosophila pseudoobscura seem, in general, to correspond to crossover values of the genetic maps. It should be taken into account, however, that in most instances we have been able to place genes only within general limits of several sections, and that we know nothing, at present, regarding the distribution within these limits.

Of the three chromosomes, the map of the third presents some evidence that crossing over occurs less frequently in the spindle fiber region than near the free end. In the salivary gland chromosome, the locus Scute is about equidistant between the loci of orange and crossveinless. On the genetic map, however, Scute is 28.8 units apart from orange but 39.6 units from crossveinless. This is apparently due to the higher frequency of crossing over in the region between Sc and cv than between Sc and or.

In regard to the second and fourth chromosomes, the evidence is less conclusive, partly due to the presence of long gaps between the loci of Smoky and bithorax in the second chromosome, and between the loci incomplete and jaunty in the fourth chromosome. Still, genes

pink and bithorax in the second chromosome appear to lie relatively farther to the right in the salivary chromosome than in the crossover map, indicating that the frequency of crossing over, in terms of the cytological map, is higher in the region between bithorax and the free end than between bithorax and the spindle attachment. The same explanation may also be applicable to the fact that the cytological location of the gene $\underline{tg}^3$ in the fourth chromosome is relatively farther to the right from its position in the corresponding genetic map.

A survey of breakage points in those translocations in the three autosomes presents an interesting picture. The frequency of breaks is not necessarily proportional to the length of a chromosome. According to Muller and Altenburg (1930) and Patterson, Stone, Bedichek and Suche (1934), the frequency of breakages due to the X-ray treatment is roughly proportional to the length of a chromosome. This is true with the V-shaped Y-chromosome of Drosophila pseudoobscura, which takes part in the translocations more frequently than any autosomes. But among the three autosomes, the third chromosome is shortest and yet more breaks were observed in this than in other two autosomes. Here, probably, a different factor is involved. According to the unpublished data of Sturtevant and Dobzhansky, inversions are found most frequently in the third chromosome in the different strains of Drosophila pseudoobscura and Drosophila miranda, Dobzhansky and Tan (~~1935~~, 1936) have found that the third chromosome underwent maximum changes between the two species. All sources of data seem to indicate that the third chromosome is more susceptible to breakages than the other two autosomes. Assuming that the origin of the different kinds of chromosome rearrangements, such as inversions, intra-chromosomal translocations, inter-

chromosomal translocations, deletions, etc., involve a similar mechanism, we may be led to believe that there is a definite inherent property in the third chromosome that rendered it more easily breakable.

In an attempt to explain the distribution of a majority of breaks in the spindle attachment region of these chromosomes in induced II-III and III-IV translocations in Drosophila melanogaster, Patterson, et al (1934) and Painter (1935) suggested that the physical basis for the exchanges of chromosomes is the close contact or proximity of the parts of chromosomes involved during or immediately after radiation. Since the spindle fiber regions of chromosomes are supposed to be closest to each other in mature spermatozoa, which are the main source of translocations obtained by X-ray, the chances of exchanges taking place in these regions should be better than in the regions away from them. This is true in third chromosome of Drosophila pseudoobscura as shown in fig. 42, in which more breaks are located near the chromocenter than in the free end region and the middle region combined. In the second and fourth chromosomes, however, such relation does not seem to exist. As a matter of fact, three of the four Y-II translocations involve breakages within sections 48 and 49, close to the middle of the second chromosome, and four out of five Y-IV translocations have breakages within the sections 91 to 94 in the fourth chromosome, far away from the spindle attachment. These observations suggest on one hand that it is not always that majority of breaks fall in the chromocenter region, and on the other hand that there may exist a definite relation between parts of particular chromosomes at the time when chromosome rearrangements are induced.

Probably, at the time when sperms are irradiated, the chromosomes may form a more or less regular pattern, so that exchanges would occur mostly in the regions where chromosomes come in contact. Such contacts may be in the spindle fiber regions, middle regions or free end regions, depending on the arrangement of the chromosomes in the pattern.

On the supposition that the loci of exchanges in chromosomes are due to proximity of contacts in the mature spermatozoa, a longer chromosome should be subject to alterations more frequently than shorter chromosomes. It is possible and probable that the high frequency of breaks in the third chromosome is due to the formation of loops, for indeed the wide occurrence of inversions in nature and presence of an inversion and three excisions among the 22 translocations obtained by irradiation furnish some evidence that loop formation is highly characteristic of the third chromosome.

