ONE type of viable chromosomal rearrangement frequently induced by X-ray treatment is a translocation in which a piece of a chromosome is taken from its normal position and inserted into some other part of the chromosomal complex. When such a rearrangement is intrachromosomal the name "transposition" is applied and when it involves two chromosomes it is called an insertional translocation. A number of transpositions and insertional translocations have been described. In this paper results will be given of studies made of five such cases in which euchromatic segments of the X chromosome of *Drosophila melanogaster* are inserted into heterochromatic regions.

Below is a list of X chromosome genes mentioned in this paper with their location on the linkage map given in parentheses (Bridges 1938b): bi, bifid (6.9); cm, carmine (18.9); ct, cut (20.0); cv, crossveinless (13.7); cx, curlex (13.6); dm, diminutive (4.6); dx, deltex (17.0); ec, echinus (5.5); fa, facet (3.0); g, garnet (44.4); N, Notch, deficiency for fa (3.0); peb, pebbled (7.3 ±) pn, prune (0.8); rb, ruby (7.5); rg, rugose (11.0); rst, roughest (1.7), rux, roughex (15.0); sc, scute (0.0+); scp, scooped (18.5 ±); shf, shifted (17.9); sn, singed (21.0); vs, vesiculated (16.3); w, white (1.5); y, yellow (0.0).

DESCRIPTIONS AND EXPERIMENTAL DATA

All five rearrangements which will be described here were found in routine experiments carried on with the purpose of inducing changes in the Notch and cut loci of the X chromosome by X-ray irradiation applied to males. All changes involving the Notch locus are given the experiment number 264 and those affecting cut the experiment number 268. Individual changes in each group are numbered in the order in which they were found; thus 264-58 indicates the 58th change affecting the Notch locus. Salivary chromosomes map designations refer to Bridges' 1938a map for the X chromosome and Bridges' 1935 maps for autosomes.

*Notch 264-58.—* N 264-58 was found on April 11, 1938 among the offspring from a mating between a white cut female and a treated yellow male. The original female had notched wings and white eyes with a few red spots. Cytological tests made by Sutton (1940) show that a section of 20 bands from 3B3.4 to 3D5.6 inclusive is inserted in an inverted order in the chromocenter of 3L proximal to 8oC. By subsequent breeding two
lines were isolated, one having the deficiency in the \(X\) chromosome and the other with a duplication in \(3L\). Cytological analysis indicates that nothing was lost in the transfer, that is, that all bands which are missing in the Notch region are inserted in the \(3L\) heterochromatin. Genetic tests show that the deficiency involves the \(w\), \(rst\), \(fa\) and \(dm\) loci, while the inserted segment, when together with recessive alleles, shows spotting for \(w\), \(rst\) and \(fa\) and the recessive mutant change in the \(dm\) locus. Neither the deficiency nor the duplication affects \(pn\) which is to the left of \(w\) nor \(ec\) which is to the right of \(dm\), indicating that these two loci are outside the limits of the \(N\) 264-58 change.

The duplication is viable both in the males and in the females. Crosses were made between females heterozygous for different lethals in the Notch and white loci and males carrying the duplication to determine if it covered the lethal effect. In all cases where the lethal effect is covered hypoploid males should appear carrying the lethal in the \(X\) chromosome and the duplication in \(3L\). The following lethals were studied:

- \(N\) 264-32, a deficiency for four bands, 3C4 to 3C7 inclusive;
- \(w\) 258-45, a deficiency for a single band, 3C1;
- \(N\) 264-8, \(N\) 264-40, \(N\) 264-46, \(N\) 264-47, all lethal Notches but without any cytologically detectable deficiencies;
- \(N\) 264-34, \(N\) 264-53, \(N\) 264-69, all translocations with one break adjacent to Notch and the other in the euchromatin of an autosome, none of them showing any detectable deficiency in the Notch region;
- \(N\) 264-62, a translocation with one break between 3C7 and 3C8 and the other in the heterochromatin of 2R, heterozygous with \(w\) and \(fa\), shows mottling for both loci;
- \(N\) 264-48, an inversion with one break following 1B6 and the other between 3C7 and 3C8;
- \(N\) 264-52, an inversion with one break between 3C3 and 5 and the other between 20B1.2 and 20C1.2, showing spotting for \(rst\), \(fa\), \(dm\), \(ec\) and \(bi\); and
- \(N\) 264-63, a transposition of a piece from 13C to chromocenter into Notch region between 3C7 and 3C9.

