IDENTIFICATION OF HALF-TRANSLOCATIONS PRODUCED BY X-RAYS IN DETACHING ATTACHED-X CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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ONCE THE detachment by X-rays of attached-X chromosomes in oocytes of *Drosophila melanogaster* carrying no Y was proved, in some cases at least, to be accomplished by means of paracentric gross interstitial deletions (MULLER and HERSKOWITZ 1954), it became reasonable to consider that all or almost all detachments represented multibreak occurrences rather than single breaks that had, in the case of the centric fragment, been followed by healing of the broken end. For it was possible that in such studies many detachments could have resulted either from gross deletion or from interchange between the attached-X and an autosome and yet had not had the connection between the detached X and the other chromosome material detected (MULLER and HERSKOWITZ 1954). Accordingly, two lines of investigation were decided upon at that time. The purpose of one was to determine the frequency-dosage relationship for detachment of attached-X's (HERSKOWITZ 1954), that of the other to determine how many detachments could be proved, by genetic methods, to be interchanges between the attached-X and an autosome. The latter type of interchromosomal rearrangement has been termed "half-translocation" (MULLER and HERSKOWITZ 1954; ABRAHAMSON, HERSKOWITZ, and MULLER 1954).

In cases of detachment of the half-translocation type, the attached-X is broken rather near its centromere, into a centric "*J*" portion and an acentric "*I*", and an autosome ("*A*") is broken subterminally (anywhere on chromosome IV being subterminal). The stump of the centric *J* then becomes "capped" by the telomere-bearing autosomal tip, and/or the acentric *I* becomes "captured" by attachment to the centric autosomal remainder, in substitution for the latter's tip. It seems likely that in some of the cases found both reciprocal types of recombinant chromosomes were formed but that only one of them became incorporated into the nucleus of the mature egg, whereas in other cases two of the broken pieces failed to join and were lost as acentric and dicentric isochromatids, while the other two pieces joined and became incorporated into the egg nucleus. In the case of a half-translocation with a long autosome (II or III), an egg receiving a capped *J* must, for survival of the zygote, receive a complete maternal autosome of the type that provided the cap, so that the zygote thereby becomes hyperploid for the autosomal tip. On the other hand an egg receiving an *I* that was captured by a long autosome must,

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for survival, fail to receive a complete maternal autosome of that type, and thus forms a zygote that is hypoploid for the autosomal tip.

Either condition may be genetically demonstrable if the tip includes the normal allele of a known genetic marker, for the marker could be "covered" by the hyperploidy or "uncovered" by the hypoploidy of the tip. Capturing would also be recognizable by X-autosome linkage. The hypoploidy associated with capturing would in addition give rise to a Minute-bristle phenotype in many cases.

In the interest of efficiency, the investigation of the frequency-dosage relation was carried out first, and the flies with detachments of the attached-X, thus produced, were used in the present, second line of study. These different cases of detachment were put through a series of genetic tests in order to identify cases of capping and capturing of the detached X by the autosomes. The results are discussed in relation to the problem of new telomere formation as well as to that of the factors involved in the production of gross chromosomal rearrangements in oocytes.

MATERIALS AND METHODS

The detachments tested were obtained from oocytes which contained, before treatment, a "snoc" attached-X chromosome having the genes *sc* ct^n *oc car* on one of the arms and *y In49* sn^{z2} on the other (MULLER 1954). No Y chromosome was present. The detachments studied were of two kinds. One type came from an attached-X which had a breakage in or near the heterochromatin proximal to the centromere and a second subterminal breakage proximal to *sc* or *y* of the same arm, which resulted in what was in effect detachment after deletion of the interstitial piece. Such cases of detachment, bearing both y^+ and sc^+ and thereby phenotypically recognizable, served as "controls" for demonstrating that the genetic tests used to detect X-autosome half-translocations would not appear to give positive results in cases that did not actually have half-translocations. The other type of detachment, bearing either y^+ or sc^+, but not both, included whatever cases of half-translocations were viable (HERSKOWITZ and MULLER 1954; HERSKOWITZ 1954).