Translocations and inversions modify the order of the genes in the chromosome, produce new linkage relations, and alter the mode of inheritance of the genetic factors located in the chromosomes involved. They need not, however, affect either the number or the kind of the genetic factors, and therefore individual heterozygous or homozygous for these chromosome rearrangements should be normal in appearance and in viability. These theoretical expectations are, however, not always realized in both Drosophila melanogaster and Drosophila Pseudoobscura. The first translocation discovered in Drosophila melanogaster (Bridges 1923, Bridges and Morgan 1923) rpoduces in heterozygous condition a dominant effect on the eye color and is lethal when

homozygous. Muller (1928a) found that a majority of induced translocations are inviable in homozygous condition. Muller's findings were corroborated by observations of Muller and Altenburg (1930), Dobzhansky (1929b, 1930a, 1931a, 1932a), Dobzhansky and Sturtevant (1931), Oliver (1932) and many others. Although some translocations are viable and normal in appearance in homozygous condition (Dobzhansky 1929a, 1931a), this is an exception rather than the rule. Not infrequently translocations produce visible external effects (Muller 1930, Burkart 1931).

As pointed out in previous sections, individuals heterozygous for translocations are mostly fully viable and fertile in Drosophila pseudoobscura, regardless of the number of chromosomes involved. This is partly because the technique used for obtaining translocations would tend to eliminate the inviable or poorly viable heterozygous translocations. But in only one case, namely T III-IV B, homozygous translocations are viable and normal in appearance.

The viability and fertility of heterozygous duplications and deficiencies depends primarily on the length of the piece involved. Duplications for more than half of the length of a chromosome, such as Y-II A, Y-II B and Y-II D, are non-viable. Deficiencies are, in general, inviable, except for a very small section. These observations seem to agree with those on Drosophila melanogaster.

The behavior of heteroploids may also vary with different regions of a chromosome. In the third chromosome of Drosophila pseudoobscura, T II-III A and T II-III B duplicating pieces, covering the sections lying close to the spindle attachment in the normal chromosome, are viable and fertile in both heterozygous and homozygous

condition, the corresponding deficiencies are however viable and fertile only in heterozygous condition. Flies carrying the duplicating piece of T Y-III A, which includes the free end of the third chromosome, are viable only in heterozygous condition, and moreover they are sterile. The deficiencies are non-viable. Since the lengths of these duplicating fragments are approximately the same, there may be a difference between the part of chromosome near spindle attachment and that near the free end with respect to the viability and fertility in duplication and deficiency.

It is, however, interesting to note that heterozygous deficiencies for even so short a piece of the third chromosome as Y-III A are lethal in pseudoobscura, and yet in Drosophila miranda males withstand a deficiency for the whole of X^2 not only without apparent harm, but even without much modification of the morphological and physiological characteristics (Dobzhansky and Tan 1936). Since the X^2 and III are partly homologous and both include the region of Y-III A translocation, the possible explanation would seem to be, as pointed out by Dobzhansky and Tan (1936), that, despite their apparent close similarity, miranda and pseudoobscura are very different in their genic balance.

Following the discovery by Muller of the induction of chromosomal rearrangements by X-rays, a large number of translocations were described in Drosophila melanogaster, and a majority of these were interpreted to be simple ones (Muller 1930, Dobzhansky 1931b). But after the introduction of the study of salivary gland chromosomes, this interpretation was proved to be erroneous, since most of the presumed simple

translocations were found to involve an exchange of very short sections of one chromosome for relatively long sections of other chromosome. Indeed, if a very short section containing no loci of known genes is transferred from one chromosome to another, the translocation in which such a transfer has taken place may appear to be a simple one, both when studied genetically and when studied cytologically in metaphase plate chromosomes. This fact has led some authors to assume that all translocations are reciprocal, and no simple translocations occur. The three cases of non-reciprocal translocations described in the previous section, especially T II-III A, which has been very carefully studied cytologically, show this extreme opinion to be erroneous likewise, though these cases are like the usual reciprocal translocations, in that they do not involve unions except at breakage points. How such a translocation which involves two breakage points in one chromosome and only one in the other can occur is not known, though Kossikov and Muller (1935) gave a hypothetical scheme for it.

