A GENETIC AND CYTOLOGICAL STUDY OF SOME X-RAY INDUCED MUTATIONS AND REVERSE MUTATIONS IN DROSOPHILA MELANOGASTER

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ABSTRACT

The literature pertaining to the problem of mutations, with special reference to reverse mutations and position effect, is reviewed. Data are presented from genetic and cytological studies of several reversions at the Dichaete and Glued loci, of forty-six cases of position effect at the white locus, thirty-six mutations at the light locus and twenty-five mutations at the straw locus. Three of the mutations at the light locus and one of the mutations at the straw locus are shown to be position effects. One case of position effect at the Bar locus and two new mutants, Antennapedia and Scarred, are described. These reversions and mutations were induced by irradiation with X-rays. The effects of adding and subtracting Y-chromosomes, and of temperature upon the light-mutant character of mutants resulting from a position effect at the light locus are demonstrated.

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PART ONE. INTRODUCTION

Mutations have been induced by ionizing radiations in a great many organisms. The most intensive and detailed work with X-rays has been done on Drosophila melanogaster. By the term mutation is generally meant, in a broad sense, any type of genotypic (as opposed to plasmatic) changes, involving qualitative or quantitative changes in the set of genes (Timoféeff-Ressovsky 1934). The genotypic changes can be classified according to the unit of change as gene mutations, chromosome mutations and karyotype mutations. In its most restricted sense, the term mutation refers to "gene mutation", which implies change in the composition of individual genes. The changes of the chromosome structure such as translocations, inversions, duplications and deficiencies are regarded as chromosome mutations or chromosomal rearrangements; while the changes of the chromosome number such as trisomics, heteroploids and polyploids can be comprised in karyotype mutations. In Drosophila, as well as in other tested organisms, gene mutations and chromosomal rearrangements are commonly induced by X-rays.

Many investigators with Drosophila or with other organisms have shown that the frequency of gene mutations induced by X-ray is directly proportional to the dosage, but that the frequency of gross chromosomal rearrangements is proportional to an exponent of the dosage. The exponent of the dose is about 1.5 at the heavy doses--1500 to 4000 r, and near 2 at lower doses. This has been explained on the assumption that the gene mutations are produced by single

ionizing particles, increasing linearly with dosage; while the gross chromosomal rearrangements involving two or more breaks are produced by separate ionizing particles, and therefore increase with a higher power of the dosage (Muller 1929, 1936, 1938, 1940; Patterson 1929; Stadler 1929, 1930; Timofeeff-Ressovsky 1929, 1930, 1931).

In view of their relation to chromosomal rearrangements, mutations at specific loci can be divided into the following three groups: (a) those which are known to be associated with relatively gross chromosomal rearrangements, (b) those which are associated with minute but cytologically detectable chromosomal rearrangements, particularly small deficiencies, and (c) those which can not be shown to belong in either of the above two groups, and are supposed to be point mutations.

Since views on the structure and chemistry of the genes themselves are, at present, to a considerable extent speculative, the nature of gene mutations remains an open question. The genes might be pictured, according to the current view of most geneticists, as self-duplicating nucleoprotein molecules or as large chemical radicals which are located in a linear order in, or connected by, a fibre protein backbone or chromosome. Different genes would differ in structure to a marked degree and their differences probably reside in the protein components, although the nucleic acids also play an important role in heredity. Therefore, one theory suggests that gene mutation is the result of a rearrangement of some of the atoms of the nucleoprotein molecule or radical which constitutes the gene.

It is also possible, at least in certain cases, that a part of the molecule might be lost. In other words, according to this theory, a gene is a distinct entity of genetical material with its own complex structure and mutation is merely a rearrangement or sometimes a loss of intramolecular material. However, the discovery that some apparent gene mutations were due to changes external to the genes, as being caused by, or associated with, chromosomal rearrangements, led Serebrovsky (1929) to advance another theory, according to which all so-called gene mutations might be nothing more than minute deficiencies or other chromosomal rearrangements, of fundamentally the same kind as the grosser ones.

If we consider the whole chromosome as a single chemical unit, the chromosomal rearrangement would involve chemical changes in the united genes and cause mutations. Since many minute rearrangements are difficult to identify, it is possible that most of the so-called gene mutations might be due actually to chromosome changes. In such a cytologically suitable organism as Drosophila, one is able to detect quite minute rearrangement involving only a few bands of the salivary gland chromosome. Thus, investigators have proven that in many cases, such as scute-19, scute-J1, scute-10, Notch, Bar and Hairy-wing, etc., the mutations concerned are substantially due to, or associated with, minute rearrangements (Muller, Prokofyeva and Raffel 1934, 1935; Bridges 1936; Demerec et al 1942). It has also been found that a linear relationship exists between the frequency of minute rearrangements, (like that of gene mutations and unlike that of gross rearrangements) and the dosage of radiation (Belgovsky

1938; Muller, Makki and Sidky 1938; Makki and Muller 1940). And a high proportion of the minute chromosomal rearrangements are actually small deficiencies. The evidence from reverse mutations and from some other sources has raised, however, certain objections to such an interpretation (Patterson and Muller 1930).

Reverse mutations indicate that the mutations can not be regarded as mere losses of genes, but that they may be due to reversible inactivations of genes, to changes in structural configurations, or to shifts of gene positions. In this connection, the problem of position effect has been of special interest to geneticists. In a great many cases in Drosophila, the coincidence between mutations and rearrangements can be explained as a position effect. The point of mutation and break of rearrangement are not always the same but are close together. The activity of a locus may be influenced by the change of its position in the chromosome. The evidence from the reversibility of mutations at several loci and that from other experiments have clearly shown such an effect. The hypothesis of position effect has been generally accepted. Although it seems too early to say, as has been suggested, that all induced mutations might be position effects, yet it is clear that further studies of reverse mutations and position effect should throw light on the nature of mutations as well as on the action of genes. To a considerable extent, the hypothesis of position effect can bridge over the gap between the aforesaid two theories dealing with the mechanism of mutations.

REVERSE MUTATIONS. Mutations occur not only from the normal alleles of the wild type to mutant alleles but also from mutants back to or toward the original normal alleles. The latter processes are known as reverse or back mutations. Reverse mutations can be induced by irradiation or occur spontaneously. The occurrence of reverse mutations is of great importance, showing that in general mutations are not mere losses or destruction of previously present genes. In several cases such as forked, miniature, pink and the white alleles in Drosophila melanogaster, the direct and reverse mutations were induced by X-rays directly one from another; they are reversible (Patterson and Muller 1930; Timoféeff-Ressovsky 1930, 1932, 1937). As Muller has remarked, it is possible to conceive of mutation as the "punching-out" or deletion of a gene, but not to conceive of "punching" it in again in the process of reverse mutation. This has been one of the more powerful arguments for the occurrence of intra-genic change in the mutation process. However, a sufficient number of cases of reverse mutations in which the original mutant could be shown to be due to a chromosomal rearrangement have occurred to rob this argument of completely general applicability. Grüneberg (1937) has described a mutant, roughest-3, in Drosophila which arose at one point of breakage in an inversion. In a reverse mutation from rst-3, it was shown that the inverted segment had been returned entirely to its normal order. Analyzing over forty cases of X-ray induced reverse or partial reverse mutations from white mottleds to normal flies, Griffen and Stone (1939, 1940) also found that in each case a new chromosomal rearrangement had occurred, but none had restored

the normal association. Other examples of essentially the same kind are provided by the experiments on Bar (Sturtevant 1925, 1928; Bridges 1936; Muller et al 1936), on curled (Panshin 1935) and on hairy (Dubinin and Sidorov 1935), which are rather more well known. All these cases have been described in terms of position effect.

POSITION EFFECT. The hypothesis of position effect was advanced by Sturtevant (1925) to account for his results at the Bar locus. He showed that two Bar alleles adjoining one another in the same chromosome have a greater effect in reducing the number of facets in Drosophila eyes than they have when they lie in the normal position of one in each homologue. This was taken to mean that the developmental effects of genes may be conditioned by their neighbors in the chromosome. Therefore, the case of Bar was the first example of position effect. Since then a rapidly growing amount of experimental evidence in favor of the existence of a position effect has been secured in Drosophila as well as in a few plants.

The cases of roughest-3, curled and of hairy, mentioned above, are examples of position effect. Studying cytologically the reversions of rst-3, Kaufmann (1942) found that in each case the roughest locus had been brought to a new position by a new rearrangement other than a reinversion. Panshin (1935) with a curled mutant, and Dubinin and Sidorov (1935) with hairy, both arising with a translocation, were able to substitute the normal alleles for the mutant alleles through crossing over. The normal alleles, when placed in the translocation, behaved as mutants, while the mutant

alleles, placed in normal chromosomes, behaved as wild types. Other loci such as yellow and achaete, scute, white, Notch, split, brown, light and cubitus interruptus are also favorable to the study of position effect. A good deal of work has been done with these loci. (Gowen and Gay 1933, 1934; Dubinin and Sidorov 1934; Panshin 1935, 1936; Schultz 1935; Demerec and Slizynska 1937; Muller and Raffel 1937, Raffel and Muller 1940; Stern et al 1943, 1944, 1946; among many others). On the whole, in every case indicating a position effect with an associated chromosomal rearrangement, it has been shown that the locus in question is near one break of the rearrangement. Moreover, in most cases the affected loci which were normally located in the euchromatic region of chromosomes have changed their neighbors with heterochromatin substituted through the rearrangements, or vice versa. Therefore, the position effect in question is very likely exerted by a heterochromatic segment upon an euchromatic locus or by an euchromatic segment upon a heterochromatic locus. In this respect, the case of Bar is somewhat different. The mutation of Bar has been cytologically proved to be due to a tandem duplication in normal order for an X-chromosome section, 16A of Bridges' map, which is composed of six bands. This section is present in triplicate in the X-chromosome of the double-Bar mutant. Therefore, not only the double-Bar but also the Bar mutant itself is due to a position effect. It is accompanied with a chromosomal rearrangement, duplication, with the locus concerned, supposed to be at 16A1-2, near one of the breaks; but this rearrangement, unlike others, takes place within the euchromatin, having nothing to do with heterochromatin

(Dobzhansky 1932, Griffen 1941, Sutton 1943). This sort of repetition for certain bands, which is responsible for a dominant mutation, has been also found in the case of Hairy-wing (Demerec 1939) and that of Star (Lewis 1940). If other cases are position effects of eu-heterochromatic or hetero-euchromatic rearrangements, then these cases of Bar, Hairy-wing and Star are purely euchromatic position effects. In connection with this, the differences of both chemical composition and physical state between heterochromatin and euchromatin have attracted much attention. They differ in breakability, extensibility, in nucleic acid content, in protein components and in genetic effects (Muller and Painter 1932; Heitz 1933; Mather 1939; Schultz 1939, 1941; Caspersson 1940).

The heterochromatin has been considered to be responsible for nucleic acid and protein synthesis, and the nucleic acid metabolism might be associated with the process of chromosome or gene reproduction. A correlation between heterochromatin or nucleic acid metabolism and variegation or mottling of somatic characters has been proposed (Caspersson and Schultz 1938, Schultz and Caspersson 1939, Schultz 1941). In many cases the variegation has been regarded as a kind of position effect peculiar to heterochromatin. The Y-chromosome is almost wholly composed of heterochromatin, and it has been demonstrated that the Y-chromosome has effects on the expression of variegation or mottling (Gowen and Gay 1934, Schultz 1935, 1939).

All the cases so far mentioned in connection with position effect have concerned Drosophila. Owing to the coincidence of both

position effect and somatic pairing in Drosophila, the possibility of some relationship between these two phenomena has long been taken into consideration (Sturtevant 1925; Muller 1935, 1941; Ephrussi and Sutton 1944). A few cases, however, have been secured in maize (Jones 1939, 1944) and in Oenothera (Catcheside 1939, 1947, ~~1946~~), indicating the existence of position effect in plants. In maize, Jones found a variable effect of the C (Aleurone color) locus on color and growth changes in the endosperm following translocations. In Oenothera, the case of Catcheside has been well investigated. The genes P^s (striped bud), P^r (rubricalyx) and S (yellow petals) produce variegated phenotypes when they are present in the translocation chromosome. When transferred by crossing over to their normal position, they produce normal phenotypes. The variegation is therefore a position effect.

The existence of position effect in Drosophila and in plants, at least in Oenothera, admits of no doubt, but the nature of the position effect and how universal it is are still questions. Also, at present, we can not deny the occurrence of gene or point mutations, since many cases leave little doubt that actual gene changes are concerned. One may argue that a number of rearrangements might be so small as to be below the limit of the resolving power of the present microscope, and the position effect concerned could hardly be distinguished from gene mutations. It is not impossible, as Goldschmidt (1946) has urged, that the visible change of order is position effect and cytologically invisible change of order is point mutation. However, any speculations and hypotheses which are

susceptible to no decisive experimental tests are barren.

The studies with X-rays of reverse mutations and position effects in Drosophila melanogaster reported herein began in 1947 at the suggestion of Dr. Edward B. Lewis.

The first five experiments were carried out on reverse mutations of several dominant and recessive mutant genes. Reversions of three dominant genes, Dichaete, Glued and Stubble, were induced, but none was found cytologically to be associated with detectable chromosomal rearrangement.

Thirteen other experiments were undertaken on position effects at the white locus. A total of fifty-eight white mottleds were found. Forty-six of them have been studied genetically, among which twenty-one were studied cytologically.

The last three experiments were performed on position effects at the light and straw loci in the second chromosome. Thirty-six light mutants and twenty-five straw mutants were secured. Most of them have been studied genetically, several studied cytologically. Three light mutants and one straw have turned out to be due to obvious position effects.

PART TWO. REVERSE MUTATIONS

Reverse mutations have been induced by X-rays at a number of loci in Drosophila.

Several cases which were striking illustrations of position effect can also be taken as examples of reverse mutations. Their reversions were not induced by irradiation but brought about by crossing over of the locus in question from an interchanged chromosome to its normal homologue. These are, as previously mentioned, the case of Sturtevant (1925) on Bar and those of Panshin (1935) on curled and of Dubinin and Sidorov (1935) on hairy, in which each mutant reverted to wild type when the locus concerned was transferred to a normal chromosome by crossing over. The case of Grüneberg (1936, 1937) with the spontaneous reverse mutation from roughest-3 to wild type also showed that the reversion was associated with a restoration to normal order of the inversion in the X-chromosome (reinversion). Nevertheless, with X-rays, Kaufmann (1942) has found that seventeen induced reversions from roughest-3 in his experiments were all brought about by various new chromosomal rearrangements other than a reinversion.

The mutants, Curly and Glazed (Suche, Parker, Bishop and Griffen 1939; Griffen and Stone 1940), which accompany inversions in the second chromosome and white mottleds (Griffen and Stone 1939, 1940) which accompany chromosomal rearrangements with one break near the white locus in the X-chromosome, have also been found to revert to

normal with changes in their chromosomal association other than a restoration.

The reversions of some "point" mutations, however, did not show chromosomal changes, and might be due to changes of the genes concerned. The induced reverse mutations of apricot, miniature and forked are examples (Griffen and Stone 1939).

Many other cases of reversions have not been studied cytologically.

Among the genes so far tested in Drosophila, the reverse mutations occur rarely at some loci, such as ec (echinus eye), th (thread arista), cu (curled wing), sr (dorsal stripe) and ca (claret eye color), and have not been induced by X-radiation at some others.

EXPERIMENTS WITH DOMINANT MUTANT GENES

MATERIAL AND METHODS. Three dominant mutant genes located in the third chromosome of Drosophila melanogaster were selected for the study of reverse mutations in this experiment. These genes were D (Dichaete wing), Gl (Glued eye) and Sb (Stubble bristle).

A balanced stock with the constitution of Gl Sb/ Cx D was established, in which Gl and Sb were present in one, and D with the balancer Cx (inversions with seven breaks in third chromosome) in the other, of the third chromosomes. Males from this stock were X-rayed (April 1, 1947) with a dose of approximately 4,500 r units and mated to virgin females from the third-chromosome multiple stock, "rucuca", which includes ru (roughoid eye), h (hairy body), th (thread arista), st (scarlet eye), cu (curled wing), sr (dorsal stripe), e^s (ebony-sooty body) and ca (claret eye)-eight recessive genes. These recessives were used as markers. F_1 flies receive one treated third chromosome from their father and one untreated third chromosome (rucuca) from their mother and would reveal any reverse mutations of Gl, Sb or D and any direct mutations of the eight recessive genes, which had occurred.

Unaffected flies would show either Gl Sb or D. Affected ones would show otherwise, i.e. Sb reversals show Gl only; Gl reversals Sb only; D reversals would be phenotypically normal; and flies with direct mutation of any one of the eight recessive genes would show up the character concerned, but less attention was paid to these direct mutations.

Whenever a fly appeared to be a reversal or a new mutant, it was mated to the flies carrying suitable markers in one of their third chromosomes and a crossing-over suppressor in the other. The stock of ru h th st cu sr e^s Pr ca/T(2;3)Mé; th st p^b p^p/D Cx; or of se rt^2 th/Mé were used for this purpose. Each reversal or mutant was, if fertile, kept in stock by balancing the affected third chromosome with a crossing-over suppressor. During the establishment of each stock, it was checked carefully with the marker genes to make sure that the phenotypical reversion or mutation of the character in question was not due to a contamination.

Salivary gland chromosomes for cytological studies were secured from larvae of the mating between the reversal or mutant and wild type flies. Temporary preparations were made following the general methods outlined by Demerec and Kaufmann (1945). Chromosome maps of Bridges (1935) were used in the study.

RESULTS. A total of 28,460 F_1 flies from the crosses of irradiated Gl Sb/Cx D males with rucuca virgin females was examined. Among them were two D-reversals, six Gl-reversals, one Sb-reversal, nine st-mutants and one cu-mutant. One of the D-reversals, two of the Gl-reversals and five of the st-mutants proved to be sterile and the Sb-reversal died from over-etherization. The other reversals and mutants have been studied cytologically.

Since the 28,460 flies examined represented the same number of the male gametes treated, and half of the latter each carried one

third-chromosome with the genes Gl and Sb, another half each carried one third-chromosome with D, the reverse mutation rate of each of these three dominant genes is, in this experiment, the number of the reversions found over half the number of the flies or gametes examined. Calculated on this basis, the rate of D reversions is 0.014 percent and that of Gl reversions is 0.042 percent and of Sb reversion is 0.007 percent. As the direct mutations could be possibly induced from either one of the third chromosomes of all the gametes irradiated, the mutation rate of st^+ is, therefore, 0.0316 percent and that of cu^+ is 0.0035 percent.

As stated above, several reversals and mutants were sterile and they could not be bred further to be examined genetically and cytologically. All the fertile reversals and mutants, however, proved to be true reversions and mutations respectively. The rate of the fertile true reversals is, then, 0.007 percent for D and that is 0.0281 percent for Gl. The rate of fertile true st mutants is 0.0141.