Detachments which were viable and fertile in the male were maintained as stocks by crossing them with attached-X females having the genotype $Y/y\,f: = ♀$. Those detachments which were lethal or incapacitating to the male were kept in heterozygous females whose other X chromosome was *y* sc^{s1} *B In49 v*. The stocks of detachments kept in the females were well balanced in those cases in which the detached X comprised the *oc*-bearing arm. In the cases having the *In49*-bearing arm there was occasional double crossing over but this did not result in the loss or unbalancing of either terminal region of the detached X, although the markers, excepting the scute S1, might become lost or change places. However, because of this situation, the possibility is not excluded that some half-translocations with the *In49* bearing arm remained unproved.

The testing procedure involved crossing flies with detachments to a series of stocks containing mutant genes located close to various autosomal termini. The mutants chosen were: *net* and al^2 for IIL, *M33a* and sp^2 for IIR, *ru* for IIIL, *bv* for IIIR, spa^{Cat} (to be referred to as *Cat*), sv^n, and *gvl* for IVR, Not only are these genes

as close to the chromosome termini as feasible, but they also have clear-cut phenotypic effects that make possible the recognition of a hyperploid condition of the given region, while at the same time this recognition is not usually prevented in cases in which there is more or less than the normal amount of heterochromatin present.

Assume, for illustration, that a fly has a detached X bearing a cap from IIR long enough to include *M33a*$^+$ (the normal allele of Minute 33a). Such a fly has two more *M33a*$^+$ loci located on the normal II chromosomes. When by means of a testcross one of the normal II chromosomes is replaced by one bearing the "haplo-insufficient" deficiency *M33a*, the Minute character will fail to show since the flies will be diploid for *M33a*$^+$.

The analysis with *Cat* is somewhat similar, since two doses of the normal allele, *Cat*$^+$, and one of *Cat* give a Cat$^+$ phenotype, and since disjunction of the free IV chromosomes is seldom prevented by the partial IV chromosome which is attached to the X. Hence, gametes containing an X capped by a *Cat*$^+$ part of IV nearly always contain a free IV, with *Cat*$^+$, in addition, if the parent from which they were derived contained two free IV's, each with *Cat*$^+$. Such gametes when united with ones (from the other parent) known to carry *Cat* result in offspring which are phenotypically Cat$^+$. In such a case, the identification of which offspring received *Cat* from the other parent is made possible by having this parent carry *Cat* (which is lethal when homozygous) balanced over another lethal dominant such as *ci*^{D}, which is expressed even when present in single dose in a triplo-IV fly. In contrast to this, flies derived from gametes that did not carry the capped X but carried one *Cat*$^+$-containing IV, united with *Cat*-containing gametes of the other parent, do manifest the Cat character.

The above account assumes that the fly with the capped X contains two free IV's. This will nearly always be true in later generations derived from the original exceptional individual in cases in which the *Cat*$^+$-containing cap on the X was not long enough to include the normal allele of Minute-IV. For such flies (produced originally from gametes in which a free IV had not disjoined from the part of IV capping the X) have a much higher viability and fertility and shorter developmental time than the Minute-IV's.

Even in the cases in which the *Cat*$^+$-containing cap did include *M-IV*$^+$, increasing numbers of the flies of later generations come to contain two free IV's, by the frequent nondisjunction whereby one free IV gets into the same gamete as the cap, since this hyperploid condition, once established, tends to be inherited in a relatively stable fashion owing to the comparative lack of influence of the cap on the segregation of the two free IV's. If however an individual with just one free IV happens to be chosen for the testcross with *Cat*/*ci*^{D} the result is less clear-cut than if two free IV's were present. Yet even here the presence of the cap will usually be recognizable. For this cap will undergo frequent nondisjunction with the one free IV. Thus many (up to 50%) of the offspring that receive the capped X from one parent and *Cat* from the other will appear Cat$^+$, while all offspring that fail to receive the capped X but do receive *Cat* will appear Cat. This will in such a case prove the presence of *Cat*$^+$ on the X.