The lethal effect was covered only in the case of \(N\) 264-53. In that case \(y\) \(N\) 264-53/In dl-49, \(y\) \(H\) \(w\) \(m^2\) \(g^4\) females were crossed with \(w\) \(Dp\) 264-58 males and the following \(F_1\) offspring were obtained:

Females: 108
Males: In dl-49, \(y\) \(H\) \(w\) \(m^2\) \(g^4\) 43
\(y\) 13
\(w\) 11
\(w\)-mottled 12

In dl-49 are the normally expected males, \(w\)-mottled and \(w\) are non-dis-
junction males with or without the duplication respectively and \( y \) males have the Notch X chromosome covered by the duplication. While the wild-type/duplication males are fully fertile the \( N/Dp \) males obtained in this experiment are sterile. Moreover under normal conditions one would expect the \( N/Dp \) class to be one half of the \( dl-49, y Hw m^2 g^4 \) class while actually it is significantly smaller. This indicates that even in this instance the duplication did not entirely cover the lethal effect of \( N264-53 \).

Notch 264-85.—\( N264-85 \) was found on April 26, 1939 among the offspring of a cross between \( y sc w \) females and X-rayed wild-type Swedish-b males. The original female had notched wings and white eyes with red spots. Cytological analysis made by SUTTON (1940) shows a complex translocation in which an euchromatic section of X from 3C1 to 6A1.2 inclusive is inserted in its normal order in the chromocenter of the fourth chromosome between 101F and 102A. In addition there is a reciprocal translocation between 2R and X. The distal segment of 2R from 60A5 to the tip is attached to X at 6B1.2 and the distal portion of X from 3B3.4 to the tip is attached to 2R at 60A3.4. The light doublet 6A3.4 is unaccounted for and it may be either deficient or it may be obscured in the preparations because of the distortion caused by the rearrangement. If this doublet were present in the segment inserted in the chromocenter it would be very difficult to find it.

The section of the X chromosome inserted into the heterochromatin of the fourth includes 145 bands. The inserted piece segregates freely from the remaining X chromosome and consequently females deficient (or hypoploid) for the segment are expected to occur as well as both females and males hyperploid for the segment. Hypoploid females were not found indicating that the deficiency for such a long sector in this region of the chromosome is a dominant lethal. Hyperploids were found both among females and among males. Hyperploid females are fertile while hyperploid males are sterile showing that this long duplication upsets the physiological balance in males to such an extent that sterility is produced. A few 264-85 males were obtained from cultures kept at 28°C. These males showed mottling for \( w, cx, cv \) and for rough eyes (presumably \( rst, fa, rg \) and \( rux \)).

Crosses were made between Notch females which were heterozygous for the insertion and males carrying recessive alleles in the loci present in the inserted segment. All Notch females from such crosses have one normal X chromosome carrying the mutant and another which is involved in the translocation. These females showed mottling for \( w, rst, fa \) and \( dm \) located in the distal end of the insertion and for \( rg, ex, cv, rux \) and \( vs \) in the proximal end. This mottling is due to instability of the loci involved. The loci of \( ec, bi, peb \) and \( rg \) in the central portion of the insertion were found to carry wild-type alleles.
Since the hyperploid males are sterile, experiments could not be made
to determine whether or not the duplication covers the lethals located in
the duplicated section of the chromosome.

_Notch 264-86._—N264-86 was found on September 18, 1939 as a single
Notch female among the offspring of a cross between a y pn female and an
X-rayed wild-type Swedish-b male. Cytological analysis made by SUTTON
(1940) shows that a section of the X chromosome from 3C7 to 3E5 inclu-
sive is inserted in its normal order into heterochromatin of 4 at 101F. This
section segregates freely from the remaining body of the X chromosome
and thus a deficiency and a duplication may be isolated. The deficiency is
viable in heterozygous females and the duplication is viable both in fe-
nales and in males.

Cytogenetic study indicates that the inserted piece is one band longer
than the deficiency, which can be accounted for by the assumption that
the chromosome was split at the time of the breakage and that both strands
did not break at the same level (DEMEREC and SUTTON 1940).