Cytological studies with these reversals and mutants showed no detectable rearrangements of salivary gland chromosomes to be associated with the phenotypical changes. Salivary-chromosome analysis of Gl and Sb by Bridges show no chromosomal rearrangement, but that of Cx D balancer, also known as In(3LR) Cx D, carries inversions with seven breaks involving the D minute inversion and both sides of the centromere of the third chromosome. The Cx inversions were still shown in the salivary-chromosomes of the D-reversal, but no

detectable new rearrangement could be found. Since the minute inversion of D was involved in the complex inversions, it could not be easily examined. An effort was made in vain to check whether the small D-inversion had been reinverted to normal order in the D reversion.

EXPERIMENTS WITH RECESSIVE MUTANT GENES

MATERIALS AND METHODS. For the study of reverse mutations of recessive mutant genes, four different experiments were carried out, in which the X-ray dosage of approximately 4,500 r units was generally applied. (120 KV, 8 ma, 15cm. 12.94 minutes, 1 mm. Al Filter)
(1) The first experiment (April 1, 1947). Male flies carrying two sex-linked recessive genes, sc^7 (scute bristle inseparable from an inversion) and w^a (apricot eye color) were irradiated and mated to virgin females carrying four other sex-linked genes, namely, y (yellow body), sc (scute bristle), w^e (eosin eye color) and spl (split bristle). F_1 females were examined for reversions of sc^7 or w^a and for mutations of y or spl.

For the other three experiments the following genes were selected to study or were used as markers. (a) In the X-chromosome: y (yellow body), ac (achaete bristle), v (vermilion eye color); (b) in the second chromosome: cn (cinnabar eye color), px (plexus veins), bw (brown eye color) and sp (speck wing axil); (c) in the third chromosome: th (thread aristae), st (scarlet eye color) and cp (clipped wing). The combinations of v bw, cn bw or bw st give colorless eyes. When white-eyed flies with any one kind of these combinations were treated with X-rays, if reverse mutation of one gene takes place, their offspring would show the eye color associated with another gene in the combination.
(2) The second experiment (Aug. 5, 1947). Male flies of v bw were irradiated and mated to y ac v bw females. F_1 flies were examined

for bw reversions and females were examined for v reversions.

(3) The third experiment (July 27, 1947). Male flies of cn bw were irradiated and mated to cn px bw sp females. F_1 flies were examined for reversions of cn or bw.

(4) The fourth experiment (Aug. 20 and 23, 1947). Males of px bw sp st were irradiated and mated to bw th st cp females. F_1 flies were examined for reversions of bw or st.

RESULTS

(1) The first experiment. F_1 females from the mating of sc^7 w^a males (X-rayed) with y sc w^e spl females totalled 7,106. One sc^7-reversal but no w^a reversal was found. This sc^7-reversal carrying w^a / w^e proved to be sterile and it could not be bred further to check whether it was a true reversal. Supposing it was a true one, the reversion rate of sc^7 in this experiment is 0.0141.

(2) The second experiment. A total of 12,096 F_1 flies was examined from the mating of v bw males (X-rayed) with y ac v bw females. No reversions of bw from these flies and no reversions of v from about 6050 females were found.

(3) The third experiment. From the mating of cn bw males (X-rayed) with cn px bw sp females, 14,432 F_1 flies were examined. No reversals of cn or bw could be found.

(4) The fourth experiment. From the mating of px bw sp st males (X-rayed) with bw th st cp females, a total of 29,195 F_1 flies were obtained. The examination for reverse mutations of bw or st also showed negative results.

In the fourth experiment, twenty two F_1 flies showing cp phenotype and about twenty three others showing slight cp characters were found and discarded without further test. It seemed somewhat unlikely that the mutation to cp had occurred at so high a rate; possibly the stocks used were heterogeneous for modifiers of this character. Three flies with th aristae were also found, probably due to mutations at the th locus.

From the above four experiments, the total number of flies examined for reverse mutations of bw was 55,723.

Table I summarizes the results obtained from all the experiments done for reverse mutations.

Table I

X-ray Induced Reverse Mutations at Some Loci in

Drosophila melanogaster (Dosage-4500 r)

Gene	Number of Tested Gametes	Number of Reverse Mutations	Percent of Reverse Mutations	Percent of Fertile Reverse Mutations
D	14230	2	0.0140	0.0070
Gl	14230	6	0.0422	0.0281
Sb	14230	1	0.0070	0.0000
sc^7	7106	1	0.0141	0.0000
w^a	7106	0	0.0000	0.0000
v	6050	0	0.0000	0.0000
cn	14432	0	0.0000	0.0000
bw	55723	0	0.0000	0.0000
st	29195	0	0.0000	0.0000

DISCUSSION AND SUMMARY

Comparatively little work has been done on induced reverse mutations. From the data thus far secured in earlier studies in Drosophila (Johnston and Winchester 1934; Timoféeff-Ressovsky 1930, 1937; Griffen and Stone 1939, 1940; Kaufmann 1942), the following four points are worthy of note.

(1) Reverse mutations are in general much rarer than direct ones at the same loci, and the frequencies of direct and reverse mutations bear no apparent relation to each other.

(2) Different genes or different alleles of one gene show a great variation in frequency of reverse mutations.

(3) No mutation which accompanies a chromosomal rearrangement has been induced to revert to wild type through point mutation.

(4) No point mutation has been induced to reverse to a normal allele through a detectable rearrangement.

There is little doubt about the first two points. Accord- to Timoféeff-Ressovsky (1937, from Kaufmann 1942), the genes, f (forked bristle) and p (pink eye color), might be exceptions, both showing a reverse mutation rate equal to or greater than that of direct mutation. However, this was very likely due to a misunder- standing. Johnston and Winchester (1934) found altogether twenty four reversions at eight loci in the X-chromosome of Drosophila. Among these twenty four flies, twelve were sterile and could not be bred further for detailed studies. In order to show that these sterile flies may not be true reversals, Johnston and Winchester

put down, in parentheses, the number of sterile flies followed with a question mark after the total number of apparent reversals found at each locus. For instance, at the f locus, of 11 apparent reversals 4 proved to be sterile, they recorded as 11(4?). Similarly, 3(2?) for sc; 4(?) for g (garnet eye color); 1(?) for car (carnation eye color), etc. Probably by mistake, Timoféeff-Ressovsky (1939) added up the numbers outside and inside the parenthesis as a total number of reversions found at each locus. Therefore, he referred to a total of 5 for sc, 5 for g, 2 for car and 17 instead of 11 for f. Perhaps the mistake as such made the frequency of reverse mutations at the f locus equal to or greater than that of direct mutations. A comparison made by Johnston and Winchester (1934) shows that at the f locus the ratio of the frequencies of direct and reverse mutations is 0.0516 : 0.0084 percent or about 6 : 1.

In the first experiment described above, nine direct mutations were induced at the st locus from 28,460 gametes irradiated, being 0.0316 percent in frequency. However, in the fourth experiment, no reversion was found at the st locus from a total of 29,195 flies examined. Timoféeff-Ressovsky (1939) found one reversal of st from 27,155 flies resulting from males treated with 5,000 r units. The frequency is, therefore, 0.0037 percent. If calculated on the basis of 4,500 r units, it will be 0.0033 percent. Thus, 0.0033 : 0.0316 or 1 : 9 is the ratio of reverse mutations to direct ones at the st locus. In other words, reverse mutations of st are nine times rarer than the direct ones.

As regards the variation of reverse mutation rates of different genes, the dominant gene Gl, in the first experiment described above, shows a higher rate than that of any reversion so far found. The reversion of D also shows a fairly high rate. The reversions of other dominants, such as B, Pm, Cy and Gla, have been induced by others (Hanson 1928; Suche, Parker, Bishop and Griffen 1939). The B-reversions of Hanson were induced at a high rate, but no records were taken about the rate of the reversions of Pm, Cy and Gla. Although these cases are too few to be taken as evidence for any conclusion, yet it may be wondered if the reversion rates of dominant genes are, in general, higher than those of recessives. The rates of direct mutations of recessive genes are, however, higher than those of dominants.

Most, if not all, of the mutations which are associated with chromosomal rearrangements are due to position effects. The "mutant genes" in question can be called position genes or alleles. The position genes, such as rst^3, w^m, Pm, Cy and Gla have been induced to revert to normal with changes in chromosomal association. Seventeen cases of rst^3 (Kaufmann 1942) and over forty cases of w^m (Griffen and Stone 1939) have been studied. It seems very likely that the position genes would not reverse to normal alleles without changing again their positions. In case of the D-reversion, the original minute inversion might have been reinverted or changed otherwise.

The reversions of point mutations have been induced at many loci, but most of them have not been checked cytologically.

Griffen and Stone (1939) have studied the salivary-chromosomes of the reversals of w^a, m and f. They found no chromosomal rearrangement to be associated with these reversions. Among the six reversals of Gl, four have been checked cytologically; there was no detectable chromosomal rearrangement in any case. It is too early to conclude that point mutations do not require chromosome change for reversions, but these few cases so far studied point to such a possibility.

It is possible that, as a rule, the position genes reverse to normal alleles by changes in position while the point-mutation genes reverse through point mutations. If these relations continue to hold as more cases are analyzed, it must be admitted that the reversal of both point and position-effect mutations depend upon changes of the same general character as those responsible for the initial mutations, although they may not show any relation to each other in frequency.

To sum up, the results obtained from the experiments described in this part are as follows:

(1) Reverse mutations were induced from the dominant genes D and Gl, and probably induced from Sb and from the recessive gene sc^7. No reversions were found from the recessive genes w^a, v, cn, bw and st.

(2) From 14,230 gametes irradiated, two apparent reversions of D, six of Gl and one of Sb were found. After breeding, one from D and four from Gl proved to be true reversions.

(3) Salivary gland chromosomes of the true reversals of D and Gl did not show detectable new chromosomal rearrangements.

(4) From 7,106 gametes irradiated, one probable reversal of sc^7 was found, which was not fertile and could not be studied genetically and cytologically. No w^a-reversal was found in the same experiment.
(5) No reversions were induced from v in 6,048, from cn in 14,432, from bw in 55,723 and from st in 29,195 gametes irradiated.
(6) The dosage of X-radiation applied in all the experiments was approximately 4,500 r units.

PART THREE. POSITION EFFECT

A number of cases of mutations in Drosophila have turned out to be due to position effects. Intensive work along this line has been done on several loci. The direct evidence indicating the existence of position effect is that the reverse changes in the phenotype of such mutants as Bar, roughest-3, white-mottleds and others have been shown cytologically to be caused by a restoration of the normal order or by a new rearrangement of the loci concerned. It has also been found that the similar changes in the arrangement of the genes are always accompanied with a similar kind of phenotypic effect.

According to Goldschmidt (1946), the position effects thus far described may be grouped in six types: (1) The Bar duplication type. A comparable case is that of Star-asteroid (Lewis 1945).
(2) Those cases where a chromosome break located near the known loci produces a phenotypic effect resembling that caused by the gene mutations. Belonging to this category are the position effects for yellow, scute, white, forked, brown, cubitus interruptus, eyeless, etc.
(3) Those mutations have been shown to be inseparable from chromosomal rearrangements, in which no point mutations have been found. Examples are the Curly, Moiré and Xasta.
(4) The rearrangements have no effect by themselves, but if combined with other mutants they exercise a modifying effect upon the type. The mutants for modification of vestigial (Green and Oliver 1940)

and those for modification of Beaded (Goldschmidt and Gardner 1942) are examples.

(5) The Dubinin effect--position effect for dominance modification. For instance, the recessive gene cubitus interruptus (ci) becomes more or less dominant when a translocation has occurred near this locus. The Plum of brown locus and other somatic mottling or variegation are regarded as variants of this type.

(6) The combination effect. An example is the following: Plum is a position effect with a break in the brown region. The combination of Plum and vermilion gives white eyes. So do the combinations of brown with vermilion, cinnabar or scarlet.

If the position effect is defined as an effect on a locus arising from the change of normal position of this locus in a chromosome, it may be questioned whether the fourth and sixth types, cited above, can be described in terms of position effect. The cases in the fourth group might be due to a modifier mutation arising with a rearrangement and this modifier, like a suppressor or an enhancer, only shows up in the presence of certain other mutant gene or genes. The so-called combination effect in the sixth group is more or less of the same sort. In this discussion, by the term position effect is meant restrictively the effect on a locus produced by change of its position through a rearrangement. Since there are two kinds of chromatin, euchromatin and heterochromatin, in chromosomes, the change in position of a locus in question may be from eu- to heterochromatin, hetero- to euchromatin or from eu- to euchromatin, or hetero- to heterochromatin. For the convenience of description, genes may be distinguished with respect to their normal location as

euchromatic or heterochromatic genes. Therefore, the change of genic position through a chromosomal rearrangement might either be that an euchromatic gene is brought to a heterochromatic or another euchromatic region, or that a heterochromatic gene is brought to an euchromatic or another heterochromatic region. This is only considering the major euchromatic or heterochromatic portions of a chromosome, and disregarding the so-called intercalated heterochromatin in the salivary-chromosomes.

Keeping this in view, the position effects so far described can be classified into three groups: (1) Eu-heterochromatic type. White mottleds, roughest-3, Plum, etc. are examples. (2) Eu-euchromatic type. Examples are the cases of Bar and of Star-asteroid. (3) Hetero-euchromatic type. Belonging to this type are the position effects for cubitus interruptus, light and straw.

The experiments performed for a study of position effects of both euchromatic and heterochromatic genes are presented as follows.

EXPERIMENTS WITH EUCHROMATIC GENES

(A) THE WHITE MOTTLEDS

The occurrence of white mottleds (w^m) in X-ray experiments is somewhat frequent. It has been found to be due to chromosomal rearrangements such as translocations, inversions or deficiencies with the white locus being brought into a heterochromatic region.

MATERIALS AND METHODS. For the study of white mottling, males from the following stocks were X-rayed with a dose of approximately 4,500 r units. (120 KV, 8 ma, 15 cm, 12.94 minutes, 1 mm. Al filter)

(a)Canton-S Wild type stock

(b) yellow-4 Stock of yellow flies which carry a long inversion in their X-chromosomes with the left break to left of yellow and the right break between fu (fused veins) and da (disarranged facets). The following three strains of y^4 flies were used.

In(1)y^4, y^4

In(1)y^4, y^4 f

In(1)y^4, y^4 cv v f

(cv = crossveinless; v = vermilion eye color; f = forked bristle)

(c) scute-4 Stock of scute flies which carry a long inversion in their X-chromosome with the left break between scute and silver, the right break between carnation and bobbed. The strain of In(1)sc^4, y sc^4 was used.

(d) scute-8 Flies carry a long inversion in their X-chromosomes with the left break between yellow and scute, the right break between bobbed and the centromere. The following two strains were used.

In(1)sc^8, sc^8

In(1)sc^8, sc^8 cv v f

(e) roughest-3 Rough-eyed flies carry a long inversion in their X-chromosomes with the left break at roughest and the right break beyond bobbed. The strain of In(1)rst^3, rst^3 was used.

Figure 1 shows diagrammatically the relative positions of the breaks of these four inversions.

Irradiated males were mated to virgin females homozygous for y (yellow body), w (white eye) and spl (split bristle). The F_1 females were examined for mottled eyes. These females would receive one treated X-chromosome from their father and one y w spl X-chromosome from their mother. A fly would be expected to reveal mottling of its eye color if the white locus in the treated X-chromosome had been brought to a heterochromatic region through a rearrangement, being over a recessive white allele in the untreated X-chromosome.

Whenever a female was found to be white-mottled (w^m), it was mated by males with the constitution of In(1)dl-49, y Hw w lz^s in their X-chromosomes. The X-chromosome with such a constitution is known as a balancer, carrying a dl-49 inversion and the yellow,

Hairy-wing, white and lozenge-spectacled genes. The female progeny with mottled-eye and Hairy-wing from this mating were selected and mated by In(1)dl-49, y Hw w lz^s males again and thus kept in stock. If the males of white-mottleds proved to be viable and fertile, a homozygous stock for w^m was made up.

Genetic Tests. In order to detect the linkage relation brought about by a translocation of the affected white locus to another chromosome, the mottled flies were mated to Cy/Pm, Sb/D Cx flies. Cy (Curly wing) and Pm (Plum eye) are dominants in the second chromosome and Sb (Stubble bristle) and D (Dichaete wing) are dominants in the third. An F_1 female with the constitution of $w^m/+$, Cy/+, D/+ was mated by white males. Pair matings or matings of one female to three males were generally made. The F_2 flies were examined for linkage of w^m with the second or third chromosome. If the mottling was due to a translocation which has occurred between the X and second chromosomes, no w^m flies would be Cy; if there was a translocation between the X and third chromosomes, no w^m flies would be D, excepting the possible crossovers in some cases. Otherwise the w^m is not linked with the second or third chromosome; there might still be a translocation between the X and the fourth chromosomes or a rearrangement within the X.

To test the linkage of w^m with the fourth chromosome, the mottled flies were mated to flies carrying w m f, ci ey characters. (m = miniature wing, f = forked bristle, in the X chromosome; ci = cubitus interruptus vein, ey = eyeless, in the fourth chromosome)

The F_1 mottled females were mated to w m f, ci ey males again, and the F_2 flies were examined for linkage. If no w^m flies carrying ci ey could be found, the w^m in question must be linked with the fourth chromosome.

Whenever a white-mottled was found to be due to a translocation which had transferred the white locus to the heterochromatic region of the second, third or fourth chromosomes, a test was made to check the effect of the w^m on the genes located in the heterochromatin. The heterochromatic loci, lt (light eye color), rl (rolled wing), stw (straw body) and ltd (lightoid eye color) in the second chromosome; in (inturned bristle) and ri (radius incompletus vein) in the third; and ci in the fourth, were selected for this purpose. For instance, in case a translocation between an X segment carrying the white locus and the heterochromatic region of the second chromosome is the cause of white mottling, the X segment might have an effect on the normal alleles of the genes located in the heterochromatin of the second chromosome, such as lt, rl, stw, etc. As a consequence a mating of such a T(1;2) mottled fly with a lt (or rl, stw, etc.) mutant fly would give lt (or other mutant phenotype) offspring if the translocation decreases the dominance of the normal allele concerned. This will be a position effect of the heterochromatic type. Similar tests with the heterochromatic genes in other chromosomes were made to the mottleds of other types of interchanges.

Cytological Analysis. To examine the salivary gland chromosomes, the mottled females (in most of the cases, no mottled male is viable or fertile) were mated to wild type, y^4, sc^4, sc^8 or rst^3 males, each being tested against the kind of X-chromosomes irradiated. Other marker genes were also used in the mating to facilitate the selection of the desired classes of larvae. The larvae were raised in an incubator at a constant temperature of 19° C.

Temporary preparations were made with the salivary glands of the larvae, which were dissected and stained with aceto-orcein following in general the methods outlined by Demerec and Kaufmann (1945). It has been found better to dissect the glands in diluted aceto-orcein (diluted with 60% acetic acid) and then transfer them to the concentrated stain. The breakage points in the salivary-chromosomes were checked with the chromosome maps of Bridges (1935, 1938).