Comparison of genetic maps of the chromosomes of different species has been possible in very few cases only due to the extreme laboriousness of this method. The best examples are Drosophila melanogaster and Drosophila simulans which were found to differ in a long inversion in the third chromosome (Sturtevant and Plunkett 1926, Sturtevant 1929). Recently these species were reinvestigated with the aid of the salivary gland chromosome method, with the result that some minor alterations of the gene alignment were detected besides the third chromosome inversion (Patau 1935, Kerkis 1936). The genetic maps of

the two species are in perfect agreement with their cytological abnormalities.

Metz (1916) and Lancefield (1922) have both suggested that one arm of V-shaped X-chromosome of Drosophila pseudoobscura corresponds to part of the rod shaped X-chromosome of such species as Drosophila melanogaster. Koller (1932b) further suggested that the other arm of the V-shaped X corresponds to a portion of one of the autosomes in those species (such as melanogaster) with a rod-shaped X-chromosome. More recently Crew and Lamy (1935) attempted to homologize part or the whole of the second chromosome (third chromosome of Crew and Lamy) of Drosophila pseudoobscura to the left arm of the V-shaped third chromosome of melanogaster, and fourth chromosome of pseudoobscura to the right arm of V-shaped second of melanogaster. In view of the facts that relatively few genes are now known in Drosophila pseudoobscura and that the two species are not crossable to effect direct test, it seems to the author that attempts to establish the homology of genetic maps of the two species are rather hazardous. Nevertheless, it is possible that a part of the third chromosome of Drosophila melanogaster is homologous to a part of the second chromosome of Drosophila pseudoobscura. This guess is based upon the evidence that most of the known genes found in the latter, such as p, gl and bi, produce similar external effects as genes reported in the former. The gene Smoky of pseudoobscura and the gene delta of melanogaster resemble each other phenotypically, and are perhaps homologous. Bare of pseudoobscura and Stubble of melanogaster, both being dominant and affecting the shortening of macrochaete, may likewise be

the same. However, the sequence of these genes is not the same in the two species. Like the chromosome differences between Drosophila pseudo-obscura and Drosophila miranda the change in sequence of genes may be due to a number of alterations within the chromosome, such as inversions or intra-chromosomal translocations.

In discussing the distribution of sterility factors in terms of cytological maps, it is interesting to note that the parts of chromosome carrying interracial inversions are likely to contain numerous or powerful sterility genes. Using testis size as an index of the degree of the departure from the normal structure of the testis leading to sterility, Dobzhansky (1936) has shown that there exist in each of the X-chromosome, the second chromosome and the third chromosome of Drosophila pseudoobscura more than one genetic factor causing the sterility of the interracial hybrids. Moreover, he found that the part of second chromosome carrying the gene Bare and the part of the third chromosome carrying the gene purple are more effective in producing sterility as compared with the part of the second carrying the gene Smoky and the part of the third carrying the gene orange. Since it is now known in the cytological maps that genes Bare and purple definitely lie within the interracial inversions of the second and third chromosomes respectively, and that the genes Smoky and orange, lying close to the spindle attachment in their respective chromosomes, are located quite far away from the inverted regions, the relation between interracial inversions and increasing effectiveness of sterility factors becomes apparent.

In so far as the direct causation of hybrid sterility by interracial inversions is concerned, it has been directly and indirectly disapproved. Directly, it has been shown by Dobzhansky (1936) that the fourth chromosome plays a part in causing the sterility of the hybrids, despite the fact that this chromosome is similar in arrangement in both race A and race B (Tan 1935b).

Indirectly, again it has been shown by Dobzhansky (1933a, 1934a) that the doubling of the chromosome complement in the interracial hybrids in Drosophila pseudoobscura does not lead to an increase in the frequency of chromosome pairing at meiosis, nor to restoration of fertility, indicating that sterility here is not due to difference in gross chromosome structure.

To account for the relation of parts of chromosomes associated with interracial inversions and the increasing effectiveness of producing sterility, two explanations seem to be possible. First, since most crossing over in the hybrid occur in the region between spindle attachment and the lower limit of inversion, the resulting part of chromosome including inverted region is in most cases considerably longer than that without it. As a consequence, the increasing effectiveness of producing sterility in the hybrids on the part of Ba region and pr region is due to the length of chromosome section involved rather than the presence of inversion. Second, the possibility that sterility is somehow indirectly connected with these interracial inversions is also not excluded. The gene mutations that cause sterility might have been in some manner connected in their origins with the origin of the inversions,

just as some mutations, or position effects, are connected with translocations and inversions.