The inserted segment includes 17 bands. When heterozygous for fa and
dm it shows mottling for both loci. Notches N264-94 and N264-97, neither
of which shows any cytological deficiency, were tested with the duplication
to determine if their lethal effect would be covered. The results of these
tests were negative. Also N264-53, which was covered by N264-58 duplica-
tion was tested with the duplication 264-86. From the cross y N264-53/dl-
49, y Hw m2 g4×w spl Dp264-86 following offspring were obtained:

- females: 364
- males: dl-49, y Hw m2 g4 176
  - w spl 41
  - w spl/Dp 30

Since y males did not appear it is evident that this duplication does not
cover the lethal effect of N264-53. The w spl and w spl/Dp classes are due
to non-disjunction.

_Notch 264-100._—N264-100 was found as a single female on December 13,
1939 among the offspring of a cross between y sc w females and X-rayed
wild-type Swedish-b males. This female had white eyes with red spots.
Cytological analysis made by SUTTON (1940) shows that a piece of the
X chromosome from 3C1 to 4B3.4 inclusive is inserted into the chromo-
center of 3 between 80C and 81F. This region is unmapped on BRIDGES’
chart. On the available cytological figures it has not been possible to de-
termine in which arm of the chromosome the insertion is located.

The inserted piece involves 45 bands. Notch females heterozygous for
w, rst, fa, dm and ec show mottling in all these loci. Genetic tests indicate
that hypoploid females are viable and fertile. Hyperploid males have not
FIGURE 1.—Section of the Bridges (1938a) salivary chromosome map on which the position of loci is indicated. Each short line above the map represents a band and two horizontal lines indicate a doublet. The limits of each of the five insertions are shown by arrows, o's stand for loci which show mottling, full *'s for loci in which recessive stable change has occurred and crosses indicate loci with wild-type alleles. (Last entry in the first column should read ct 268–37 instead of ct 264–37.)
been found among a large number of flies examined in the various tests. Since these tests include crosses with a number of different stocks as well as cultures raised at low and at high temperatures the non-appearance of males suggests that males carrying the duplication are not viable.

Cut 268-37.—ct268-37 was found on November 7, 1939 as a single ct female among the offspring from a cross between ct g females and X-rayed y males. Breeding tests showed that this change in the cut locus was connected with a lethal effect. This may be due either to a lethal allele of ct or, since this is an insertional translocation, the lethal may have been induced in connection with any one of the three breaks. Cytological study made by SUTTON (1940) shows that a piece of the X chromosome from 5D3.4 to 7B1.2 inclusive is inserted in an inverted position in the heterochromatin of 3L between 40F and 41A. In the inserted piece all bands which are missing in the X chromosome are accounted for. The insertion includes a section of 76 bands.

Hyperploid females and males and heterozygous hypoploid females are viable and fertile. Hypoploid females show a strong minute bristle character and have a lower viability. Genetic tests show that rg, cx and cv which are on the left side of the rearrangement and sn which is on the right side are not affected and thus are not located in the inserted segment. It has been already mentioned that ct shows a recessive change. It is known that the cut locus is represented by bands 7B3-5 (DEMEREC and HOOVER 1936) and since one of the breaks occurred adjacent to and to the left of 7B3 the locus ct is not located in the inserted segment. Tests with rux and vs show that recessive changes producing mottling have occurred in these loci. Tests with shf and cm indicate that the insertion carries wild-type alleles at these loci.

DISCUSSION

Experimental evidence presented in the foregoing pages is summarized graphically in figure 1. That figure shows the pertinent section of the salivary map of the X chromosome which has been copied from the revised Bridges' map (BRIDGES 1938a). The position of loci is shown as determined by work in this laboratory which is still unpublished.

An extensive study of mottling in the loci of the white-Notch region is now under way, and the results will soon be ready for publication. In the course of this study mottling has been observed in the following loci: pn, w, rst, fa, dm, ec, bi, rg, cx, cv, rux and vs. Results indicate that mottling occurs in conjunction with a chromosomal rearrangement when a certain locus is brought into the proximity of a specific region of a chromosome. As has been shown by SCHULTZ (1936) and later confirmed by a number of investigators heterochromatin is effective in inducing mottling. My studies indicate (Demerec in press) that not all regions of the heterochromatin
are equally potent, and also that certain sections of the heterochromatin do not induce mottling at all. On the other hand these studies suggest that all autonomous loci might show mottling given a suitable environment.