RESULTS. A total of fifty eight white-mottleds was found from 45,255 flies or gametes examined in thirteen X-ray experiments. Forty six of these mottleds have been studied genetically and among these twenty one have been studied cytologically. Symbols, w^{m48-1}, w^{m48-2}, to w^{m48-46} are used for these mutants since they are white mottleds found in 1948.

Tables II, III and IV give the general results of these experiments.

Table II

X-ray Induced White Mottleds in Drosophila melanogaster

(Dosage--4,500 r units)

Males X-rayed	Date of Exp't	Number of F_1 ♀♀	Number of w^m (No. fertile)	Percent of w^m
Canton-S	Jan. 22, '48	2494	2 (1)	0.08
	April 6, '48	3484	2 (1)	0.06
y^4	Jan. 29, '48	1125	5 (5)	0.44
	May 31, '48	3691	3 (3)	0.08
y^4 f	June 8, '48	3875	3 (3)	0.08
	July 29, '48	950	3 (3)	0.32
y^4 cv v f	Jan. 29, '48	1888	3 (2)	0.16
y sc^4	April 6, '48	2695	3 (3)	0.11
	May 31, '48	6451	3 (1)	0.05
	July 15, '48	6603	1 (1)	0.02
sc^8	June 15, '48	2516	0 (0)	0.00
sc^8 cv v f	July 29, '48	6285	10 (8)	0.16
rst^3	June 15, '48	3198	20 (16)	0.63

Table III

White Mottleds Induced in Drosophila melanogaster

(Summary of Table II)

Males X-rayed	Number of F_1 ♀♀	Number of w^m (No. fertile)	Percent of w^m	Percent of Fertile w^m
Canton-S	5978	4(2)	0.07±0.03	0.03±0.02
y^4	11529	17(16)	0.15±0.04	0.14±0.03
sc^4	15749	7(5)	0.04±0.02	0.03±0.01
sc^8	8801	10(8)	0.11±0.04	0.09±0.03
rst^3	3198	20(15)	0.63±0.14	0.47±0.12
Total	45255	58(46)	0.13±0.03	0.10±0.02

The Frequency of White Mottleds

The frequencies of white mottleds induced from different stocks are shown in Tables II and III. As has been remarked, the five kinds of males irradiated differed from each other in the gene sequence of their X-chromosomes. The Canton-S males carried the normal gene sequence while the y^4, sc^4, sc^8 and rst^3 males carried respectively three different long inverted segments in their X-chromosomes. (Fig. 1) The frequency of white mottling induced from y^4, ~~sc^4~~, sc^8 and rst^3 flies is higher than that from the normal flies. It is especially high from the rst^3 males where a segment of heterochromatin had been brought to the vicinity of the white locus by the rst^3-inversion. A computation of chi-square (X^2) made upon the observed numbers of the mottleds, with the 47/45,255 ratio of the total flies examined as expected frequencies, shows a highly significant value. This will be discussed at the end of this part. However, two points should be mentioned here. First, the rst^3 and the first series of sc^8 males were irradiated at the same time. The conditions should be exactly the same during the application of radiation to these two kinds of flies, but their frequencies of induced mottleds are quite different, i.e. 0.63 percent for rst^3 and 0.00 for sc^8. This difference can hardly be ascribed to unexpected external factors occurring during the course of irradiation. Secondly, the untreated rst^3 flies do not show white mottling. For this point, the untreated rst^3 males were mated to y w spl virgin females and their F_1 female offspring were examined for mottling. In about 3,000 flies with the constitution of rst^3 against w, no white-mottled could be found.

The Distribution of Breaks and Reunions

The results of genetic tests and cytological analysis with the white mottleds are shown in Table IV and Figures 2-17.

The salivary gland chromosomes of each white-mottled, of those cases so far studied cytologically, involve a rearrangement having one break adjacent to the right or left of the white locus, 3C2-3 in the X-chromosome, and the other break in the heterochromatin of the X, second, third or fourth chromosome. Thus, either the white locus has been brought to a heterochromatic region or a heterochromatic segment has been brought to the neighborhood of the white locus through the rearrangment. Table IV shows the distribution of breaks and reunions of the chromosomal rearrangements in a majority of the cases and Table V summarizes the same results.

Table IV

Cytological Analysis and Genetic Test of White Mottleds of *Drosophila melanogaster*

White Mottled	Culture Number	Mottled Male	Cytological Analysis	Genetic Test
$w^{m4}8-1$	C-S 16	inviable	T(1;4) 3E5/101	Linked with 4 Position effect on ci locus Position effect on rst and ec loci
$w^{m4}8-2$	C-S 42	viable fertile	Insertional translocation 4(prob. 101-102F) in normal order to X 3B	Not linked with 4 (duplication?) No effect on ci
$w^{m4}8-3$	y^4 15	inviable	In(1) y^4 X 3C8, 19E-F	Not linked with autosomes
$w^{m4}8-4$	y^4 23	inviable	T(1;3) y^4 X $3C_3$ 3L hetero	Linked with 3 No effect on in, etc.
$w^{m4}8-5$	y^4 24	inviable	In(1) y^4 X $3C_7$ to base	Not linked with autosomes
$w^{m4}8-6$	y^4 25	viable infertile	T(1;2) y^4 X $3C_3$ 2L base	Linked with 2 No effect on lt, etc.
$w^{m4}8-7$	y^4 26	inviable	T(1;2) y^4 X $3C_6$ 2L base	Linked with 2, 3 No effect on lt, etc.
$w^{m4}8-8$	y^4 29	inviable	T(1;3) y^4 X $3C_3$ 3R base	Linked with 3 No effect on in, etc.

Table IV (Cont'd.)

w^{m48-9}	y^4 45	viable fertile	In(1) y^4 X $3C_3$ to base	Not linked with autosomes
w^{m48-10}	y^4 46	inviable	T(1;3) y^4 X 3C 3R base	Linked with 3 No effect on in, etc.
w^{m48-11}	y^4 cv 3	viable infertile	In(1) y^4 X $3C_3$ to base 2-3 entangled	Linked with 2,3 No effect on lt, rl, stw, in, etc.
w^{m48-12}	y^4 cv 48	viable infertile	T(1;2;3) y^4 X 8C/3L 65C y^4 X 3C/2R base	Linked with 2,3 No effect on lt, rl, stw, in, etc.
w^{m48-13}	y^4 f 23	viable infertile	Insertional T y^4 X $3C_2$ to 19F in inverted order into 3L $62D_3$	Linked with 3 No effect on in, etc. No w^m fly with a duplication of the inserted X piece was found
w^{m48-14}	y^4 f 30	viable fertile	In(1) y^4 X $3C_3$ to base	Not linked with autosomes
w^{m48-15}	y^4 f 47	viable fertile	T(1;3) y^4 X 2F 3L 80B	Linked with 3 No effect on in, etc.
w^{m48-16}	y^4 f 11	viable infertile		Not linked with autosomes
w^{m48-17}	y^4 f 13	viable fertile		Not linked with autosomes
w^{m48-18}	y^4 f 16	viable infertile		Not linked with autosomes
w^{m48-19}	y sc^4 2	inviable	In(1) sc^4 X 3C to 19E	Not linked with autosomes

Table IV (Cont'd.)

w^{m48}-20	y sc^4 17	inviable	T(1;2) sc^4 X $3C_3$ 2R base	Linked with 2 No effect on lt, etc.
w^{m48}-21	y sc^4 26	viable infertile	Undetermined	Duplication(?)
w^{m48}-22	y sc^4 29	inviable	In(1) sc^4 X 3C to 1C	Not linked with autosomes
w^{m48}-23	y sc^4 66	viable infertile		Linked with 3 No effect on in, etc.
w^{m48}-24	sc^8 cv 11	inviable		Linked with 2 No effect on lt, etc.
w^{m48}-25	sc^8 cv 14	viable infertile		Not linked with autosomes
w^{m48}-26	sc^8 cv 25	inviable		Not linked with autosomes
w^{m48}-27	sc^8 cv 27	viable, very few infertile		Linked with 3 No effect on in, etc.
w^{m48}-28	sc^8 cv 29	inviable		Linked with 3 No effect on in, etc.
w^{m48}-29	sc^8 cv 33	inviable		Linked with 3 No effect on in, etc.
w^{m48}-30	sc^8 cv 35	inviable		Not linked with autosomes
w^{m48}-31	sc^8 cv 39	viable infertile		Linked with 2 No effect on lt, etc.
w^{m48}-32	rst^3 9	viable		Linked with 3 No effect on in, etc.

Table IV (Cont'd.)

w^{m48-33}	rst^3 11	viable	T(1;4) rst^3 X 3C 4 101		Linked with 4 Position effect on ci locus
w^{m48-34}	rst^3 12	inviable			Not linked with autosomes
w^{m48-35}	rst^3 13	viable			Linked with 3 No effect on in, etc.
w^{m48-36}	rst^3 17	inviable	T(1;2) rst^3 X $3C_3$ 2L base		Linked with 2 Position effect on lt locus
w^{m48-37}	rst^3 21a	viable			Undetermined
w^{m48-38}	rst^3 21b	viable			Linked with 3 No effect on in, etc.
w^{m48-39}	rst^3 22	viable			Not linked with autosomes
w^{m48-40}	rst^3 27a	viable			Not linked with autosomes
w^{m48-41}	rst^3 27b	viable			Linked with 3 No effect on in, etc.
w^{m48-42}	rst^3 30	viable			Not linked with autosomes
w^{m48-43}	rst^3 34	inviable			Linked with 3 No effect on in, etc.
w^{m48-44}	rst^3 46a	inviable			Not linked with autosomes
w^{m48-45}	rst^3 46b	viable			Linked with 3 No effect on in, etc.
w^{m48-46}	rst^3 50	viable infertile	T(1; 2; 3) rst-3 X 3C 2R base		Linked with 2, 3 No effect on in, etc. Position effect on ltd locus No effect on rl, stw, etc.

Table V

Types of Chromosomal Rearrangement in White-Mottleds of *Drosophila melanogaster*

	In(1) or Insertion of X-piece to Chromocenter	T(1;2)	T(1;3)	T(1;4)	Undetermined
Canton-S	0	0	0	2	0
y^4	8	3	5	0	0
sc^4	2	1	1	0	1
sc^8	3	2	3	0	0
rst^3	5	2	6	1	1
Total	18	8	15	3	2

Of the forty-six cases studied, eighteen are due to rearrangements within the X-chromosome and chromocenter; eight due to X-2 translocations; fifteen X-3 translocations; three X-4 translocations and two cases undetermined. The breaks and reunions in the fourth chromosome are much rarer, but the two mottleds from Canton-S stock are both due to X-4 translocations. This difference may be due to the differences of the X-chromosomes irradiated. The data collected in Bridges and Brehme's book (1944) from the previous studies of white mottleds show the number of different types of rearrangements as follows: In(1) and insertions into chromocenter, three; T(1;2), eight; T(1;3), six; and T(1;4), eleven, of a total of twenty-eight cases. All of them were induced from the flies with a normal gene sequence in their X chromosomes. The X-4 translocations obviously constituted a large proportion.

White Mottling with Euchromatic Breaks Only?

In one case of the white mottleds, for which the symbol w^{m48-13} is used, the salivary gland chromosomes show that a piece of the y^4 X-chromosome carrying the bands from $3C_2$ to 19 F is inserted in inverted order to the left arm of third chromosome at 62 D_3 region (Fig. 12, 12a, 12b). Does this inserted segment carry the white locus? It is a very important point concerning both the phenomenon of white mottling and the location of the white locus in the salivary gland chromosome. At the joint to 3L, two bands, $3C_1.2$, in the inserted X-piece are clearly seen, leaving no room for doubt.

If the white locus is in $3C_2$, as Schultz has determined, then the white mottling in this case is due to another euchromatic break in 3L in addition to the break in $3C_2$ in the X chromosome. And this should be considered as a position effect of eu-euchromatic type, having nothing to do with heterochromatin. In attempting to clear up this point, several tests were made. Supposing the above statement is correct, it is possible to get a fly carrying a normal X-chromosome with the inserted X-piece as a duplication segment. The following experiments were planned on this assumption.

(a) y^4 f/y Hw w lz^s, w^m +/+ ♀(mottled) X y w spl, +/D Cx ♂

F_1 y^4 f/y w spl, w^m +/D Cx ♀(mottled) X y w spl ♂

F_2 y w spl, w^m +/+ ♀(duplication) X y w spl ♂

(b) y^4 f/y Hw w lz^s, w^m +/+ ♀(mottled) X Gl/D Cx ♂

F_1 y^4 f/+, w^m +/D Cx ♀ X Gl/D Cx ♂

F_2 look for +, w^m +/D Cx ♂ (duplication)

or +, w^m +/Gl ♂ (duplication)

No flies carrying such a duplication piece were found. These two tests might not be good since the D Cx inversions can not prevent crossing over in the 3L left portion into which the X-piece has been inserted.

(c) y^4 f/y Hw w lz^s, w^m +/+ ♀(mottled) X sc^7 w ♂

F_1 y^4 f/sc^7 w, w^m +/+ ♀(mottled) X y Hw w lz^s ♂

F_2 look for y Hw w ♀♀ (y^4 f-Df/y Hw w lz^s, +/+) (deficiency)

non-y Hw w^m ♀♀ (sc^7 w/y H w w lz^s, w^m +/+) (duplication)

sc^7 w^m ♂♂ (sc^7 w, w^m +/+) (duplication)

One non-y Hw w^m female was found from this experiment but it was very weak and could not be bred further to be verified as a fly with the duplicated X-piece. The experiment was repeated, but no suspected duplication flies could be found again.

Location of The White Locus in Salivary-chromosome.

The alternative explanation for the above case is that the white locus is not at $3C_2$ and the inserted X-piece does not carry the white locus. The white locus which is left in the remaining portion of the y^4 X-chromosome, may be at $3C_3$. This $3C_3$ band left in the deficient chromosome has been brought to the heterochromatin of X base since the segment from $3C_2$ to 19F was deleted and inserted to the tip of 3L. If this be the case, the white mottling is due, as in the usual case, to the effect of heterochromatin on the white locus.

Demerec and Sutton (1942) place the white locus at $3C_{2-3}$ and Prokofyeva-Belgovskaya (1941) places it at $3C_3$. In several cases in the experiments described above, the salivary-chromosomes have shown clearly that the break in the X-chromosomes is to the right of the $3C_3$ band (Fig. 7, 8, 8a, 10, 14). It is very likely that the white locus is at $3C_3$.

Position Effect of w^m on Heterochromatic Genes

The tests made to detect the effect of w^m on the normal alleles of lt, rl, stw, ltd, in, ri and ci genes in the cases of x-2, X-3 and X-4 translocations yield positive results in only four strains. These have been shown in Table IV and the following is the summary.

Table VI

Position Effect of w^m on Heterochromatic Loci in Drosophila melanogaster

Strain of w^m	Linkage	Cytology	Locus Affected
w^{m48-1}	X-4	$3E_5$/101	ci
w^{m48-33}	X-4	3C/101	ci
w^{m48-36}	X-2	$3C_3$/2L hetero.	lt
w^{m48-46}	X-2	3C/2R hetero.	ltd

The offspring from the crosses of these four strains of white mottleds with ci, lt and ltd mutant flies show ci, lt and ltd characters respectively. This is a position effect of euchromatin upon the heterochromatic loci, being opposed to that of heterochromatin upon an euchromatic locus such as in the case of white mottling.

(B) THE BAR^{48} MUTANT

B^{48} occurred as a single female, in the progeny of one of the X-ray experiments, described above, with y sc^{4} males. Its eyes looked like that of the combination of B and lz^{g} (lozenge-glossy eyes), very narrow with a few facets on its shining surface. This dominant mutant is sex linked and is also linked with the second chromosome. Cytological evidence proved it to be an X-2 translocation with one break in the sc^{4} X at 15F, just to the left of 16A, the Bar locus, and with the other break in 2L at 33B. Thus it turned out to be a case of position effect, being more or less like the recessive mutant Baroid of Dobzhansky (1932, 1936). As the translocation has two breaks, both in euchromatic regions, this is a case of position effect of eu-euchromatic type.

The genetic test and cytological analysis with this mutant will be described in more detail in the fourth part of this thesis, under the title of "Mutations".

EXPERIMENTS WITH HETEROCHROMATIC GENES

(A) THE LIGHT MUTANTS

The gene lt (light eye color) has been known to be located in heterochromatin of the left arm of the second chromosome. In the case of Plum-2, which is inseparable from $In(2LR)Pm^2$ with one break in the euchromatin of 2R and the other break in the heterochromatin of 2L, the dominant mutant behaves as an allele to both mutants brown and light. (Schultz and Dobzhansky 1934; Schultz 1936). The Pm/lt compound shows more dilute eye color and more pronounced variegation than does that of Pm/bw. These have been interpreted as position effects of these two loci, light and brown, which are respectively near the two breaks of the inversion. Since the brown, in euchromatin, has been brought to a heterochromatic region and light, in heterochromain, has been brought to an euchromatic region, the position effect of the brown locus is of the eu-heterochromatic type and that of the light locus is of the hetero-euchromatic type.

Schultz (1936) has demonstrated the effect of the Y-chromosome on the expression of variegation in Pm^2/lt bw flies by changing the number of Y-chromosomes. From XO male to XXYY female, the expression of variegation varies in such a manner that more Y's give more light eye color and less Y's give more brown. Thus, the additional Y suppresses the variegation of brown in the same manner as it does that of other euchromatic loci such as white-Notch (Growen and Gay 1934); but it enhances the expression of the heterochromatic locus, light.

In addition to the mutant w^{m48-36}, where a position effect on the lt locus has been proved genetically and the cytological evidence shows that one break is near the light locus, the experiments done on this locus are presented as follows.

MATERIAL AND METHODS. Males from a mutant stock, b (black body color, in the second chromosome), were irradiated with an X-ray dosage of about 4,500 r units (120 KV, 8 ma, 15 cm, 12,94 minutes, 1 mm. Al filter) and mated to virgin lt females. Their F_1 offspring were examined for lt or lt-variegation. Whenever a lt or lt-variegated fly was found, it was mated to flies carrying Cy(2L), dp^2 b pr in one of their second chromosomes. Cy(2L), dp^2 b pr is known as a balancer for 2L, in which Cy (Curly wing) is associated with the In(2L)Cy and dp^2 (dumpy wing), b (black body) and pr (purple eye) are included. The Cy b flies (b lt-mutant/Cy(2L), dp^2 b pr) of the next generation were selected and mated to each other to keep a balanced stock, if the new mutant is homozygous lethal. Otherwise, a homozygous stock of the mutant is established.

The mutants that occurred might be due to chromosomal rearrangements, such as translocations, inversions or deficiencies, with one break near the lt locus, which has thus changed its neighborhood to be near euchromatin (position effect), or they might be due to deficiencies or point mutations of the locus.

Genetic Tests. To test the linkage of the new light mutants with chromosomes other than the second, the following crosses were made and their F_2 offspring were examined.