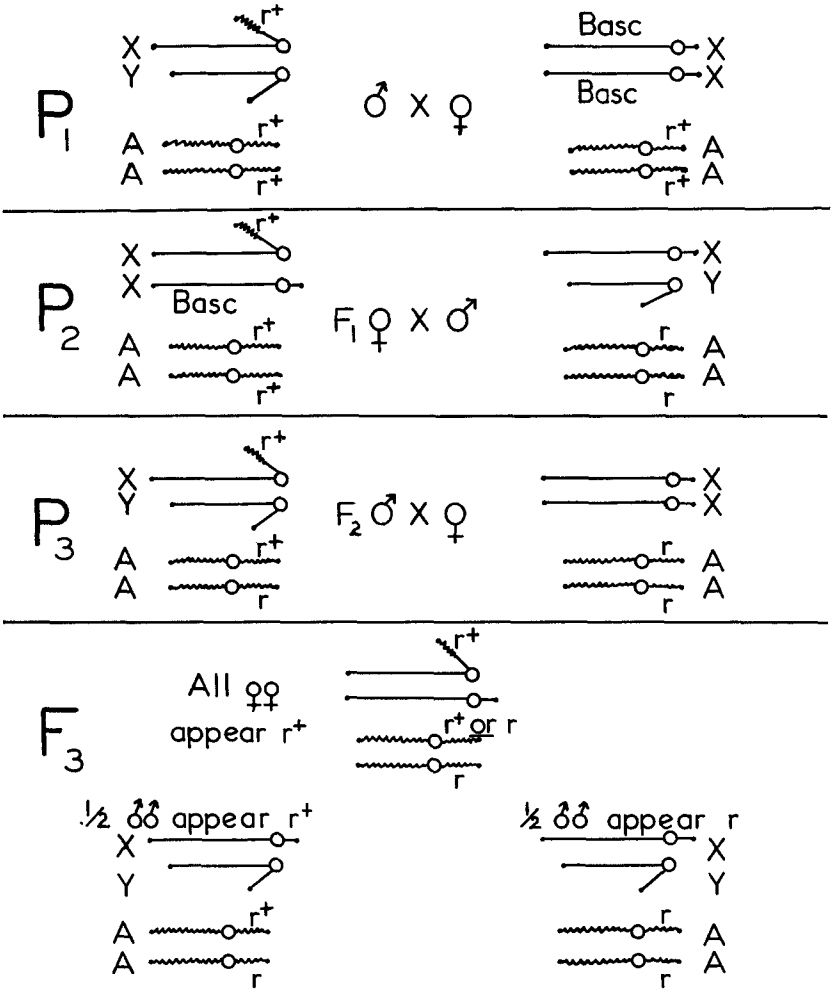

FIGURE 1.—Diagram of matings to detect the presence of an autosomal gene r^+ contained in a telomere-bearing cap which has joined to the stump of the centric arm of a broken attached-X chromosome. The X chromosome in question was present in the P_1 male. X = X chromosome carrying either *sc* ct^n *oc* (*car*) or *y In49* sn^{x2} (*car*), Y = Y chromosome, A = autosome normally carrying the locus of r^+ or *r*, *Basc* = X chromosome rarely giving crossovers with a wild type X, containing sc^{S1} *B In-S apr* sc^8, 0 = centromere, · = telomere. All flies are assumed to carry two complete A's in addition to the hyperploid segment capping the X (see text for discussion of another situation which applies only to chromosome IV).

If the X tested did not carry an r^+-containing cap, in the F_3, one half the females would appear r and one half r^+, the expectation for males being, however, unchanged.

If the normal allele (r^+) of a recessive marker (r) is present in hyperploid condition as a cap on the X it can be demonstrated as follows. When the detached X is maintained in the male (fig. 1), males are crossed to "Basc" females and the heterozygous Basc F_1 females mated to r/r males. Non-Basc (F_2) sons carry the detached X and all are therefore $r^+/r^+/r$. When these males are backcrossed to r/r females all daugh-

ters are phenotypically r+ while one half the sons appear r+ and the other one half r. If, on the other hand, the detachment does not carry an r^+-containing cap the offspring from the last cross would have one half of each sex appearing r+ and the remainder r. If the cap on the X were from IV, even if the P_3 male had only one free IV instead of two, all F_3 females would still appear r+, while all the males would in this case be r.