SUMMARY

1. The genetic maps of the three autosomes of Drosophila pseudoobscura are shown in figure 42.

A. The second chromosome is 92.9 units long, and the sequence of genes is Smoky, bithorax, pink, Bare, glass and pauciseta.

B. The third chromosome is 68.4 units long, and the sequence of genes is orange, Blade, abrupt, Jagged, Scute, purple and crossveinless.

C. The fourth chromosome is 69.2 units long, and the sequence of genes is incomplete, jaunty, tangled, Curly and Rough.

2. Twenty-two translocations have been obtained with the aid of X-ray. They involve different combinations of chromosomes, exclusive of the X-chromosome, as follows:

Frequency of Translocation	Nature of Translocation
4	Y-II translocations
2	Y-III "
5	Y-IV "
3	II-III "
3	III-IV "
2	Y-III-IV "

Frequency of Translocation	Nature of Translocation
1	II-III-IV translocation
1	Y-II-III-IV "
1	Y-II-III-IV-V "

3. Most of the translocations that involve the Y-chromosome give viable individuals heterozygous for duplications. They were tested for the suppression of genes; this method is very efficient for the localization of genes on the cytological maps.

4. Two of the II-III translocations and one of the III-IV translocations are non-reciprocal. They involve excisions of pieces of the third chromosome near the spindle attachment, including the gene orange. The excised piece is intercalated into the second chromosome in the case of the II-III translocations, and into the fourth chromosome in the case of the III-IV translocation. Since the duplication flies in all the above three cases suppress the gene orange, the locus for it has been determined to lie within the limits of a few bands in section 65 of the third chromosome.

5. In reciprocal translocations, the heteroploid flies carrying duplication for a section of one chromosome necessarily carry also a deficiency for a section of another chromosome. Such flies are completely inviable in all cases studied. The genetic tests for breaks here, therefore, are limited only to crossing over experiments.

6. In only one case, namely T III-IV B, individuals homozygous for the translocation are viable, fertile, and normal in appearance.

7. The salivary chromosome configurations of all heterozygous translocations were studied for the determination of the exact nature

of the translocations and of the points of breakage.

8. By combining the genetic data and cytological data for 22 translocations, the salivary chromosome maps of the three autosomes are established, as shown in figure 42, with the various breaks of translocation indicated by the lines above.

9. Due to the irregular distribution of breaks and genes along the genetic maps, most genes are located in the salivary chromosomes only within general limits of several sections.

10. Comparisons of genetic and cytological maps of Drosophila pseudoobscura have established the following facts:

A. That the genes are arranged in a linear sequence.

B. That the morphological positions of the loci in the three autosomes roughly correspond to crossover values on the genetic maps.

C. That the lengths of the salivary chromosomes are fairly proportional to the lengths of the genetic maps.

D. That the genes lying near the spindle attachment are relatively ~~much~~ farther apart in terms of the cytological than in terms of the genetic map.

E. That parts of the second and the third chromosome which are more effective in producing sterility in A/B hybrids are located in the regions involving interracial inversions.