P_1 b lt-mutant/Cy(2L) dp^2 b pr ♂ or ♀ X y f, bw, e, ci ey ♀
or bw, e, ci ey ♂

F_1 b lt-mutant/bw, +/e, +/ci ey ♂ X y f, bw, e, ci ey ♀

F_2 if the lt-mutant in question is due to:

T(2;3) flies would be either bw, e; or non-bw, non-e, regardless of other characters.

T(2;4) flies would be either bw, ci ey; or non-bw, non-ci non-ey, regardless of other characters.

T(1;2) all females would be bw
all males would be non-bw

T(Y;2) all males would be bw
all females would be non-bw

The flies with the constitution of y f, bw, e, ci ey used in the above crosses carry y (yellow body) and f (forked bristle) in the attached X-chromosomes, bw (brown eye) in the second, e (ebony body) in the third and ci (cubitus interruptus vein) and ey (eyeless) in the fourth chromosomes.

Deficiency for a segment of a chromosome is generally lethal in homozygous condition. The lethal lt-mutants, suspected to be possible deficiencies, were mated to rh (roughish eye), Bl (Bristle beaded), Alu (Alula fused to wing), Jag (Jagged wing), M(2)D (Minute bristle), rl (rolled wing), stw (straw color) and ltd (lightoid eye color) flies respectively. These mutant genes are located in the second chromosome to the left or the right of the centromere. If a mutant is deficient in one or several of these loci, the allelic gene or genes would be expressed in heterozygous condition. This deficiency test usually gives precise information for the location of genes.

A certain proportion of the lt mutations might be due to inversions. If an inversion occurred in the second chromosome, the crossing over in the inverted portion should be suppressed or reduced. For a rough test, the mutant flies were mated to flies carrying dp (dumpy wing), b (black body), pr (purple eye), c (curved wing), and px (plexus vein) genes which are located in the second chromosome. F_1 females of the constitution b lt-mutant/dp b pr c px were mated to the males of dp b pr c px again. The F_2 flies were classified and counted to check roughly the crossing over values between these loci.

If a lt-mutant is due to a position effect, as in the case of Pm/lt bw (Schultz 1936), it may be possible to demonstrate the effect of adding or subtracting a Y-chromosome on the expression of the "light" character. To check a Y effect, the following crosses were made.

(1) b lt-mutant/Cy(2L) dp^2 b pr ♂ X y Hw v^o f/w m f/Y, lt ♀
(XXY ♀)

F_1 Compare the eye color of XX, lt/lt-mutant ♀♀ and
XXY (Hw f), lt/lt-mutant ♀♀

A mating of lt males from the stock room with the XXY lt females was made as control. The XXY females carrying yellow, Hairy wing, vermilion, forked in one X and white, miniature, forked in the other show Hairy-wing and forked-bristle characters.

(2) b lt-mutant/Cy(2L) dp^2 b pr ♂ X y^2 su-w^a w^a bb, lt/+ ♀
(without Y, showing bobbed)

F_1 Examine the eye color of XO, lt/lt-mutant ♂♂

A cross of lt ♂ (from stock room) X $\widehat{XX}$ bb, lt/+ ♀ was made as control. The attached X-chromosomes of the females bear yellow, suppressor of white-apricot, white-apricot and bobbed in homozygous condition. Since a normal allele of bb is carried in Y, the bobbed character only shows up in the absence of a Y-chromosome.

All the matings were made at 25° C.

It has also been known that temperature exerts effect on the expression of white mottling. The mottling is suppressed at high temperature. Temperature might also have an effect on the expression of the "light" character of these mutants. To check for a temperature effect, the mutants were mated to lt-mutants from the stock room. Each mating was made in two sets, one set raised at 19° C and the other set at 28° C. The eye color of F_1 "light" flies was examined and compared with that of the females from the above tests.

Cytological Analyses. Several lt-mutants were selected for cytological studies. These mutants had been shown genetically to be 2-3 translocations or of other interest. Flies of these strains raised at 19° C. Temporary preparations of the salivary glands of these larvae were made with aceto-orcein. The salivary gland chromosomes were studied with Bridges' map (1935).

RESULTS. A total of 5,280 F_1 flies were examined from the crosses of irradiated black males with light females in two experiments. Thirty-six flies showing the lt (or lt-variegated) character have been found. Ten out of these thirty-six mutants proved to be sterile, and another five mutants lost their mutant character before tests could be completed. Symbols lt^{48-1} to lt^{48-21} are used for the remaining lt-mutants.

Tables VII and VIII show the general results.

Table VII

Frequency of X-ray Induced lt-Mutants in

Drosophila melanogaster (Dosage-4,500 r units)

Males X-rayed	Date of Experiments	Total Number of F_1 flies	Number of lt-mutants (No. fertile)	Percent of lt-mutants	Percent of Fertile lt
b	July 29, '48	2682	15(12)	0.56±0.14	0.45±0.13
b	Sept. 2, '48	2598	21(14)	0.81±0.17	0.54±0.14
TOTAL		5280	36(26)	0.68±0.11	0.49±0.10

Table VIII

Lt-mutants, X-ray Induced, in Drosophila melanogaster

(Dosage--4,500 r units)

lt Mutant	Culture Number	Sex, When Found	Linkage To Other Chromosome	Cytology	Other Remarks
lt^{48-1}	A 3	♀	2-3	T(2;3) 2L hetero. 3L 64B,C	Homo. lethal Position effect
lt^{48-2}	A 4	♀	2-3	T(2;3) 2L 23E,F 3R 86 E In(2) 2L left of 40A-2R 47A,B	Homo. lethal
*	A 5a	♀	none		(Lost)
*	A 5b	♀ lt^{v}	none		(Lost)
lt^{48-3}	A 8	♂	none		Homo. lethal
lt^{48-4}	A 10a	♀	none		
*	A 10b	♀	2-3	Normal	(Lost)
lt^{48-5}	A 12	♂ lt^{v}	none		Homo. lethal
lt^{48-6}	A 13	♀	none		
*	A 15	♂	none		(Lost)
lt^{48-7}	A 18a	♀	none	In(2) 21F-39E_3 Df(2) 39E_3 to 40B_1 or further	Minute, homo. lethal Df. for rh locus

Table VIII (Cont'd.)

lt^{48-8}	A 18b	♂	none		Homo. lethal
lt^{48-9}	B 2	♂	none		Homo. lethal
lt^{48-10}	B 8	♀	none		Homo. lethal
lt^{48-11}	B 9a	♀	none		Slight Minute Homo. lethal
lt^{48-12}	B 9d	♂ lt^{V}	2-3	T(2;3) 2L hetero. 2R 50C 3R 93B,100A_4	Position effect
lt^{48-13}	B 10	♂ lt^{V}	none		Homo. lethal
lt^{48-14}	B 11c	♂ lt^{V}	none		Homo. lethal
lt^{48-15}	B 13	♂	none		Minute, homo. lethal Df for rh locus
lt^{48-16}	B 16a	♀	none		Homo. lethal
lt^{48-17}	B 16b	♂	none		Homo. lethal
*	B 17	♀	none		(Lost)
lt^{48-18}	B 18a	♀	none	Normal	
lt^{48-20}	B 19	♀ lt^{V}	2-3	T(2;3) 2L hetero. 3R 100B_3	Homo. lethal Position effect
lt^{48-21}	B 20	♀	none		
lt^{48-19}	B 18a	♀	none		Homo. lethal Inversion

The Frequency of lt Mutations

As shown in Table VII, the frequency of induced mutations at the lt locus is remarkably high. Some sex-linked genes such as white, scute, forked, etc. are known to be induced at high rates. However, their rates are low in comparison with the frequency of these lt mutants. Table IX shows the comparison made between the frequencies of the mutations at these loci and that at the stw locus, being calculated on the basis of 4,500 r units. The induced mutations at the stw locus will be described later.

Table IX

Induced Mutation Rates of Some Loci in Drosophila melanogaster

Mutation	Dosage	Percent of Mutation	Investigator	Dosage	Percent of Mutation
w^+---w	4800 r	0.051	Timoféeff-Ressovsky 1933	4500 r	0.048
w^+---w^x	4800 r	0.076	"	4500 r	0.071
sc^+--sc	3975 r	0.052	Moore 1934	4500 r	0.058
ec^+--ec	3975 r	0.154	"	4500 r	0.174
v^+---v	3975 r	0.052	"	4500 r	0.058
g^+---g	3975 r	0.052	"	4500 r	0.058
f^+---f	3975 r	0.052	"	4500 r	0.058
lt^+--lt				4500 r	0.492
stw^+-stw				4500 r	0.169

(w^x signifies any new alleles of w locus)

There are six lt-variegated flies among the twenty-six fertile lt-mutants (Table VIII). These may belong to another category of mutations at the lt locus. The frequency of the remaining twenty lt-mutants, from a total of 5,280 flies examined, is, then, 0.378±0.08, which is still very high.

No interpretation can easily be made for such a high frequency of mutations at the lt locus. It may be due to a peculiar property of heterochromatin since the lt locus is in heterochromatin and the evidence from previous studies indicates that the X-ray induced breaks in heterochromatin are more frequent (Bauer, Demerec and Kaufmann 1938).

Types of Chromosomal Rearrangements

In linkage tests, five lt mutants, lt^{48-1}, lt^{48-2}, lt^{48-12}, lt^{48-20} and culture no. A 10b, show a linkage relationship between the second and third chromosomes. Cytological examination proves that four of them are 2-3 translocations among which one mutant, lt^{48-2}, also carries an inversion. The other mutant, A 10b, lost its mutant character before cytological analysis could be made.

Two mutants, lt^{48-7} and lt^{48-15}, show a M (Minute bristle) character; deficiency tests prove that each is deficient in a segment including certain other loci in addition to the lt locus (lt^{48-7} has also been examined cytologically).

The mutant, lt^{48-19}, shows, in the inversion test, that a long inversion including parts of both 2L and 2R has occurred, since

there is no crossing over in the major part of the whole second chromosome.

Altogether, as far as the evidence from various tests goes, there are four 2-3 translocations, two inversions and two deficiencies. Others are perhaps due to minute inversions, deficiencies or "point" mutations.

The salivary gland chromosomes of several lt mutants, which have been analyzed, are shown in Figures 18-22. The mutant lt^{48-1} carries a translocation between 2L and 3L, with the break in 2L in heterochromatin and that in 3L at 64B,C (Fig. 18). The mutant lt^{48-2} shows an inversion from 2L base (probably to left of 40A) to 2R 47A,B and a translocation between 2L and 3R with breaks at 23E,F and 86F respectively (Fig. 19). The mutant lt^{48-12} has a translocation between 2L, 2R and 3R, with breaks in 2L in heterochromatin, 2R at 50C and 3R at 93B and $100A_4$ (Fig. 21), thus the three interchanged chromosome-arms are (a) 2L base, 3R 100A to tip; (b) 2R base to 50C,3R 93B to 100A, 2L from heterochromatic region to tip; and (c) 3R base to 92B, 2R 50C to tip. The mutant lt^{48-20} carries a translocation between 2L and 3R with breaks in 2L in heterochromatin and 3R at $100B_3$ (Fig. 22, 22a).

The analysis of the salivary-chromosomes of the mutant lt^{48-7}, which has shown genetically a deficiency for certain loci in its 2L, shows that they appear to have carried an inversion in 2L from 21F to $39E_3$ and a deficiency in 2L base from $39E_3$ to $40B_1$ or further (Fig. 20, 20a).

Position Effect at the lt Locus

Six lt mutants showed, when they were first found, light-variegation with dark patches or spots on the background of "light" color. They were suspected to be due to position effects. After genetic and cytological studies, two of them have turned out to be actual position effects. They are lt^{48-12} and lt^{48-20}. As described above, each of these two mutants carries a 2-3 translocation with one break in the heterochromatin of 2L, probably at or near the lt locus, and the other break at the tip of 3R. Thus, in each case, the neighborhood of the lt locus has been changed from heterochromatin to euchromatin of 3R tip (Fig. 21, 22). The new euchromatic neighborhood decreases the dominance of the heterochromatic lt^+, as does inversely the heterochromatin to the euchromatic w^+, and consequently the combination of this affected lt^+ locus over a recessive lt allele gives a lt or lt-variegated phenotype. In lt^{48-12}, the flies homozygous for the translocation also show lt-variegation.

As described above, the mutants lt^{48-1} and lt^{48-2} have also been shown cytologically to be 2-3 translocations. In lt^{48-1}, one break of the translocation is at the base of 2L, probably near the lt locus, and the other break is in 3L at 64B,C (Fig. 18). Thus the neighborhood of the lt^+ has changed from heterochromatin to the euchromatin of 3L 64B,C. The lt mutation in this case is, no doubt, also due to a position effect.

In lt^{48-2}, the translocation between 2L and 3R perhaps has nothing to do with the lt mutation, since the breakage point in 2L is

far away from the heterochromatin or the lt locus (Fig. 19). In addition, this mutant carries an inversion with one break at 2L base to left of 40A and the other break in 2R at 47A,B. The break in 2L is still not in heterochromatin. The tests for the effect of the Y-chromosome and that of temperature upon the lt-mutant character give no strong indication to verify that a position effect is present in this mutant.

In lt^{48-7}, an inversion from 21F to $39E_3$ has occurred in 2L, and this inverted chromosome is also deficient in a segment from $39E_3$ to $40B_1$ or further (Fig. 20, 20a). If the lt locus is involved in the deficient segment, the lt-mutation is simply due to deficiency of this locus. As the results from other tests show(for instance, no effect of the Y-chromosome or of temperature can be demonstrated), it is very likely that this mutant is merely due to the deficiency.

The Y-Chromosome Effect

All the lt-mutants have been checked for the effect of an additional Y-chromosome upon the expression of their lt-mutant character. In a majority of cases, the results are negative. To some mutants, such as lt^{48-2}, lt^{48-3}, lt^{48-11}, lt^{48-13} and lt^{48-14}, an additional Y exerts some, but not a very striking effect. However, in lt^{48-1}, lt^{48-12} or in lt^{48-20}, an additional Y-chromosome makes the eye color much lighter than that of the same mutant with the normal number of sex chromosomes. The lt-variegation does not show up when an extra Y is present. For some unknown reason, the body color of

lt^{48-12} also looks lighter in the presence of an additional Y. The constitution of such a fly is y Hw v^{o} f/w m f/Y, lt/lt^{48-12}.

In the opposite case, the effect of subtracting a Y from such mutants as lt^{48-1}, lt^{48-12} and lt^{48-20} is to make the eye color darker and more pronounced in lt-variegation with dark spots, appearing more nearly wild type.

In comparison with the effect of the Y-chromosome in white mottleds, it seems clear that its effect goes in an opposite direction in these lt mutants as far as the intensity of eye color is concerned. In other words, an extra Y darkens the eye color in white mottleds but it lightens the eye color in these lt mutants. The lt-variegation is, however, not enhanced by an additional Y-chromosome. These mutants, when over a lt allele, show some variegation with somewhat dark patches, not condensed spots, on the light-colored background. With an extra Y being present, the eye color becomes much lighter and no variegation can be seen. This is probably because the extra Y increases the lt area until it includes the whole eye, thus having no dark areas. In agreement with this relation, the mutants on subtracting a Y-chromosome show a darker color with variegation of dark spots, appearing more like wild-type.

Temperature Effect

These lt-mutants were also raised at 19° and 28° C to check for a temperature effect upon their lt eye color.

In ordinary lt mutants, the eye color is slightly darker at low temperature and slightly lighter at high temperature. To

these three mutants, lt^{48-1}, lt^{48-12} and lt^{48-20}, which have shown the Y effect on their lt-character, the effect of high temperature, 28° C, acts in a different manner in that it darkens the eye color. Especially in lt^{48-1} and lt^{48-20}, the lt-color appears very dark with variegation approaching wild-type.

High temperature suppresses the variegation of eye color in white-mottleds and thus makes the eye appear more normal. Here, the high temperature does not suppress lt-variegation but similarly makes the eye color darker and more normal-like.

As a control test, a lt-mutant from the stock room was checked for the effect of Y-chromosome and of temperature with negative results.

(B) THE STRAW MUTANTS

The gene stw (straw color) is located in the heterochromatin of the right arm of the second chromosome.

No work on position effect for straw had previously been done. The data on mutations and position effect secured from the studies with X-rays at this locus are presented in the following.

MATERIAL AND METHODS. Males carrying pr cn (purple and cinnabar eye color, in the second chromosome) were X-rayed with a dose of approximately 4,500 r units (120 KV, 8 ma, 15 cm, 12.94 minutes, 1 mm. Al filter). Irradiated males were mated to virgin straw-3 females and F_1 flies were examined for mutations of stw (or stw-variegation). Whenever a stw-mutant was found, it was mated to flies carrying Cy(2L) dp^2 b pr in one of their second chromosomes and their offspring with the constitution of pr cn stw-mutant/Cy(2L) dp^2 b pr were bred for genetic and cytological studies.

All the genetic tests carried out with the lt-mutants were similarly applied to these stw-mutants.

For deficiency tests, the stw-mutants which are homozygous lethal were mated to lt, rl, M(2)S4, M(2)S10, blt (blot wing), ap^4 (apterous wing), Xa (Xasta wing), pk (prickle bristle), tk (thick leg), tuf (tufted bristle) and ltd flies.

In testing the effect of the Y-chromosome, females of the composition XX(Hw f)Y stw and $\widehat{XX}$ bb, stw/+. were used.

To study cytologically the salivary-chromosomes, a few strains of stw-mutants were chosen and mated to stw^3 flies. Temporary preparations with the salivary glands of their larvae were made and compared with the maps of Bridges (1935, 1939).

RESULTS. From 8,277 F_1 flies of the crosses pr cn ♂♂ (X-rayed) X stw^3 ♀♀, twenty-five stw-mutants were found. One fly carried straw bristles and hairs only on the left side of its thorax, but its straw offsprings are wholly straw. No stw-variegation has been observed.

Table X

Frequency of X-ray Induced stw-mutants in Drosophila melanogaster (Dosage-4,500 r units)

Males Rayed	Date of Exp't	Total of F_1 flies	Number of stw-mutants (No.fertile)	Percent of stw-mutants	Percent of fertile stw-mutants
pr cn	Sept.2,'48	8277	25(14)	0.30±0.06	0.17±0.04

As shown in Table X, the frequency of induced mutations at the stw locus is also high. The percentage of the fertile stw-mutants is, however, just as high as that of ec-mutations (see Table IX).

The symbols stw^{48-1} to stw^{48-12} are used for these mutants.

So far as the evidence from linkage tests shows, in two mutants, stw^{48-9} and stw^{48-12}, the second and third chromosomes are linked. Cytological studies prove that a 2-3 translocation is

present in each case and that an additional inversion has occurred in the stw^{48-9}.