The diagrams in figure 1 demonstrate that all loci included in the insertions $N_{264-86}$ and $N_{264-100}$ show mottling. In the case of $N_{264-58}$ all loci included in the insertion are affected, but only $w$, $rst$ and $fa$ exhibit mottling while $dm$ shows a recessive mutant change. Such recessive changes are frequently observed in chromosomal rearrangements involving euchromatic segments. As a rule the locus which happens to be adjacent to the break is affected, and only in exceptional cases is the locus changed which is separated from the break by several bands. The length of the sensitive region within which a break may induce a change varies with the locus. A majority of the loci studied have a very short sensitive region.

The available evidence indicates that the sensitive region of the $dm$ locus is of medium size and therefore the right break of $N_{264-58}$ may well be within it and may be responsible for the recessive change in $dm$. In that case since the insertion is inverted the region of the heterochromatin of $3L$ between $80C$ and the centromere is effective in inducing mottling while the region to the left of $80C$ is not effective. However, in salivary gland chromosomes that region has the characteristic appearance of heterochromatin. A similar situation is found in $ct_{268-37}$ where a section of the X chromosome is inserted in an inverted position into heterochromatin of $3L$ to the right of $80C$ in approximately the same position as in $N_{264-58}$. Here again the region close to the centromere induces mottling while the region to the left of $80C$ does not. Since the cytological evidence indicates that the $ct$ locus is not included in the inserted segment, the recessive change in $ct$ is probably caused by a break in its proximity and a subsequent fusion to euchromatin.

A good illustration of the extent of the influence of heterochromatin is found in $N_{264-85}$. Here a long segment of 145 bands is inserted in an inverted position in the heterochromatin of the fourth chromosome. Mottling is induced in the loci on each side of the inserted segment but the loci in the center are not affected. On the right side the effect stops between $rb$ and $rg$. It is known that $rg$ is located in the section between the 22nd and 81st band from the right end and therefore the effect on the right side extends through at least 22 bands and may reach as far as $80$ bands. It appears probable that the effect on the right side extends through a longer distance than the effect on the left side. It is of interest to note that the right side is attached close to the centromere. On the left side the effect stops between $dm$ and $ec$. Since $dm$ is located in the 13th and 14th band from the left break and $ec$ is the doublet represented by the 27th and 28th band from the break, the effect on the left side extends through at least 14 bands and it stops before the 27th band is reached.
Evidence is available showing that the effect in this region of the chromosome may spread to a longer section than 14 to 26 bands. In $N_{264-52}$, which we have in our collection, a section from 3C4 to 20B1.2 inclusive is inverted and thus the $N$ region is brought into the proximity of heterochromatin. In this case mottling is evident in $rst$, $fa$, $dm$, $ec$ and $bi$ and therefore the effect extends at least 50 bands from the break.

The material presented here indicates that certain regions of heterochromatin are not effective in inducing mottling. The data show that the region to the left of 8oC was ineffective in both $N_{264-58}$ and $ct_{268-37}$. In these cases that region is moved away from the centromere by insertions. It may be argued that the increase in the distance from the centromere may be responsible for the non-appearance of mottling. However, the inserted segment in $N_{264-85}$ is much longer than either in $N_{264-58}$ or $ct_{268-37}$ and in spite of that the heterochromatin is effective in producing mottling. I am inclined to think that the quality of the heterochromatic region as well as its relation to the centromere and its quantity are all important factors in determining mottling. The evidence in support of this view was discussed in a paper read at the Seventh International Genetics Congress in Edinburgh (Demerec in press).

Changes which occurred in the inserted segments are best visualized by analyzing biological effects produced by them. The most striking and at the same time the most characteristic effect is mottling. As an illustration I will use the mottling observed at the white locus. In all cases mentioned in this paper the females heterozygous for the insertion and the white gene have eyes prevailingly cream or very light cherry with smaller or larger spots colored dark cherry or red (wild type). Judging from the appearance and the distribution of spots it seems probable that red spots originate through changes from light color into dark color which have occurred during the development of the eye. If this is expressed in terms of genes it may be said that in light regions the action of the wild-type allele of the white gene, which was present in the segment before insertion, is partially or totally suppressed. Total suppression produces white background and partial suppression light color. In red spots the activity of the gene is again fully restored. It is important to note that in the case of large red spots all facets in a spot are red, indicating that when the restoration of activity occurs it persists among the daughter cells.