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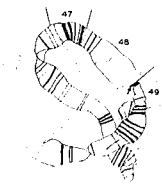
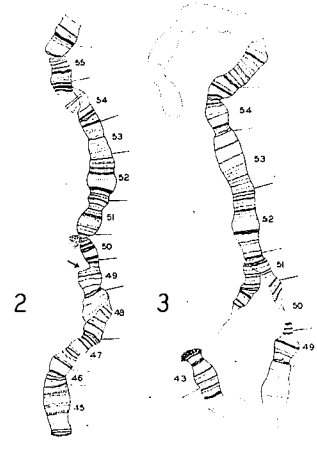
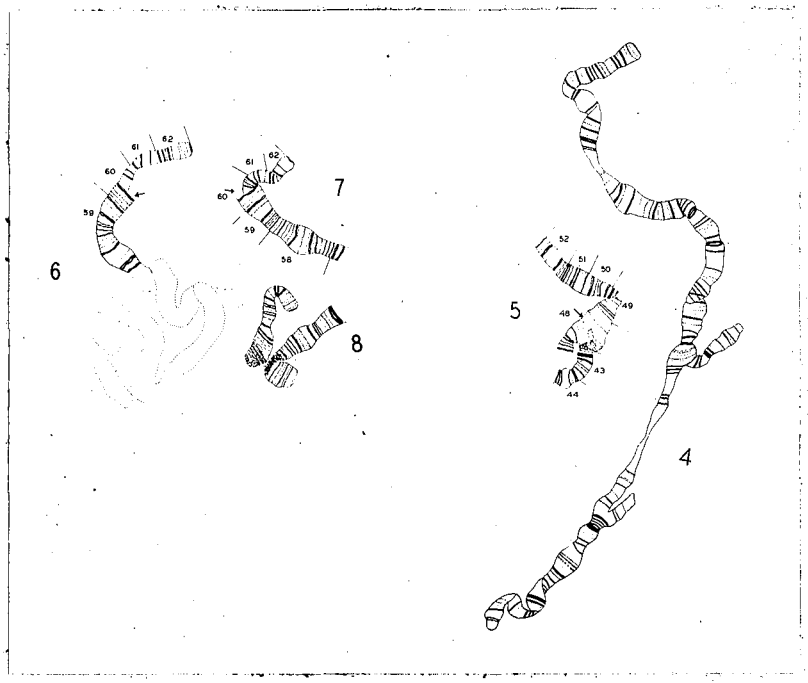


1

Figure 1.— From a larva heterozygous for translocation Y-II A



Figures 2 and 3.— From two cells of a larva heterozygous for translocation Y-II B



Figures 4 and 5.-- From two different larvae heterozygous for translocation Y-II I

Figure 6.-- From a larva heterozygous for translocation Y-II C

Figures 7 and 8.-- From a single cell of a larva heterozygous for translocation Y-II C

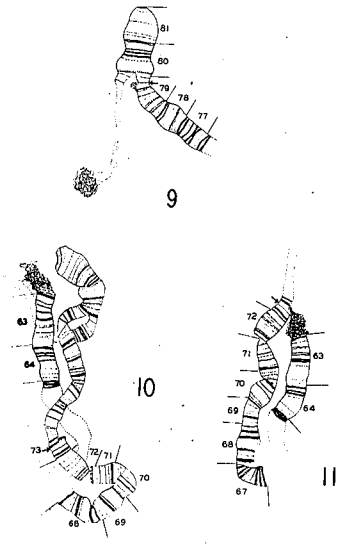


Figure 9.-- From a larva heterozygous for translocation Y-III A

Figures 10 and 11.-- From two separate cells of a larva heterozygous for translocation Y-III B

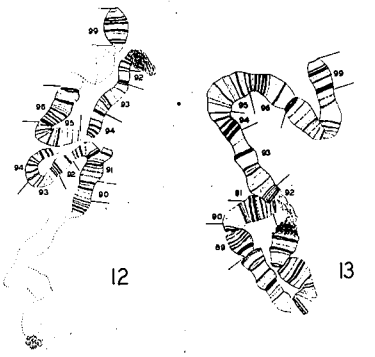
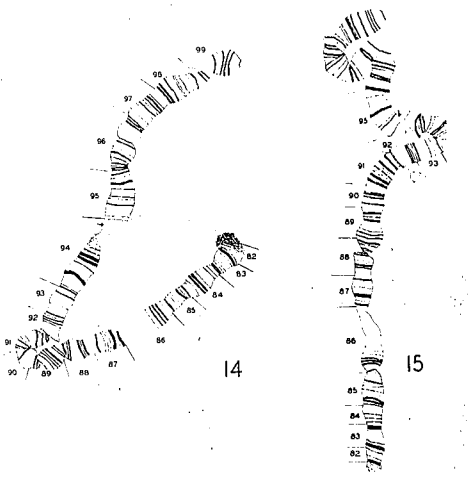
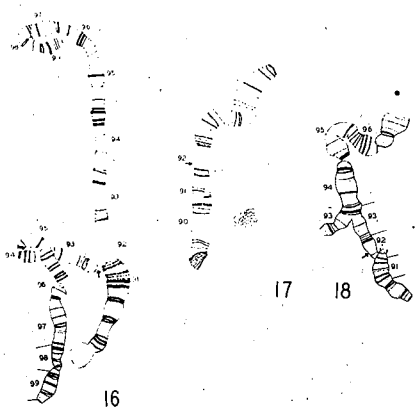