The translocation of 2-3 in stw^{48-9} with breaks in 2L at 34D and in 3R at 89E seems to have nothing to do with the stw- mutation, while the inversion in its 2R base from $41D_4$, just to right of $41D_3$, to left of 42B is probably the cause of the mutation (Fig. 23, 23a). This minute inversion has brought the euchromatin of 42A to the heterochromatin, adjoining $41D_3$. Thus the neighborhood of $41D_3$ has been changed from heterochromatin to euchromatin, the latter caused the mutation. If this be the case, the mutation is due to a position effect. The band $41D_3$ is, therefore, very possibly the locus of straw.

In stw^{48-12}, the translocation between 2L and 3R is rather complicated, with the tip of 2L being inserted into the chromocenter. No detectable change in the base of 2R can be found.

Table XI

X-ray Induced stw-Mutations in Drosophila melanogaster (Dosage-4500 r units)

stw Mutants	Culture Number	Sex, When Found	Linkage to Other Chromosome	Cytology	Other Remarks
*	s 7	♀	None		(Lost)
stw^{48-1}	s 8	♀	None		Homo. lethal
stw^{48-2}	s 9	♂	None		
*	s 12	♀	None		(Lost)
stw^{48-3}	s 16	♀	None		Df.for blt^+, ap^+, tk^+, etc. Homo. lethal
stw^{48-4}	s 20	♂	None		Df.for blt^+, ap^+, etc. Homo. lethal
stw^{48-5}	s 21b	♂left thorax straw	None		Homo. lethal Slight Minute
stw^{48-6}	s 23b	♂	None		
stw^{48-7}	s 27	♂	None		Minute, homo. lethal Df.for blt^+, ap^+, etc.
stw^{48-8}	s 30	♀	None		Homo. lethal
stw^{48-9}	s 32a	♀	2-3	T(2;3) 2L 34D 3R 89E In(2) $41D_4$-- 42B	Homo. lethal
stw^{48-10}	s 33a	♂	None		Homo. lethal
stw^{48-11}	s 35	♂	None		
stw^{48-12}	s 36	♂	2-3	T(2;3) 2L/3R	Homo. lethal

Deficiency tests with these stw mutants indicate that stw^{48-3}, stw^{48-4}, stw^{48-5} and stw^{48-7} are deficient in certain loci, probably including the stw locus. Table XII shows the results.

Table XII

Deficiency Tests of Some stw-mutants of

Drosophila melanogaster

LOCI TESTED	rl	M(2)S2	M(2)S4	M(2)S10	blt	ap^4	Xa	pk	tk	tuf	ltd
stw-MUTANTS											
stw^{48-3}	-	-	1		blt	ap	Extreme	-	tk	-	-
stw^{48-4}	-	-	1		blt	ap	Not extreme	-	-	-	-
stw^{48-5}	1?	1	1	1	-			-	-	-	-
stw^{48-7}	1?	1	1	1	blt	ap	Extreme	-	-	-	-

For some unknown reason, the mutants stw^{48-5} and stw^{48-7} are lethal with rl. The cross pr cn stw^{48-7}/Cy(2L) dp^2 b pr ♂♂ X rl ♀♀, or reciprocal, gives no normal-like flies (pr cn stw^{48-7}/rl). The cross pr cn stw^{48-5}/Cy(2L) dp^2 b pr ♂♂ X rl ♀♀, or reciprocal, gives very few normal-like flies, for instance, in two such matings the number of Cy and normal-like flies is 193 and 1; 147 and 4. In another mating, some extreme rl flies appear at the end of the examination and counting of the F_1 flies. This mutant, stw^{48-5}, was, when first found, a male with straw bristles and hairs on the

left side of its thorax. However, its straw progeny (stw-mutant/ stw^3) are wholly straw.

Three stw mutants, stw^{48-3}, stw^{48-4} and stw^{48-7} are deficient for the blt locus. According to Schultz, blt is allelic to Xa and ap^4. These three mutants were, therefore, also tested with ap^4 and Xa. The results are shown in Table XII. Being over ap^4 allele, all these three mutants show apterous-characters; while over Xa, stw^{48-3} and stw^{48-7} flies show very extreme form of Xasta wings and with eyes somewhat bigger and brighter red in color, but stw^{48-4} appears no more than an Xa mutant, leaving some doubt about the allelic relationship of Xa to blt or ap^4.

The mutant stw^{48-3} is also deficient for the tk locus but not for pk. This shows that the pk locus is probably at some place to the right of tk.

In summary, the rearrangements so far detected among these stw-mutants are two 2-3 translocations, one inversion and four deficiencies. Only in one case, stw^{48-9}, is the stw-mutation obviously due to a position effect.

Neither the Y -chromosome nor temperature has a striking effect on the expression of the stw-mutant character of these mutants.

DISCUSSION AND SUMMARY

A considerable amount of work has been done on the position effect in Drosophila melanogaster. Previous studies have been reviewed briefly in the first part and at the beginning of this part. This part has presented the genetic and cytological studies of the forty-six white mottleds, and of three lt-mutants, one stw-mutant, among other lt and stw mutants, which are due to position effects.

The frequencies of the white-mottleds induced from the rst^3 X-chromosomes and of the mutations induced at the lt locus are remarkably high in comparison with those of other induced mutations. However, it is clear, the mutation rate of a gene depends on the particular gene or allele in question, on the species, strains, and on the genetic constitution of the organism. Some genes are stable, others mutate frequently. Some genes are stable in certain individuals, but may mutate frequently in others when combined with certain other genes. The white mottleds induced from rst^3 males and the mutations at the lt locus of black flies probably can be simply taken as extremely unstable cases. Nevertheless, in white mottleds, each mutant must have a chromosomal rearrangement with one break near the white locus and the other in heterochromatin, involving at least two breaks.

The rst^3 inversion in the X-chromosome involves a left break at roughest, $3C_4$, near white, and the right break in heterochromatin probably at 20B, close to the centromere. Thus, the heterochromatin of the X-chromosome base had already been brought to the vicinity of the white locus in those flies before irradiation. It would be

natural for one to assume that the high frequency of white-mottleds induced from rst^3 flies might be, in the majority of cases, due to small changes which made the white locus closer to the inverted heterochromatic segment, or that the rst^3 flies themselves might have already carried some white-mottling characters. Disproving the second point, a total of about three thousand F_1 flies from the crosses of unirradiated rst^3 males with w m f females were examined with negative results as to the occurrence of white-mottling. Regarding the first point, the genetic tests show, as listed in Table IV, that in fourteen cases, from w^{m48-32} to w^{m48-46}, there are only five mottleds with arrangements within their rst^3 X-chromosomes, but two X-2, six X-3, one X-4 translocations. Cytological evidence from one X-4 and two X-2 linked mottleds proves that each with one piece of the X-tip, involving the white locus, has been translocated to 4, 2L or 2R heterochromatin, rather than having undergone a minute change within the X-chromosome (Fig. 15, 16, 17).

Therefore, what might possibly be the cause which is responsible for the high frequency? The breakability of heterochromatin and the susceptibility of a new union point to induced breakage are worthy of note. Sitko (1938) presented evidence that the region of new unions established as a result of inversion and translocation are more susceptible to induced breakage than such regions in the normal chromosomes. Somewhat similar evidences obtained by Tzubina (1939), in her studies of cubitus interruptus, by Griffen and Stone (1940), in their studies of the w^{m5} translocation, also indicate a "weak attachment" of the original breakage and union point. On the

other hand, according to Bauer, Demerec and Kaufmann (1938), the X-ray induced breaks in heterochromatin are more frequent than that in euchromatin. It is very likely, then, that a frequent breakage at the region of the left union point of the rst^3 inversion in the X-chromosome, probably especially in the heterochromatin at that region, plus the frequent breakages in heterochromatic regions of all the chromosomes, might be responsible for the high frequency of the occurrence of white-mottleds from irradiated rst^3 flies. However, studying cytologically the reversions of rst^3 , Kaufmann (1942) found no indication that the positions of the breaks were determined by preexisting "weak" spots in the chromosomes.

It can also be noted that different frequencies may be observed in different experiments with the same kind of flies. Table II shows that in one experiment with y^4 flies the percentage of white mottleds is 0.44 while in another it is 0.08; and in one experiment with y^4 f flies it is 0.32, in another 0.08. The percentage of 0.44 or 0.32 is unusually high while that of 0.08 is about the average rate. It would seem, therefore, that the high frequency of white mottleds induced from rst^3 flies in one experiment can hardly be taken as a characteristic rate for this particular genetic composition.

Returning now to the property of heterochromatin, it has been known that the heterochromatin is the center of nucleic acid synthesis, either directly or indirectly as sources of protein precursors. It forms a large amount of thymonucleic acid, it forms or affects the composition of the nucleoli. The nucleic acid metabolism in heterochromatin is correlated with the variegation or mottling

of somatic characters (Heitz 1939; Caspersson and Schultz 1938; Schultz and Caspersson 1939). So far as the cytological evidence goes, the white mottling is really a kind of position effect peculiar to heterochromatin. Nevertheless, the effect of heterochromatin exerted on an euchromatic locus shows, sometimes, specificity. For instance, in some w^{m48} mutants the heterochromatin extends its position effect to the white locus from 2F, $3C_7$, $3C_8$ or $3E_5$ (Table IV), over several or some nineteen bands of the salivary gland chromosome. And Demerec (1940) found that position effect can extend over fifty bands. In rst^3, however, the heterochromatic region of 20B which has been brought so close as only one or two bands next to the white locus has no effect on the expression of white mottling. It seems clear that there must be a qualitative difference within the heterochromatic material and that certain parts of the heterochromatin are specific in producing white mottling.

In the case of w^{m48-1}, the X-4 translocation involves a break in the X-chromosome at $3E_5$. Several loci, such as roughest, facet and echinus, are known to lie between the white locus and this breakage point. To test the position effect on these three loci, matings of w^{m48-1} with roughest-3, facet and echinus flies were made respectively, from which the offspring are roughest or echinus but not facet. This means that in this case the loci of roughest and echinus, but not that of facet, are also affected by the heterochromatin of the fourth chromosome. As the facet locus has not been affected, the heterochromatin concerned may not be specific in exerting position effect on this locus, or, this euchromatic locus may not be specific to response to the effect of this heterochromatin. Another possibility

is that the result may be related to the usual slight (or no) expression of facet in females.

The specificity of heterochromatin or that of an euchromatic locus in the position effect is perhaps a problem of chemistry. Although the general chemical nature of heterochromatin and euchromatin, such as nucleic acid and protein content, have been known to some extent, yet the present state of knowledge is far from being complete enough to interpret the position effect in terms of chemistry.

Other properties of heterochromatin, in addition to those mentioned above, have been known as follows. Heterochromatin has been suggested to be a region in which the same gene is duplicated many times. These regions are regarded as genetically inert. A large portion of the mitotic X-chromosome is made up of heterochromatin as "blocks", but in salivary-chromosomes there is very little heterochromatin in the X (Muller 1944). The proportion of heterochromatin to euchromatin in autosomal chromosomes of the salivary gland is also much smaller than that in mitotic prophase chromosomes. The frequency of crossing-over per unit of cytological length is lower in heterochromatic regions; it can be increased by high temperature (Mather 1939). These characteristics can hardly be applied to an interpretation of the position effect.

Besides the position effect of heterochromatin to euchromatic loci, there is a position effect of euchromatin to the heterchromatic loci or genes, such as lt, ltd, stw and ci in the second or fourth chromosome. The mutants, lt^{48-1}, lt^{48-12}, lt^{48-20} and w^{m48-36}, are examples of position effects at the lt locus; while w^{m48-46} is an example at the ltd locus; stw^{48-9} an example at the

stw locus; and w^{m48-1} and w^{m48-33} are examples at the ci locus. In all these cases, cytological analyses show that an euchromatic segment has been brought near each of these loci concerned, by a chromosomal rearrangement (Fig. 2,15,16,17,18,21,22,23,23a).

An additional Y-chromosome enhances the light character of the lt position mutants but exerts no effect on the stw position mutant. In the lt position mutants, the light-colored eyes show some variegation or mottling. It seems, therefore, that the variegation or mottling of somatic characters is not a position effect peculiar, as has been suggested, to heterochromatin, but possibly to the interaction of certain euchromatic genes and heterochromatin or of certain heterochromatic genes and euchromatin. Probably both euchromatin and heterochromatin have some specificity in a certain interaction to reveal a certain position effect.

The localized chemical reactions between the products of nearby genes through diffusion (Sturtevant 1925; Muller 1935; Offerman 1935) and the competition of two genes for the same substrate (Waddington 1939) or the interference of alleles which differ in capacities for combining with a common substrate and differ in efficiencies in converting the substrate to a common product for ci locus (Stern et al 1943) have been considered to be responsible for position effects. Other interpretations, such as changes of structural interrelations of genes in an integrated larger chemical unit (Muller 1935), changes in shape of genes (Muller 1935, 1946), and physical distortion of the gene due to somatic pairing in structural heterozygotes (Ephrussi and Sutton 1944) are structural hypotheses of

position effects. None of these interpretations is entirely satisfactory in all respects.

In w^{m48-2}, w^{m48-9}, and lt^{48-12}, the position effects can not be explained as due to structural heterozygosis since the flies homozygous for the rearrangements concerned are white-mottled and lt-variegated respectively.

In a recent paper, Goldschmidt (1946) has discussed in considerable detail the position effects in Drosophila melanogaster. He concluded that an explanation of the position effect requires an abolition of the over-simplified classic theory of the gene. The chromosome has an orderly serial structure, and it reproduces itself as a unit. Taking all the basic facts of both position effect and point mutation into consideration, he assumed that the visible change of chromosome structure is a position effect and the invisible change of chromosome structure is a point mutation.

More recently, Stern (1948) has presented a paper in a symposium on genes and cytoplasm discussing the results he and his associates have obtained from their studies of the position effect at the ci locus. In conclusion, he made the following statement giving a general picture of the present state of the problem of position effect. "The genetic analysis, in spite of its lack of biochemical precision, remains at present a more delicate tool for the probing of immediate genic action than even the most advanced methods of the microanalyst. But the vagueness of the geneticist's results lets us look forward eagerly to the time when the biochemist has caught up with him."

By way of summary, the following points resulting from the studies of position effects at the white, light and straw loci, may be mentioned.

(1) A total of fifty-eight white-mottleds was found in 45,255 female progeny of irradiated Canton-S, y^4, sc^4 and rst^3 males. Forty-six of them proved to be fertile. Genetic tests were made with all these forty-six mottleds, and cytological study was made with twenty-one of them.

(2) The frequency of induced white-mottleds is remarkably high from rst^3 males. Possible explanations have been discussed briefly. The progeny of untreated rst^3 flies do not show the white-mottled character.

(3) Linkage tests show that eighteen white-mottleds are due to rearrangements within the X-chromosomes, eight due to X-2, fifteen X-3, three X-4 translocations, and two cases were undetermined. Cytological analysis with their salivary-chromosomes shows that one break of each of the rearrangements in the X-chromosome is near or at the 3C region and the other break is in the heterochromatin of the X, second, third or fourth chromosome. The location of the white locus in the salivary-chromosome has been discussed.

(4) The position effect, of a translocated X-piece, on the heterochromatic genes which are located near one breakage point of the rearrangement has been found in four cases. The affected loci are lt, ltd in the second chromosome and ci in the fourth chromosome.

(5) Thirty-six light-mutants were induced in 5,280 progeny of the irradiated black males. So far as the genetic and cytological evidence goes, there are four 2-3 translocations, two inversions and two deficiencies, in these mutants.

(6) Three light-mutants, of which two are due to 2-3 translocations and one due to an inversion, have turned out to be due to position effects. In each case, an euchromatic segment has been brought to the heterochromatin of 2L, probably near the lt-locus. These mutants show some light-variegation when over a lt allele or in homozygous condition. An additional Y-chromosome enhances the light-character and subtracting a Y suppresses it. The effect of high temperature suppresses the light-character, making the eyes appear more nearly wild type.

(7) Twenty-five straw-mutants were found in 8,277 progeny of irradiated pr cn males. As genetic and cytological studies show, these mutants carry the following different rearrangements: two 2-3 translocations, one inversion and four deficiencies.

(8) One straw-mutant carrying a 2-3 translocation and a small inversion in the base of its 2R is obviously a position effect, since an euchromatic segment has been brought to the heterochromatin through the inversion. No effects of the Y-chromosome or of temperature could be demonstrated upon straw mutants.

(9) Deficiency tests with straw-mutants show that the pk locus is probably somewhere to the right of tk, and that there is some doubt about the allelic relationship of Xa to blt or ap^4.

(10) The X-ray dosage applied in all the experiments was about 4,500 r units.

PART FOUR. MUTATIONS

The term mutation has been discussed in the first part. Without doubt, position effects are mutations in the broad sense of the word. Merely as a matter of convenience, all the data secured from the studies of the white-mottleds and of the light- and straw-mutants have been collected in the third part under the title of position effects, and the results from some studies with other mutations will be presented in this part.

In the experiments for white mottling, the following dominant mutants were induced.

Males Rayed	Induced Mutation	Number of Mutations
Canton-S	brown-dominant	1
y^4	brown-dominant	1
	brown-variegated	2
$y\ sc^4$	brown-dominant	2
	brown-variegated	2
	Bar^{48}	1
sc^8 cv v f	Antennapedia	1
	Scarred eye	1

The mutants Bar^{48}, Antennapedia and Scarred have been studied genetically and cytologically. Antennapedia and Scarred are new mutants.

(A) THE ANTENNAPEDIA MUTANT

Antennapedia was found, on Aug. 13, 1948, as a single female among 6,085 female progeny of the cross sc^8 cv v f ♂♂(X-rayed) X y w spl ♀♀. The antennae of this mutant appeared as leg-like structures with aristae still present near their distal portion (Fig. 27, 27a,27b,27c). The name "Antennapedia" is suggested for this mutant type.

Such a phenomenon as the disappearance of an organ and the development of another organ in its place has been known as "hereditary homoeosis" (homoeosis = Bateson's term for replacement of a segmental structure by another one of the series.)(Bridges and Dobzhansky 1933; Goldschmidt 1945). The homoeotic mutants discovered in Drosophila are bithorax, bithoraxoid (Bridges and Morgan 1923), tetraptera (Astaurov 1929), aristapedia (Balkaschina 1929, Sturtevant 1929), proboscipedia (Bridges and Dobzhansky 1933), antennipedia (Sturtevant 1940), tetraltera (Goldschmidt 1940), podoptera (Goldschmidt 1945), Hexaptera (Herskowitz 1949) and one strain, with wing-like antenna and arista, of pointed-wing (Bridges 1923).

This new mutant, Antennapedia, belongs to the class of hereditary homoeosis. When it was found among other sc^8 cv v f/y w spl females, the Antennapedia fly was mated, as were the white mottleds, to y Hw w lz^s males. The mating was made successfully although the mutant was not very fertile. The Antennapedia character appeared in the two kinds of males, sc^8 cv v f and y w spl, and in the two kinds of females, sc^8 cv v f/y Hw w lz^s and y w spl/y Hw w lz^s, of the F_1 generation. This indicated that the mutation is dominant and not sex-linked.