The question now arises concerning the mechanism involved in the suppression and the restoration of the activity of the affected genes. Two possibilities are evident. (1) We may be dealing here with real chemical changes in genes which are unstable and revert to the original state. In this case it would be assumed that the change in the position of the locus produced a reversible chemical change in the gene and that reversions occur during the ontogeny of the fly whenever conditions are suitable. (2) It is also possible
that because of the shift in position the action of the gene is suppressed without any change in the chemical constitution of the gene itself. In such a case also certain physiological conditions arising during the ontogeny of the fly would reestablish the activity of the gene and thus bring about the development of wild-type spots. I intend to discuss the evidence in favor and against these two possibilities in another paper where experiments dealing with 35 cases of mottling will be described. In this paper I am only considering the facts which are specific to the material described here.

From a theoretical standpoint the experiments in which the covering effect of duplications was tested are of particular interest. Tests with duplication $N_{264-58}$ showed that of 12 lethals connected with the Notch phenotype only one, namely $N_{264-53}$, was covered by the duplication and in addition one lethal white tested was not covered. One of the Notches and the white were cytologically detectable deficiencies while all other Notches had a full complement of bands in salivary chromosomes. Similarly neither of the three lethal Notches tested which included also $N_{264-53}$ was covered by $N_{264-86}$ duplication. These results show (1) that these duplicated segments inserted in the heterochromatin were not only unable to cover a physical deficiency for a small part of the duplication but in the majority of cases they were unable to cover a biological deficiency which is expressed as a lethal; (2) that there is a difference between the Notches in regard to lethality and (3) that there is a difference between similar duplications in their ability to cover the lethal effect of loci which are present in duplication.

The inserted segment of $N_{264-58}$ shows mottling for $w$ and for $fa$, which is considered an allele of $N$, but was not able to cover a deficiency affecting either of these two loci. It has been shown by Poulsion (1940) that the abnormalities in development which bring about the death of the hemizygous flies are evident in very early embryonic development, during blastoderm formation, in the case of Notches and somewhat later in the case of white lethals. This indicates that in early embryonic stages the function of white and facet-Notch genes in the inserted segment of $N_{264-58}$ is not adequate to cover deficiencies in these loci. However, the wild-type spots which appear on the eyes of flies indicate that their function is normal in some of the cells during late stages of development.

Another interesting fact brought out in these experiments is the difference in viability of duplications. It has been shown that males carrying the $N_{264-85}$ duplication are viable although they are sterile, while the males carrying the $N_{264-100}$ duplication have never been found and presumably this combination is lethal. Both duplications have identical left limits. The $N_{264-100}$ segment includes 45 bands and the $N_{264-85}$ segment includes in addition to these same bands 100 others. It is evident that the lethal effect
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of N264-100 cannot be due to the unbalancing effect of the total number of
genes present in the duplicated segment, since the total number of genes in
N264-85 duplication is much larger. The lethal effect could be brought
about only through the unbalance of the genic system caused by the activ-
ity of the genes located in the duplicated segment, which activity is de-
termined by the position of the segment in the gene system.

SUMMARY

Description is given of five insertional translocations in which euchro-
matic pieces of the X chromosome are inserted into heterochromatin. Fig-
ure 1 summarizes the results of cytogenetic analysis.

As a rule the loci brought into the proximity of heterochromatin show
mottling. When the inserted segment is long, as in the case of N264-85
where it includes 145 bands, the loci on both ends of the segment show
mottling while the loci in the center are not affected.

In N264-58 and ct268-37 segments are inserted in 3L to the right of 80C
in an inverted position, and in both cases the loci farthest from the centro-
mere do not show mottling.

A number of Notches and one lethal white were tested with duplications
N264-58 and N264-86. In all but one Notch the duplication did not cover
the lethal effect.

The inserted segment in N264-85 includes all bands of the segment in
N264-100 and in addition 100 bands more. In males the duplication for
N264-100 is lethal while the duplication for N264-85 is not, indicating that
in this case the length of the segment is not responsible for the lethal effect.

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