Figure 12.— From a larva heterozygous for translocation Y-IV A

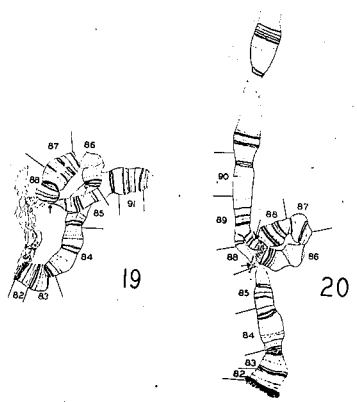
Figure 13.— From a larva heterozygous for translocation Y-IV B



Figures 14 and 15.— From two separate cells of a larva heterozygous for translocation Y-IV C



Figures 16-18.-- From larvae heterozygous for translocation Y-IV E



Figures 19 and 20.-- From two separate larvae heterozygous for translocation Y-IV

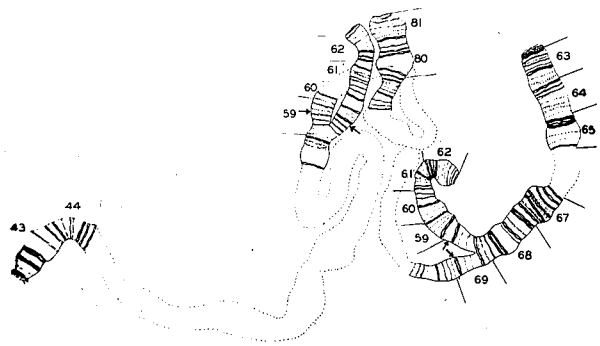


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Figure 21.— From a larva heterozygous for translocation II-III C

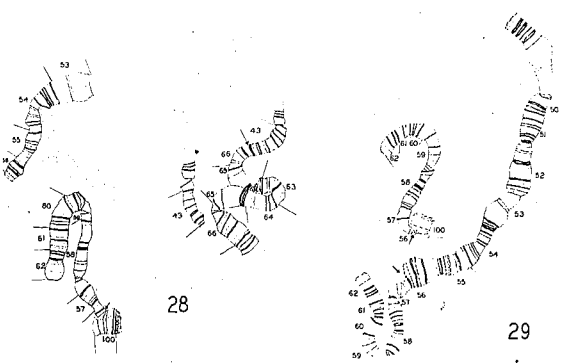

Figure 28.-- From a larva hyperploid for II_III B translocation and heterozygous for II_V part.

Figure 29.-- From a larva heterozygous for II-III-(V) translocation, showing exchanges between II and V chromosomes.

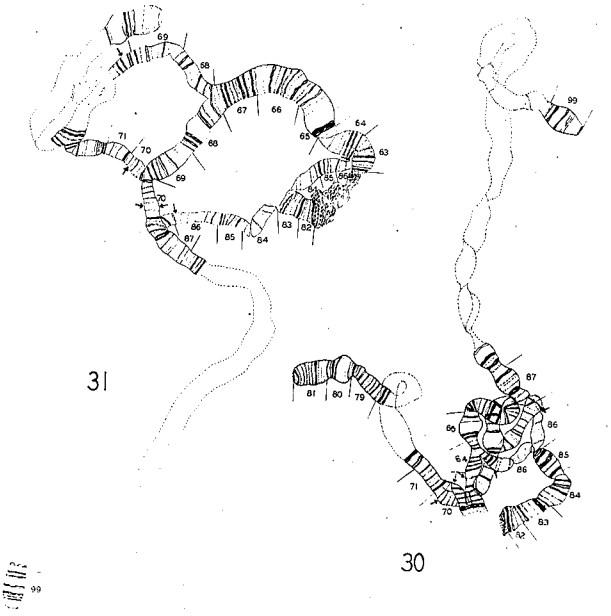

Figure 30.-- From a larva heterozygous for translocation III-IV A

Figure 31.-- From a larva hyperploid for translocation III-IV A and heterozygous for 'Arrowhead' inversion.