Genetic Test

To test its autosomal linkage, the Antennapedia flies were mated to PM/Cy,Sb/D Cx flies. As described in the third part, the Plum and Curly are dominants in second chromosomes and the Dichaete and Stubble dominants in thirds. The F_1 female carrying Antennapedia and Cy, Sb was crossed to wild type male. Table XIII shows the results obtained from three such pair matings.

Table XIII

P_1 Antp ♂♂ X Pm/Cy, D/Sb ♀♀

F_1 Antp/Cy, Sb ♀ X + ♂

F_2 flies (sex-linked genes disregarded in table)

	Antp	Cy Sb
Mating		
A	12	11
B	7	8
c	30	35
Total	49	54

As the data show, the Antennapedia is completely linked with the second and third chromosomes. This must be due to an interchange having occurred between these chromosomes. The mutant has been kept, therefore, balanced over Cy Sb or Pm Sb. It is lethal when homozygous.

The mutant flies proved to be not very fertile and the Antennapedia-character shows some fluctuation in expression. It seems

that D and Pm enhance it. Temperature has no effect on its expression, but this mutant is very infertile at 28° C.

Some extreme types of Antennapedia-character can usually be found, especially in individuals heterozygous for Pm or D, in which the antenna is differentiated into three distinguishable segments, corresponding to coxa-trochanter, femur and tibia of a leg. The latter two segments are usually fused (Fig. 27b, 27c). In some instances, the arista is replaced by a tarsus-like structure with two claws at its tip. This makes the antenna-arista resemble a complete leg (Fig. 27e). This may be of interest to the morphologists as direct evidence indicating that the insect antenna is homologous to a leg. From this point of view, this mutant is of considerable importance.

An attempt was made in vain to combine this mutant with aristapedia. This is because the gene aristapedia, in 3R, can not be transferred to the translocated third chromosome of the Antennapedia, since crossing over in 3R is inhibited by the translocation. This point has been proved by cytological evidence which will be presented later. Flies with the constitution of Antennapedia/aristapedia show the Antennapedia character; aristapedia has no intensifying effect.

The Antennapedia character has not yet been found to overlap wild type. In connection with this, one fact may be mentioned here. When the mutant was first found and mated by y Hw w lz^s males, the mating was carefully watched and transferred twice to a new bottle at an interval of about four days. Since the mutant was not very fertile, in order to save the mutant character from possibly overlap-

ping wild type, the F_1 non-Antennapedia flies were also collected in one bottle regardless of their difference in sex-linked characters. Their offspring were examined roughly for abnormal antennae. Most of the flies had normal antennae, but a few flies showed the basal portion (frontal cranium) of the antennae thickened, among which was one fly with three aristae on its left side, one in the normal position and two lying side by side beneath the antenna. This fly was mated to its brothers, no abnormal antennae could be found in its offspring. It remains a question whether this was an effect of Antennapedia.

Cytological Analysis

In examining the salivary gland chromosome, the mutant was mated to p^P (pink-peach eye color) flies. F_1 males with the constitution of Antp/p^P were mated to p^P females again. Since the larvae homozygous for p^P had colorless Malpighian tubes, the other kind of larvae, heterozygous for Antp/p^P could be identified for cytological study. Temporary preparations of their salivary glands were made following the general methods described in the foregoing parts.

The configuration of the salivary-chromosomes of this mutant shows a reciprocal translocation between 2L and 3R, involving several inversions. The breaks in 2L are at 22B and 38F, while those in 3R at 83E and 98A. One of the interchanged chromosome is, therefore, from 2L base to 38F, 3R 98A to tip; the other is from 3R base to 83E, 2L 22B inversely to 38F, 3R 98A inversely to 83E, then 2L 22B to tip. (Fig. 24, 24a). It is very likely that an inversion or several inversions were originally present in the 3R.

No genes have been known to be located near the breakage

points. It can only be regarded, at the present, as a mutation, Antennapedia, being associated with, or inseparable from, a translocation. The symbol is T(2;3)Antp. As its mutant character is dominant and striking and its chromosomal rearrangement is complicated, this mutant is undoubtedly useful as a balancer for 2L and 3R.

Recently, Le Calvex (1948) found a mutant with its arista replaced by a tarsus-like segment and its antenna normal. It has shown cytologically that an inversion occurred in its 3R with the breaks approximately at $84A_5$ and $92A_5$. The mutation is dominant and homozygous lethal. It has been supposed to be an allele of aristapedia, being associated with the inversion in 3R. The symbol $In(3R)ss^{aR}$ is used for it by Le Calvez.

The antenna and arista are two portions of one unit structure, the antennal appendage in Drosophila. Antennapedia and aristapedia-dominant are different mutations; one exerts a phenotypic effect primarily upon the antennae while the other acts only on the aristae, but both have breaks of a rearrangement in 3R. It can also be noted that almost all the homoeotic mutations thus far found in Drosophila melanogaster have occurred in 3R (Table XIV). Most of them have a phenotypic effect upon the differentiation and development of appendages. It seems natural to question whether these mutations, at least some of them, will turn out to be position effects. This awaits further investigations.

Table XIV

Homoeotic Mutants In Drosophila

Homoeotic Mutation	Symbol	Locus	Cytology	Authority
bithorax	bx	3-58.8		Bridges, Morgan 1923
bithoraxoid	bxd	3-58.8±		Bridges, Morgan 1923
bithorax-dominant	bx^D	3-58.8	Normal	Hollander 1937 Bridges 1937
tetraptera	ttr	3-51.3		Tshetverikov 1925 Astaurov 1929
proboscipedia	pb	3-47.7		Bridges, Dobzhansky 1923
tetraltera	tet	3-48.5	Identical to IN(3R)C $92F-100F_{1-2}$	Goldschmidt 1940 Villee 1942
podoptera		3-58.5		Goldschmidt 1945
Hexaptera		2		Herskowitz 1949
aristapedia	ss^a	3-58.5		Balkaschina 1929 Sturtevant 1929
Aristapedia	ss^{aR}		In(3R)ss^{aR} $84A_5-92A_5$	Le Calvez 1948
antennipedia		2L(4 of D. affinis)		Sturtevant 1940
Antennapedia	Antp		T(2;3) 2L 22B, 38F 3R 83E, 98A	

(B) THE SCARRED MUTANT

The Scarred, dominant mutant, was found, as was the Antennapedia described above, in the X-ray experiment with sc^8 cv v f males, on Aug. 15, 1948. It was a female among others with the constitution of sc^8 cv v f/y w spl but showed its eyes deformed. This mutant character proved to be not sex-linked.

The eye of this mutant is more or less elliptic in shape with an indentation covering its inner one third area. This indentation area with a shining surface and a few facets present looks like a scar from a heavy wound (Fig. 28). The wings are spread at about 45° from the body axis. The name "Scarred" is suggested for this mutation.

Genetic Test

For an autosomal linkage test, the Scarred mutant was mated to Pm/Cy, Sb/D Cx flies. F_1 females carrying Scarred and Cy, Sb were mated by wild type males. F_2 progeny of two such matings are shown in Table XV.

Table XV

P_1 Scar ♂ X Pm/Cy, Sb/D ♀

F_1 Scar/Cy, Sb ♀ X + ♂

F_2 flies (sex-linked genes disregarded in table)

Mating	Scar	Cy Sb	Cy
A	55	40	1
B	89	81	0
Total	144	121	1

As the data in Table XV show, the Scarred mutation is almost completely linked with the second and third chromosomes. The one Cy fly that appeared in F_2 is probably due to crossing over of the Sb locus. This mutant stock has been kept balanced over Cy or Cy Sb. It is lethal when homozygous. High temperature, 28° C, enhances its mutant-character.

Cytological Analysis

To examine its salivary gland chromosomes, the Scarred mutant was mated to lt (light eye color) flies. F_1 Scar/lt males were mated to lt females again. The F_2 larvae with yellow Malpighian tubes were dissected. The other kind of larvae homozygous for lt, which had colorless Malpighian tubes, was discarded. Temporary preparations with the salivary glands of the larvae heterozygous for Scar were made following the general methods.

The salivary-chromosomes of this mutant show a translocation between 2L and 3R, involving one break in 2L at 27E and three breaks in 3R at 91F, 95A and 96A. It is very likely that an inversion was originally present in the 3R. It might be the In(3R)Payne (from $89C_{1,2}$ to 96A), but here the inverted segments are from 91F to 95A and from 95A to 96A. One interchanged chromosome of this translocation is from 2L base to 27E, then 3R 95A inversely to 91F, 96A to tip; the other interchanged chromosome is from 3R base to 91F, 96A inversely to 95A, then 2L 27E to tip (Fig. 25).

No genes have been known to be located near the breakage points. This is a mutation associated with the translocation, T(2;3)Scar. This mutant is useful in balancing certain loci in 2L and 3R.

(C) THE BAR^{48} MUTANT

The B^{48} was found July 27, 1948 as a single female from 6,603 female progeny of irradiated y sc^{4} males mated with y w spl females. Its eyes looked like that of a combination of Bar and lozenge-glossy, being as narrow as that of double Bar but with fewer facets, averaging 12, on a shining surface. This mutant female was mated by y Hw w lz^{s} males. In F_1, the Bar character occurred only in y sc^{4} males and y Hw females, showing the gene to be sex-linked.

Genetic Test

Since the Bar character was linked with the irradiated X-chromosome, the mutant gene concerned was thought to be possibly an allele of the Bar. Females of the mutant were, therefore, mated to Bar males. Their offspring with this mutant gene over Bar appeared, however, exactly like the new mutant, Bar having no effect on it.

To test whether the mutation be also linked with autosomes, the females of y sc^{4} B^{48}/y Hw w lz^{s} were mated by Pm/Cy, Sb/D males. F_1 females carrying y sc^{4} B^{48} and Cy, D were crossed with wild type males. Table XVI shows the F_2 flies.

Table XVI

P_1 y sc^4 B^{48}/y Hw w lz^s ♀ X Pm/Cy, Sb/D Cx ♂

F_1 y sc^4 B^{48}, +/Cy,D ♀ X + ♂

F_2 flies

	Mating A	Mating B	Total
B^{48} ♀	9	5	14
y sc^4 B^{48} ♂	2	2	4
B^{48} D ♀	12	3	15
y sc^4 B^{48} D ♂	1	1	2
Cy ♀,♂	15	12	27
Cy D ♀, ♂	12	7	19
y sc^4 Cy ♂	0	1	1

It is shown in the table that the mutant is linked with the y sc^4 X-chromosome as well as the second chromosome. The one male carrying y sc^4 Cy is probably due to a crossing over at the tip of the X-chromosome just to left of the sc^4 inversion.

This mutant is lethal when homozygous, and the males are completely sterile. In stock, there is great fluctuation in expression of the mutant character. Many flies have wide eyes, with a number of facets, like those of wide Bar flies. If these wide-bar females are mated by any kind of males other than those in the stock, the eyes of their offspring may again be narrow-bar. This may be due to a suppressor or modifier present in homozygous condition in the stock.

The combination of this mutant with ey^D (eyeless-dominant, in the fourth chromosome) gives extreme eyeless, very small round eyes

with rough facets, but does not give extreme bar eyes. In other words, this mutant enhances ey^D but ey^D exerts no effect upon this mutant character.

No striking effect of temperature has been demonstrated.

Cytological Analysis

To analyze the salivary-chromosomes, the mutant female, y sc^4 B^{48}/y Hw w lz^s, was mated by y sc^4 car m w^a males (car = carnation eye color). The female larvae carrying yellow Malpighian tubes, supposed to be y sc^4 B^{48}/y sc^4 car m w^a but not y Hw w lz^s/y sc^4 car m w^a since white-alleles give colorless Malpighian tubes, were dissected and their salivary gland chromosomes were studied cytologically.

The salivary-chromosomes of this mutant show a translocation between the sc^4 X-chromosome and 2L. The break in sc^4 X is at 15F, just to the left of 16A, and that in 2L at 33B. One of the two interchanged chromosomes is from sc^4 X base to 15F, then 2L 33B to tip; the other one is from 2L base to 33B, then sc^4 X 16A to 19E (Fig. 26, 26a). The Bar locus has been suggested to be at $16A_{1-2}$ (Griffen 1941, Sutton 1943), and in the case of Baroid (Dobzhansky 1932, Bridges 1936) the X-2R translocation had one break in X between the halves of the heavy doublet $16A_{1-2}$. Baroid has been, therefore, interpreted as a position effect (Dobzhansky 1936). This mutant, with a sc^4 X-2L translocation as described above, can be regarded as another example of position effect at the Bar locus. The neighborhood of $16A_{1-2}$ has changed to be 2L 33B instead of the original 15F. And possibly, from

the cytology of this mutant, the Bar locus is at $16A_1$.

It turns out clearly that this mutant is a dominant Bar allele. Since it was found in 1948, the name B^{48} is suggested for this mutant. It is inseparable from the sc^4 X-2L translocation. Its full symbol is $T(1;2)B^{48}$.

PART FIVE. GENERAL SUMMARY

The observations, in the studies of reverse mutations and position effects, presented in the second and third parts of the paper have been summarized at the ends of those two parts. Here, a brief summary of the entire paper will be presented.

The literature pertaining to the hypotheses or experimental data of mutations, with special reference to reverse mutations and position effects, was reviewed in the first and second parts.

Five X-ray experiments were performed for reverse mutations of several dominant and recessive mutant genes. Reversions of two dominant genes, Dichaete and Glued, and apparent reversions of the dominant gene, Stubble, and of the sex-linked recessive gene, sc^7, were induced and the reversals of Dichaete and Glued were studied cytologically with no detectable chromosomal change. No reversions of the recessive genes, vermilion, cinnabar, brown and scarlet were observed.

Thirteen experiments with Canton-S, y^4, sc^4, sc^8 and rst^3 males irradiated for induced position effects at the white locus were undertaken, and fifty-eight white-mottleds were found. Forty-six of these white mottleds have been studied genetically, among which twenty-one were studied cytologically. In each case, one break of a rearrangement occurred near the white locus and the other break was in heterochromatin of the X, second, third or fourth chromosome. The white mottling is thus due to the neighborhood of the white locus being

changed from euchromatin to heterochromatin. The frequency of white-mottling induced and the location of the white locus in the salivary gland chromosome were discussed.

Three experiments for induced mutations, including position effects, at the light and straw loci in the second chromosome were carried out, in which thirty-six light-mutants and twenty-five straw-mutants were induced. Most of them have been studied genetically and several studied cytologically. Three light-mutants and one straw-mutant proved to be position effects. In each of these cases, a chromosomal rearrangement has occurred with one break in the heterochromatin of 2L or 2R, probably near the light or straw locus, and the other break in euchromatin of the same chromosome or of a third chromosome. Thus, the heterochromatic neighborhood of the light or straw locus has been changed to a euchromatic one by the rearrangement. This is a position effect of the opposite kind to that at the white locus. The light-mutant character of these light mutants due to position effects can be enhanced by adding a Y and suppressed by subtracting a Y-chromosome. High temperature also suppresses it. No effect of Y-chromosomes or of temperature upon the straw-mutant character has been demonstrated.

In the study of white-mottleds, several cases of position effect at some other loci, such as Bar in the X-chromosome, light and lightoid in the second, and cubitus interruptus in the fourth, were found and studied cytologically. The case of Bar, for which the symbol B^{48} is suggested, has shown that a translocation occurred between the sc^{4} X-chromosome and 2L, with one break in the X at 15F, just to the left of 16A.

From all the twenty-one experiments with X-rays, a number of mutations, other than those mentioned above, such as scarlet, curled, clipped, brown-dominant, Plum, Scarred and Antennapedia, were also induced. Antennapedia and Scarred, which are dominant mutants, have been studied genetically and cytologically. Both are due to 2-3 translocations and are useful as balancers for 2L and 3R. Antennapedia is also an important homoeotic mutant indicating that the antenna is homologous to a leg in insects.

The X-ray dosage applied in all the experiments was approximately 4,500 r units.

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EXPLANATION OF FIGURES

Fig. 1 -- Diagram showing the relative positions of the breaks of four inversions in the X-chromosome. The solid line indicates euchromatin, and wavy line heterochromatin.

Fig. 2 to 26a -- Photographs of aceto-orcein preparations of the salivary gland chromosomes. The chromosomes are labeled X (X-chromosomes; y^4 X, sc^4 X, sc^8 X and rst^3 X are X-chromosomes carrying the inversions concerned. See Fig. 1), 2L and 2R (the left and right arms of the second chromosomes), 3L and 3R (the left and right arms of the third chromosomes), and 4th (the fourth chromosomes). Other numbers and letters indicate the divisions and subdivisions respectively of the chromosomes (Bridges' maps, 1935, 1938).

Fig. 2 -- w^{m48-1} T(1;4). Fourth chromosome (prob. from 101 to 102F) translocated to X 3E. x1200

Fig. 2a -- w^{m48-1} T(1;4). X-tip translocated to 4th 101. x400

Fig. 3 -- w^{m48-2} Insertional translocation of 4th (prob. 101-102F) in normal order into X 3B. x1200

Fig. 4 -- w^{m48-3} In(1). y^4 X 3C to 19F inverted; inverted segment paired with its normal homologue. x1200

Fig. 4a -- w^{m48-5} The same as Fig. 4. x1200

Fig. 5 -- $w^{m4\beta-5}$ In(1). y^4 X 3C inverted to heterochromatin. Upper half of the unpaired portion is inverted y^4 X-base; lower half is normal y^4 X-base. x1200

Fig. 5a -- $w^{m4\beta-5}$ In(1). y^4 X 3C inverted to heterochromatin. Upper half of the unpaired portion is normal y^4 X-base; lower half is inverted y^4 X-base. x1200

Fig. 5b -- $w^{m4\beta-5}$ In(1). y^4 X 3C inverted to heterochromatin. Left half of y^4 X-base is normal; right half is inverted. x1200

Fig. 6 -- $w^{m4\beta-7}$ T(1;2). y^4 X 3C/2L base. x1800

Fig. 6a -- $w^{m4\beta-7}$ T(1;2). y^4 X 3C/2L base. Left half of y^4 X unpaired portion is normal; right half is connected to 2L base. x1200

Fig. 7 -- $w^{m4\beta-8}$ T(1;3). y^4 X 3C/3R base. Left, y^4 X unpaired portion is normal; right, y^4 X base to 3C is connected to 3R base. x1200

Fig. 8 -- $w^{m4\beta-9}$ In(1). y^4 X 3C inverted to base. Inverted segment paired with its normal homologue. x1200

Fig. 8a -- $w^{m4\beta-9}$ In(1). y^4 X 3C inverted to base. Left, unpaired portion of y^4 X-base is normal; right, y^4 X 3C to base is inverted. x1200

Fig. 9 -- $w^{m4\beta-10}$ T(1;3). y^4 X 3C/3R base. Segments of y^4 X 3C to base are paired. x1200

Fig. 10 -- w^{m48-11} In(1). y^4 X 3C to base inverted. Right, unpaired portion of y^4 X-base is normal; left, y^4 X 3C to base is inverted. x1200

Fig. 11 -- w^{m48-12} T(1;2;3). y^4 X 8C/3L 65C; y^4 X 3C/2R base. (2R not present in photograph, detached as a result of squashing). x960

Fig. 11a -- w^{m48-12} The same as Fig. 11, showing the base of y^4 X. Upper half of y^4 X is normal. x960

Fig. 12 -- w^{m48-13} Insertional translocation of y^4 X $3C_2$-19F in inverted order into 3L $62D_3$. Left half of 3L is normal. x1200

Fig. 12a -- w^{m48-13} The same as Fig. 12. x1200

Fig. 12b -- w^{m48-13} The same as Fig. 12, showing the translocated y^4 X-piece paired with its normal homologue. x1200

Fig. 13 -- w^{m48-15} T(1;3). y^4 X 2F/3L 80B . Upper, y^4 X from tip to 2F attached to chromocenter (3L base; 3L-y^4 X translocated chromosome); lower, y^4 X from base to 2F attached to 3L base (y^4 X-3L translocated chromosome). x1200

Fig. 14 -- w^{m48-20} T(1;2). sc^4 X 3C/2R base. Upper half of sc^4 X is normal. x1200

Fig. 14a -- w^{m48-20} T(1;2). sc^4 X 3C/2R base. Left half of unpaired 2R-base is connected to sc^4 X 3C region (sc^4 X-2R translocated chromosome); right half of 2R-base is normal. x1200

Fig. 15 -- w^{m48-33} T(1;4). rst^3 X 3C/4th 101. x960

Fig. 16 -- w^{m48-36} T(1;2). rst^3 X 3C/2L base. Chromocenter, rst^3 X-tip attached to 2L base (2L-rst^3 X translocated chromosome); right, one normal rst^3 X and one rst^3-2L translocated chromosome. x600

Fig. 17 -- w^{m48-46} T(1;2;3). rst^3 X 3C/2R base. Chromocenter, rst^3 X-tip attached to 2R base (2R-rst^3 X translocated chromosome). X600

Fig. 18 -- lt^{48-1} T(2;3). 2L hetero./3L 64B,C. Upper left, 2L base connected with 3L 64B,C (3L-2L translocated chromosome); chromocenter, 3L tip attached to 2L base (2L-3L translocated chromosome). x600

Fig. 19 -- lt^{48-2} T(2;3) and In(2). Right, translocation: 2L 23E,F/3R 86E; lower left, inversion : 2L left of 40A to 2R 47A,B. x1200

Fig. 20 -- lt^{48-7} In(2) and Df(2). Inversion : 2L 21F to $39E_3$; deficiency: 2L $39E_3$ to $40B_1$ or further (see Fig. 20a). x600

Fig. 20a -- lt^{48-7} The same as Fig. 20, showing only the deficiency. x1200

Fig. 21 -- lt^{48-12} T(2;3). 2L hetero./2R 50C/3R 93B , $100A_4$. Three interchanged chromosomes (or arms of chromosomes) are : (1) 2L base, 3R 100A to tip; (2) 2R base to 50C, 3R 93B to 100A, 2L base to tip; and (3) 3R base to 92B, 2R 50C to tip. x600

Fig. 22 -- lt^{48-20} T(2;3). 2L hetero./3R $100B_3$. Two interchanged chromosomes are: (1) 2L base, 3R $100B_3$ to tip; and (2) 3R base to $100B_2$, 2L base to tip. (see Fig. 22a). x600

Fig. 22a -- lt^{48-20} The same as Fig. 22. x600

Fig. 23 -- stw^{48-9} T(2;3) and In(2). Translocation : 2L 34D/3R 89E; inversion : 2R $41D_4$ to 42B. (see Fig. 23a). x600

Fig. 23a -- stw^{48-9} The same as Fig. 23, showing only the inversion. x1800

Fig. 24 -- Antennapedia. T(2;3)Antp. 2L 22B and 38F/3R 83E and 98A. Two interchanged chromosomes are : (1) 2L base to 38F, 3R 98A to tip; and (2) 3R base to 83E, 2L 22B inversely to 38F, 3R 98A inversely to 83E, then 2L 22B to tip. (see Fig. 24a). x400

Fig. 24a -- Antennapedia. The same as Fig. 24.

Fig. 25 -- Scarred eye. T(2;3)Scar. 2L 27E/3R 91F, 95A and 96A. Two interchanged chromosomes are : (1) 2L base to 27E, 3R 95A inversely to 91F, 96A to tip; and (2) 3R base to 91F, 96A inversely to 95A, then 2L 27E to tip. x400

Fig. 25a -- Scarred eye. The same as Fig. 25, showing diagrammatically the translocation. (see Fig. 25).

Fig. 26 -- Bar^{48}. $T(1;2)B^{48}$. sc^4 X 15F/2L 33B. Two interchanged chromosomes are : (1) sc^4 X base to 15F, 2L 33B to tip; and (2) 2L base to 33B, sc^4 X 16A to 19E. x600

Fig. 26a -- Bar^{48}. The same as Fig. 26, showing the sc^{4} X-2L translocated chromosome. x1200

Fig. 27 to 28. Camera-lucida drawings.

Fig. 27 -- Antennapedia, dorsal view, showing the leg-like structure of the antennae with aristae still present near their distal portion. x16

Fig. 27a -- Antennapedia, ventral view. x16

Fig. 27b -- Antennapedia, showing three segments of the antenna, corresponding to coxa-trochanter, femur and tibia of a leg. Left, dorsal view; right, ventral view. x21

Fig. 27c -- Antennapedia. The same as Fig. 27b. Ventral view. x21

Fig. 27d -- A fore leg of Antennapedia ♂ (normal). x21

Fig. 27e -- Antennapedia, showing the arista being replaced by a tarsus-like structure with two claws at its tip. Right, dorsal view; left, ventral view. x21

Fig. 27f -- A normal antenna of Drosophila. x21

Fig. 28 -- Scarred eye, showing the eye with an indentation covering its lower one third area. This indentation area with a shining surface and a few facets present looks like a scar. x22

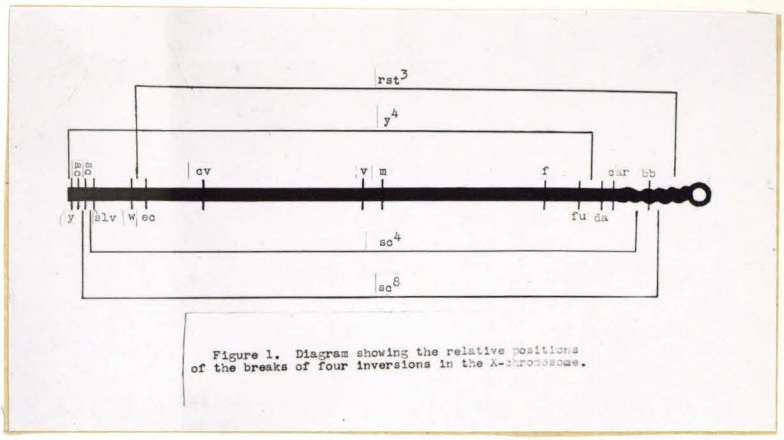


Figure 1. Diagram showing the relative positions of the breaks of four inversions in the X-chromosome.

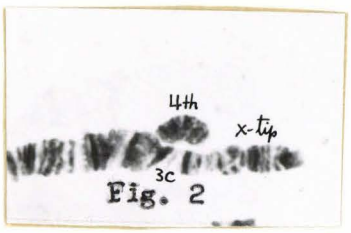


Fig. 2

Fig. 2a

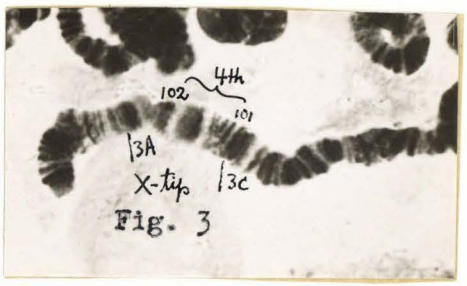
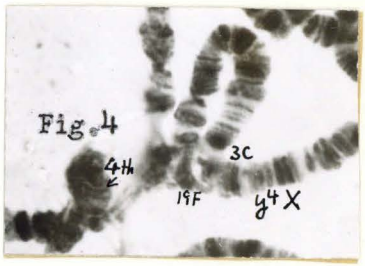


Fig. 3



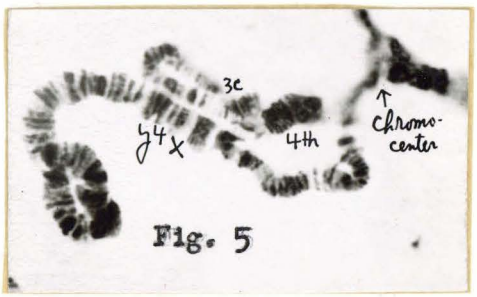
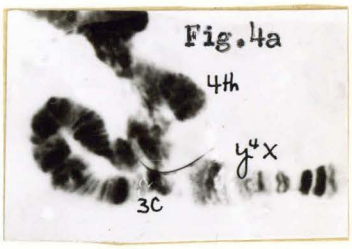


Fig. 5



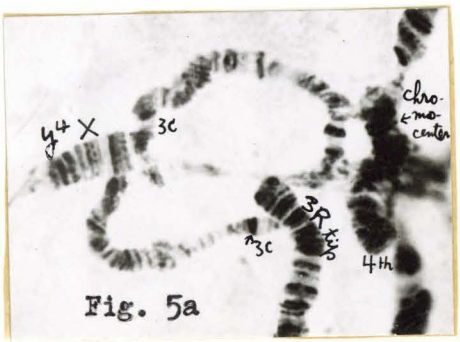
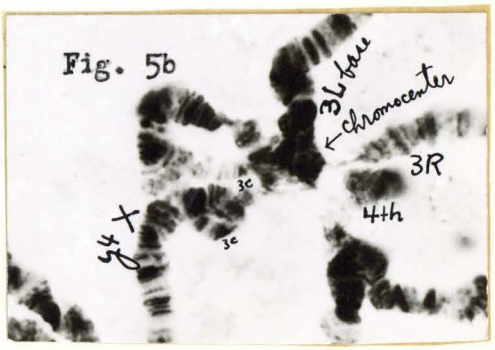