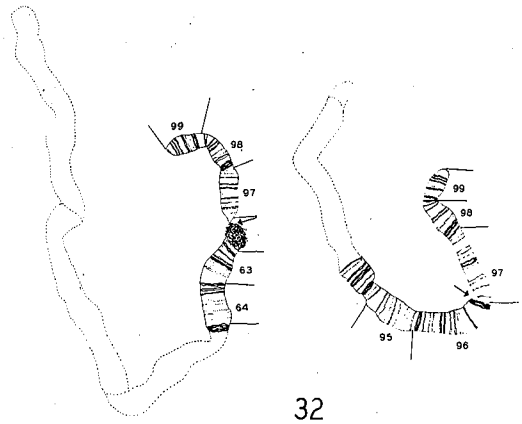

Figure 32.-- From a larva heterozygous for translocation Y-III-IV A.

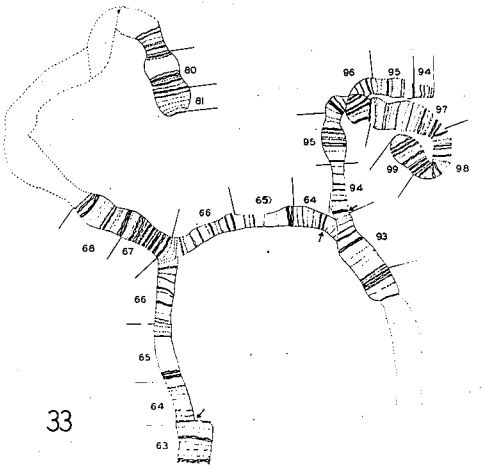

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Figure 33.-- From a larva heterozygous for translocation Y-III-IV B.

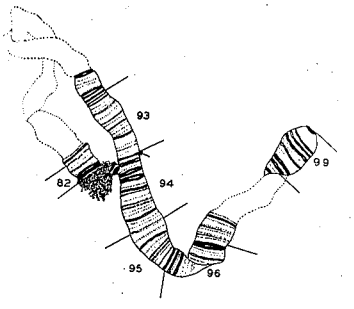

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Figure 34.-- From a larva hyperploid for translocation Y-III-IV B, showing a triplicate piece of the fourth chromosome attached to the Y-chromosome at chromocenter.

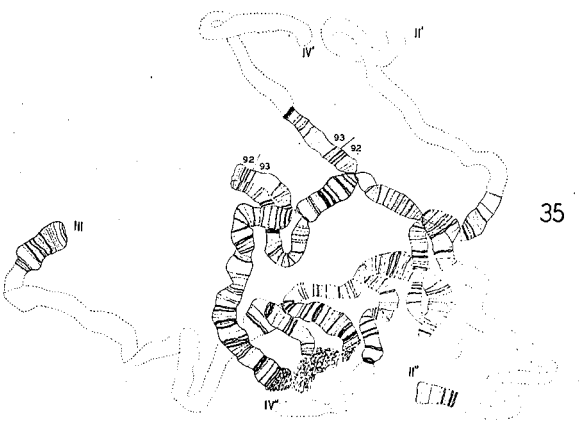

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Figure 35.— From a larva heterozygous for translocation II-III-IV A.

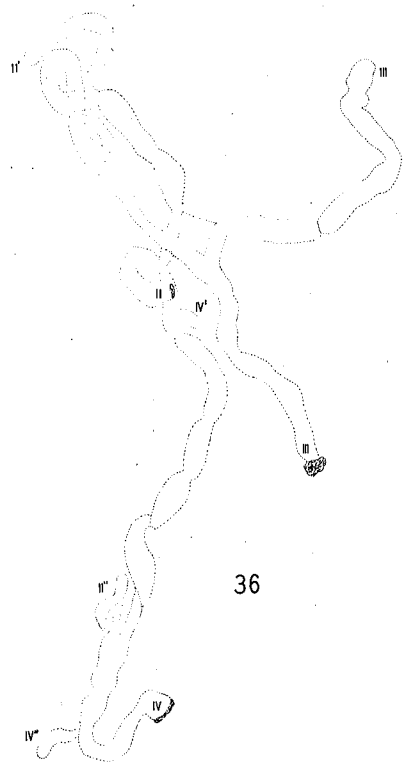


Figure 36.-- An outline sketch drawn from a larva heterozygous for translocation Y-II-III-IV A.