Fig. 5a



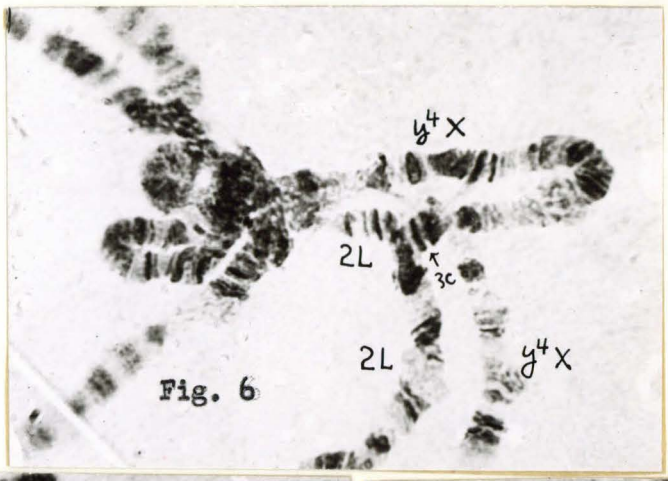


Fig. 6

Fig. 6a

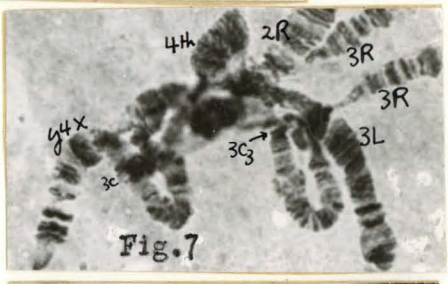


Fig. 7

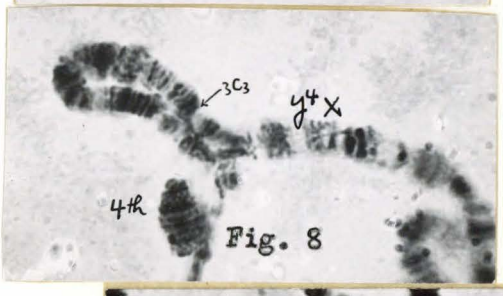


Fig. 8

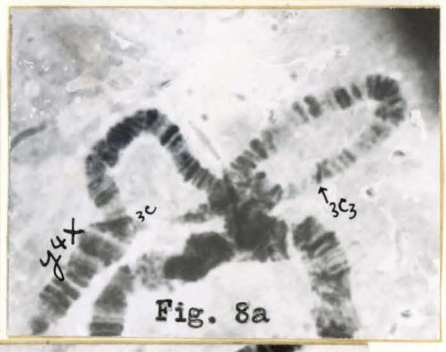


Fig. 8a

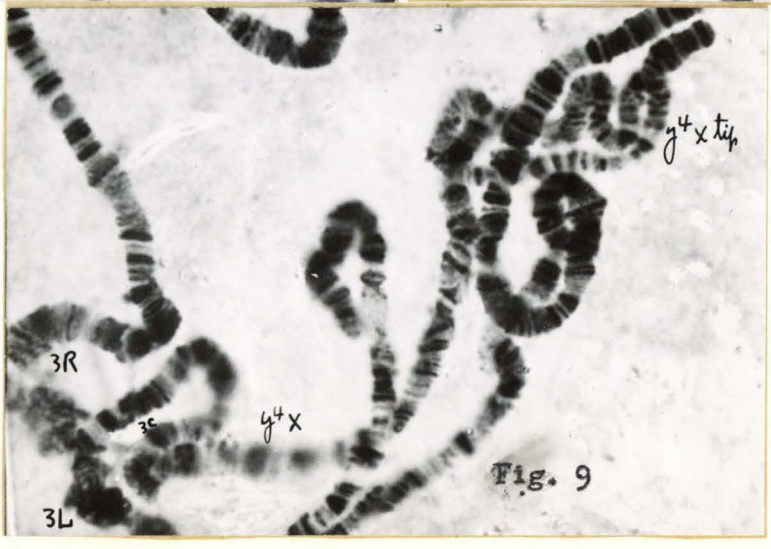


Fig. 9

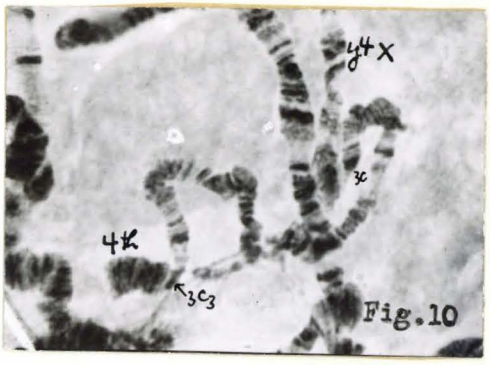


Fig. 10

Fig. 11a

Fig. 11

Fig. 12

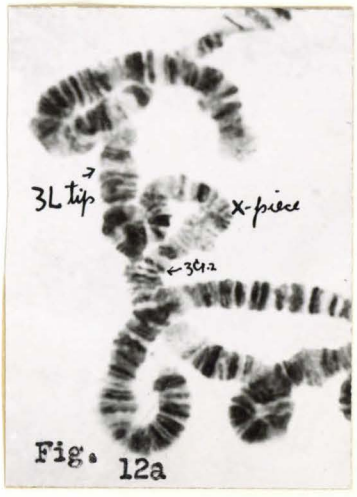


Fig. 12a

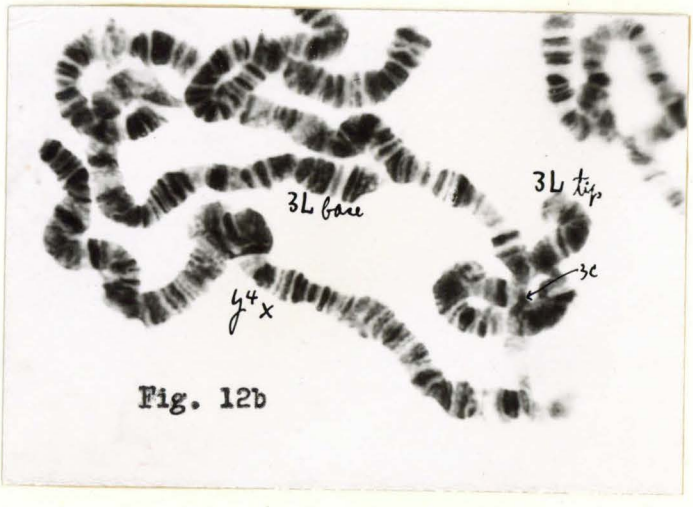


Fig. 12b

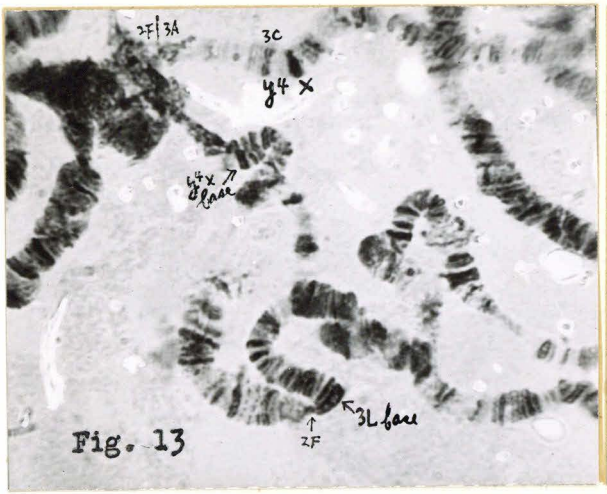


Fig. 13

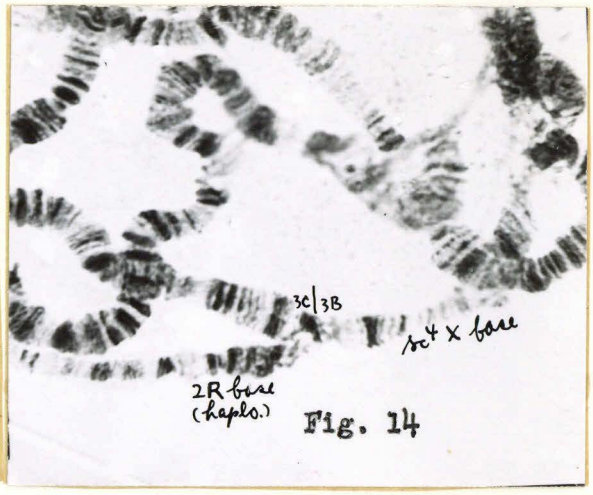


Fig. 14

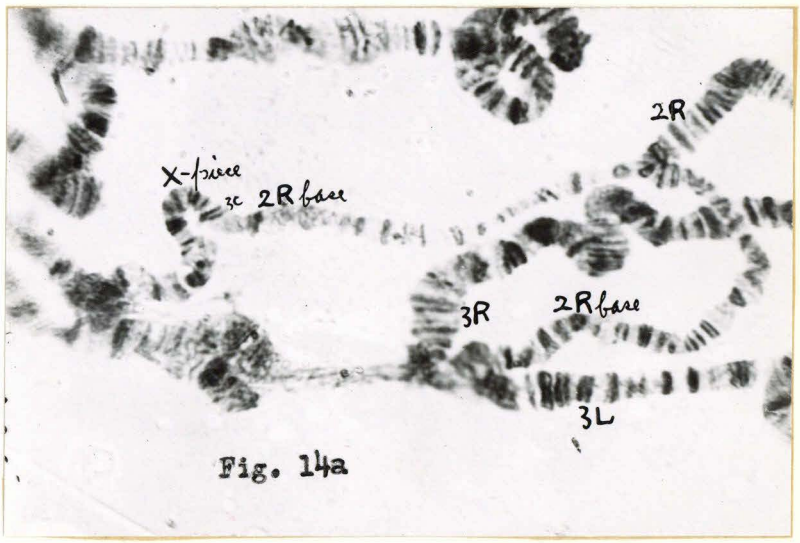


Fig. 14a

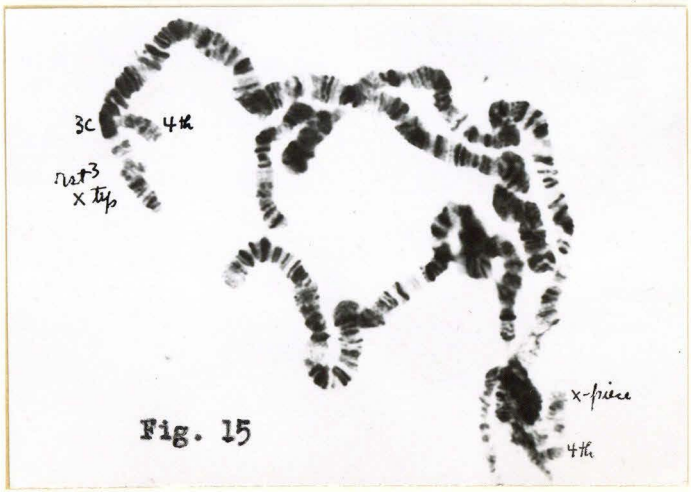


Fig. 15

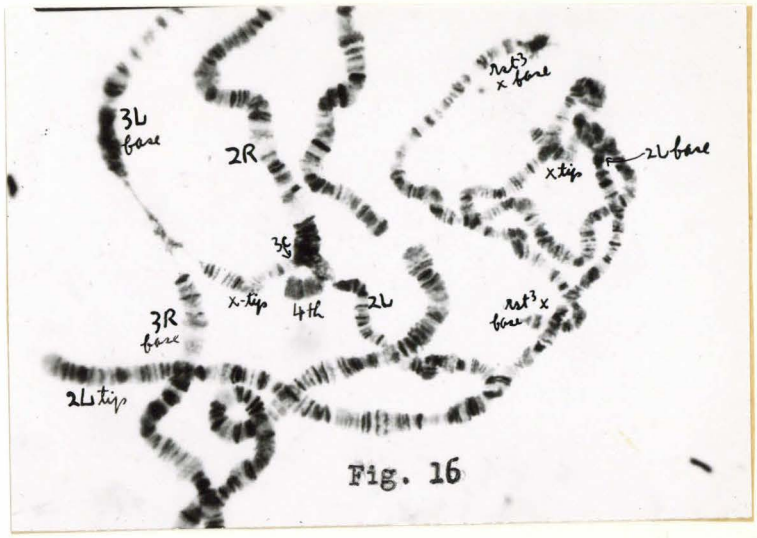


Fig. 16

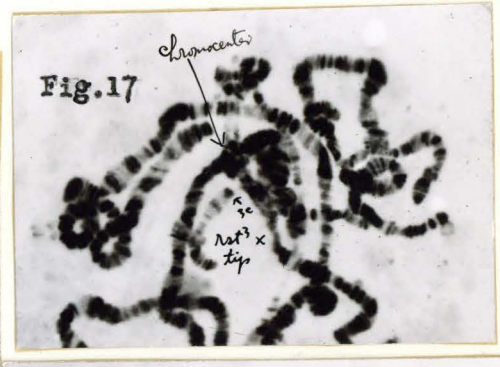


Fig.17

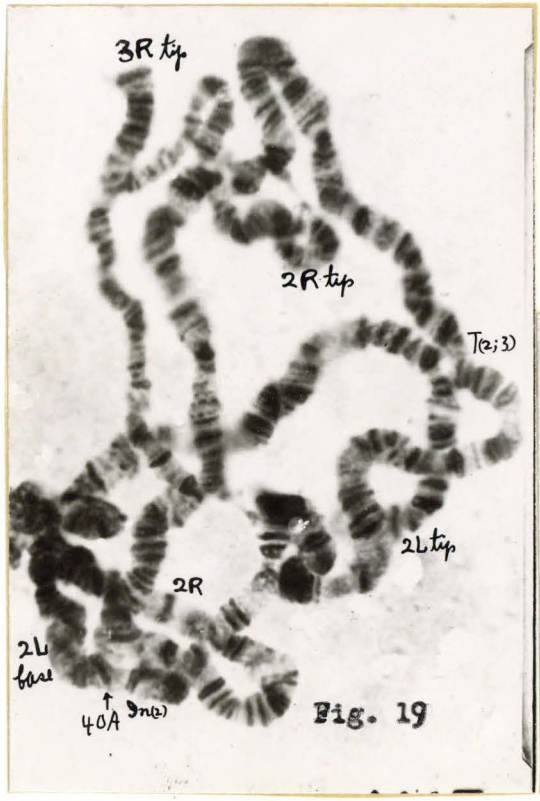


Fig. 19

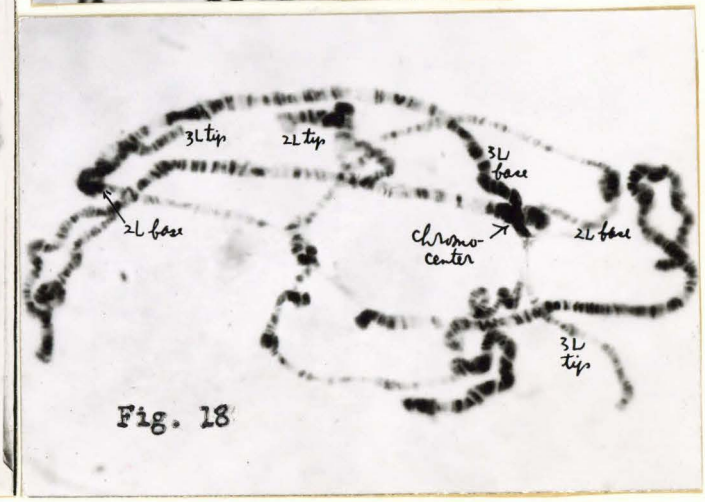


Fig. 18

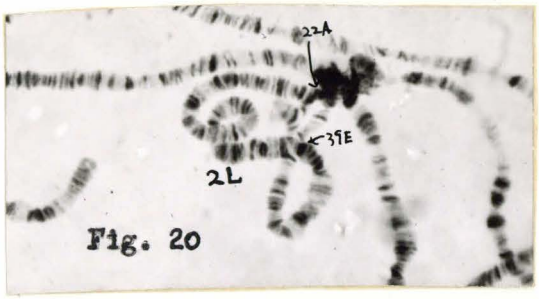


Fig. 20

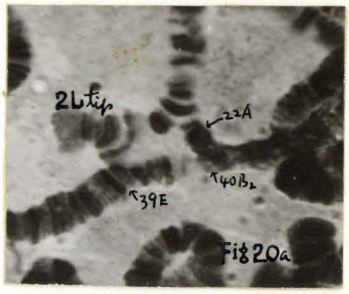


Fig. 20a

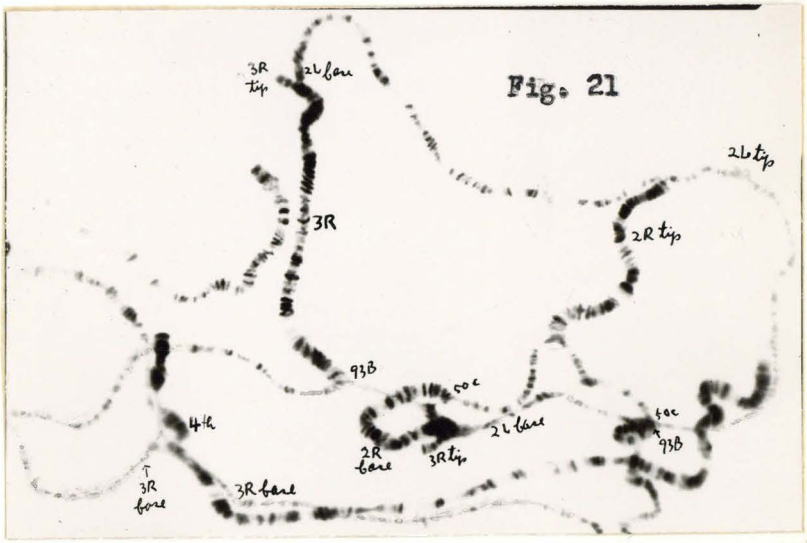


Fig. 21

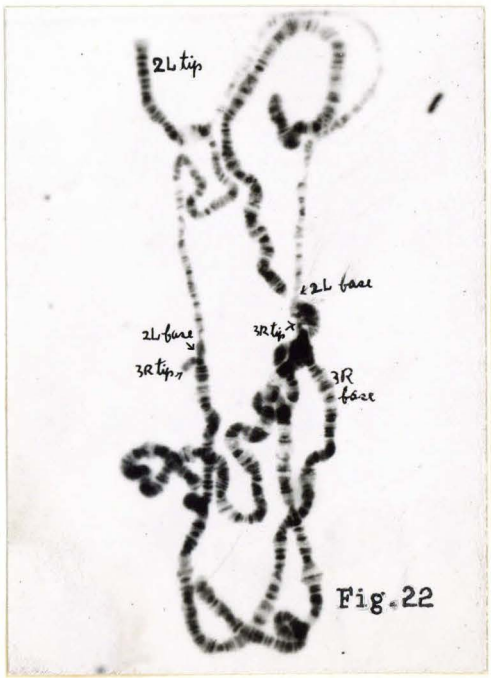


Fig. 22

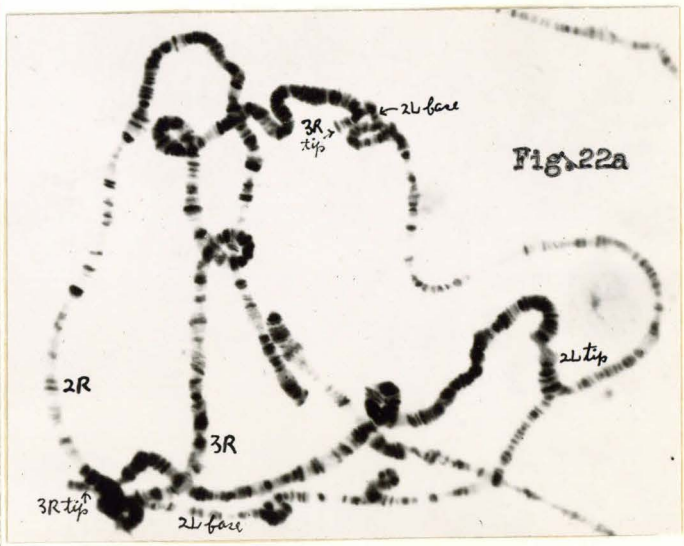


Fig. 22a

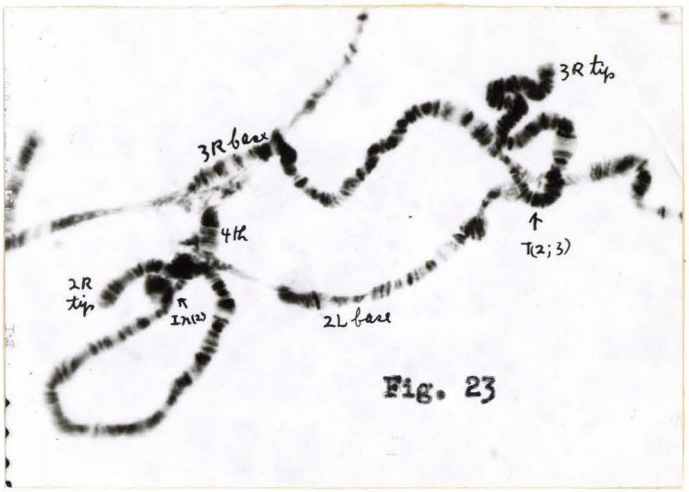


Fig. 23

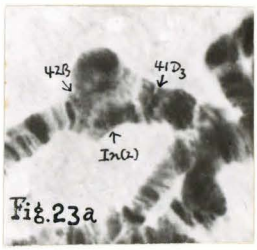


Fig. 23a

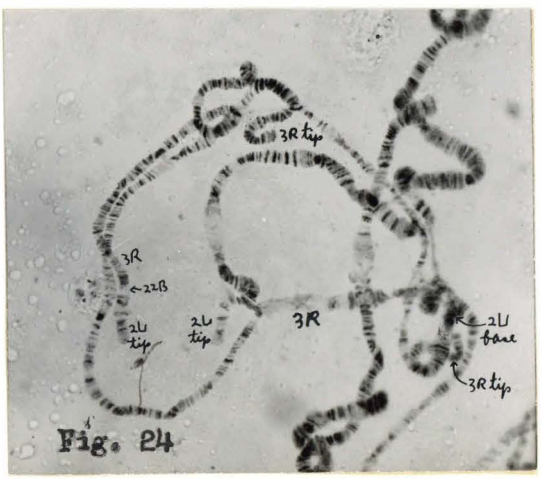


Fig. 24

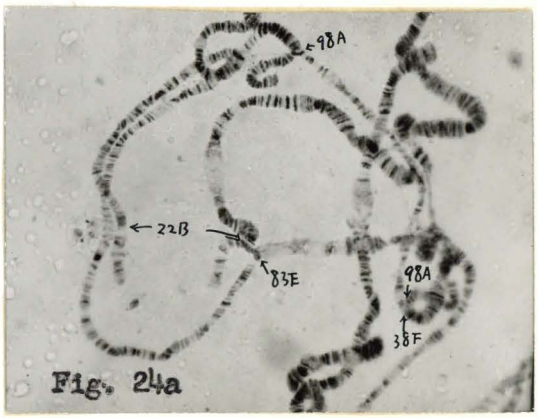


Fig. 24a

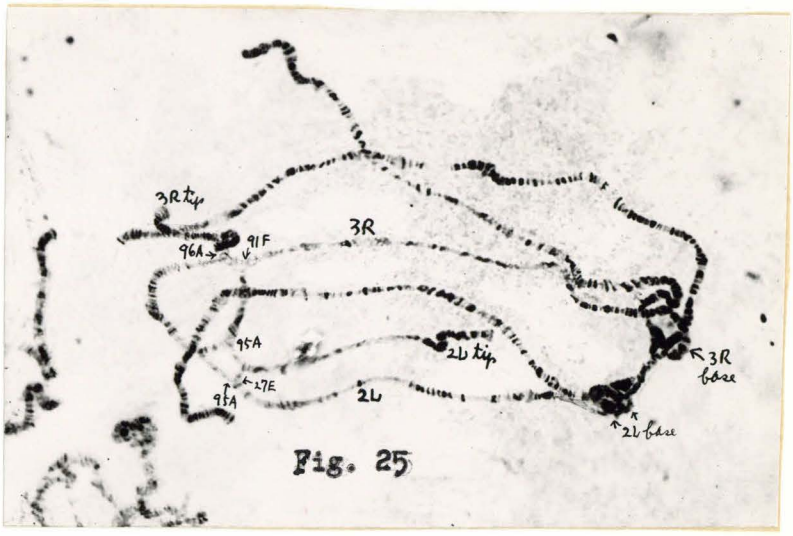


Fig. 25

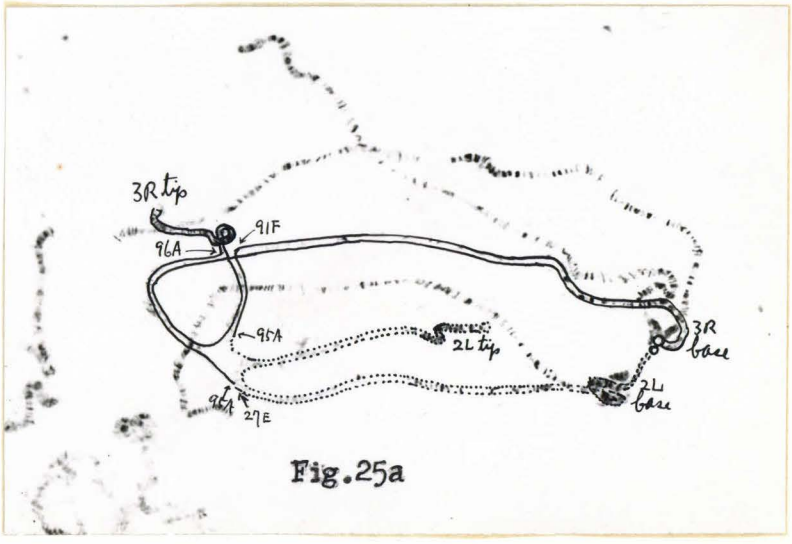


Fig.25a

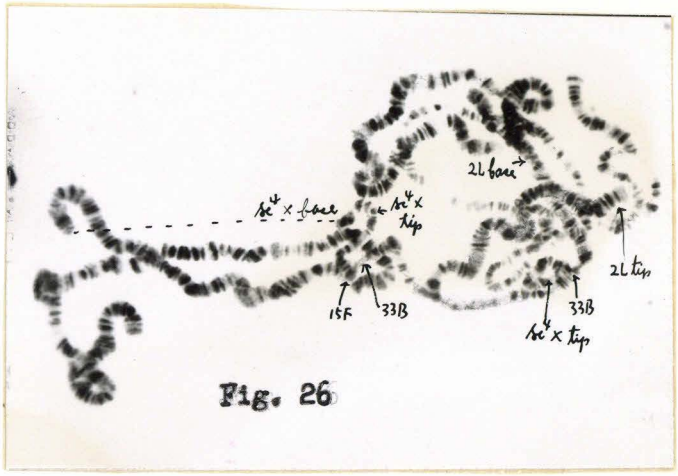


Fig. 26

Fig. 26a

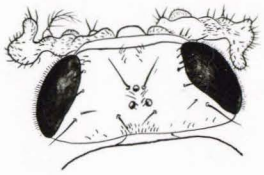


Fig. 27

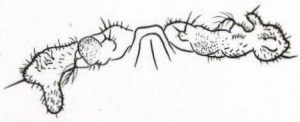


Fig. 27a

Fig. 27b

Fig. 27c

Fig. 27d

Fig. 27e

Fig. 27f

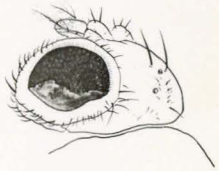


Fig. 28