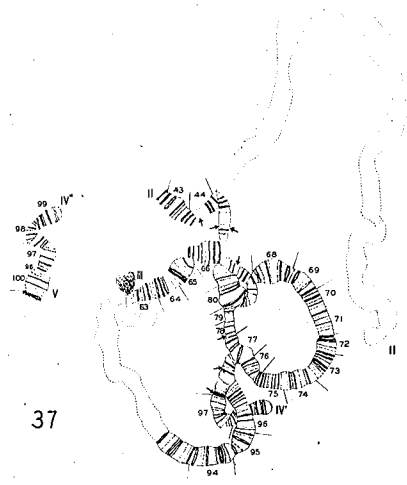
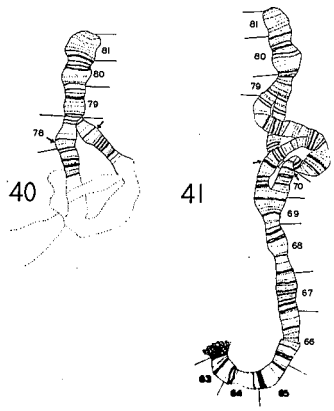


Figure 37,— From a larva heterozygous for translocation Y-II-III-IV-V A.

Figures 38 and 39.— From larvae of A/B hybrids, showing interracial inversion in the second chromosome.



Figures 40 and 41.— From larvae of A/B hybrids showing interracial inversion in the third chromosome.

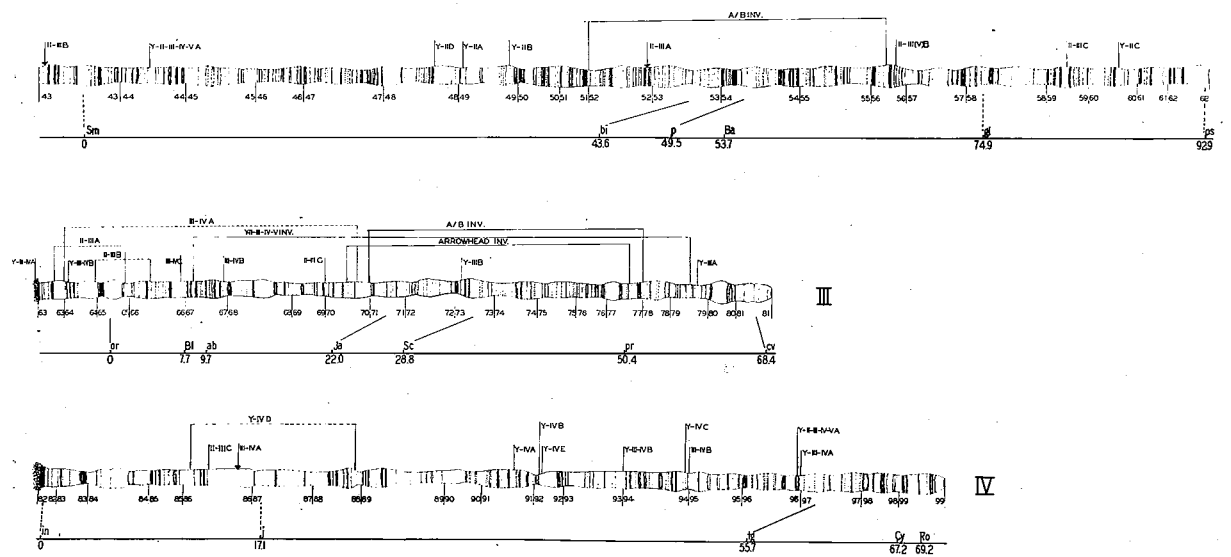


Figure 42.—II- A cytological map of euchromatic area of the second chromosome showing the typical morphology as this appears in salivary glands.

III- A cytological map of euchromatic area of the third chromosome showing the typical morphology as this appears in salivary glands.

IV- A cytological map of euchromatic area of the fourth chromosome showing the typical morphology as this appears in salivary glands.

The crossover scale of genetic map is shown below each chromosome and translocation breakage points are marked by the lines above the chromosomes.

The dotted lines above the chromosomes represent limits of excisions.

The solid lines above the chromosomes represent limits of inversions.