When the detached X is carried in the female along with *y sc*^{s1} *B In49 v* (fig. 2), these females are crossed to *r/r* males. If the detached X has an r^+-containing cap, present in hyperploid condition, the F_1 non-Bar females carrying the detached X are all $r^+/r^+/r$. When these F_1 are backcrossed to *r/r* males, three fourths of their fe-

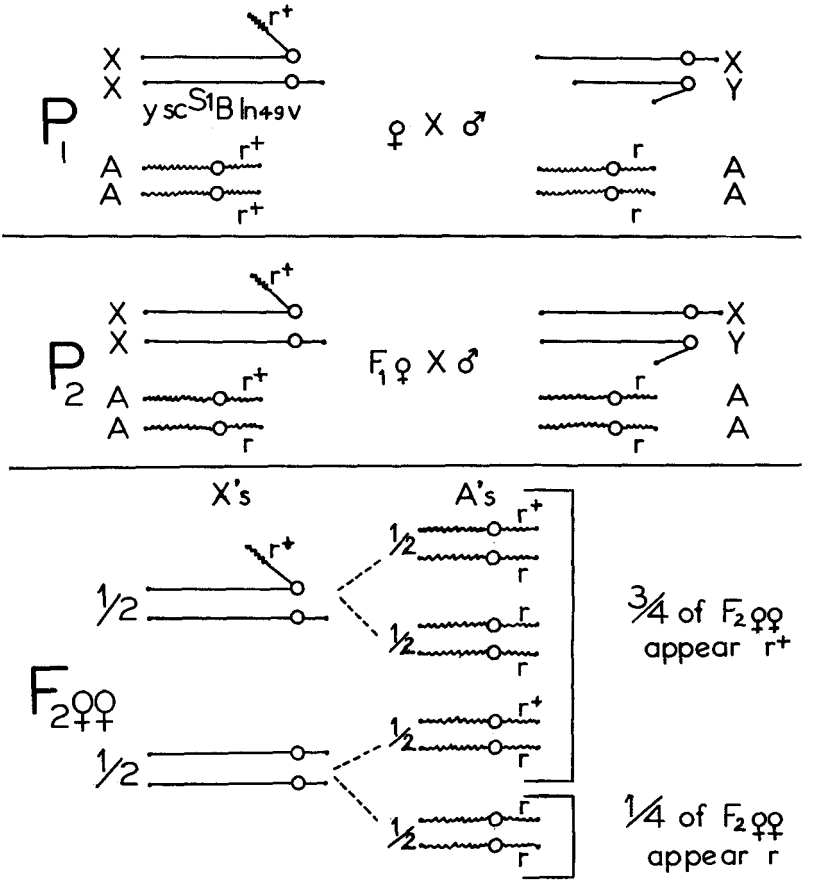

FIGURE 2.—Diagram of matings to detect the presence of an autosomal gene r^+ contained in a telomere-bearing cap which has joined to the stump of the centric arm of a broken attached-X chromosome. The X chromosome in question was presented in the P_1 female whose other X contained *y sc*^{S1} *B In49 v*; crossovers were produced rarely when the X tested carried *sc ct*^{n} *oc* (*car*) and occasionally when it carried *y In49 sn*^{z2} (*car*). The other symbols used are the same as defined in the legend to figure 1. All flies are assumed to carry two A's in addition to the hyperploid segment capping the X (see text for discussion of another situation which applies only to chromosome IV).

If the X tested did not carry an r^+-containing cap, in the F_2, one half of the females would appear r+, the rest r.

male offspring (all of this with the detached X and half of the others) will appear r^+ and one fourth r. This is not true of the male offspring since the males with the capped X are seldom able to live. For this reason only F_2 females were scored. If, however, the detached X did not carry an r^+-containing cap one half of each sex appearing from this cross would look r^+, the rest r. Were the cap on the X derived from IV, and had the P_2 females only one free IV instead of two, one half the females and males would appear r^+, the other one half of each would appear r, and it would not be possible from these results to prove the detachment was a half-translocation. But this situation would be infrequent in view of the previous discussion and the fact that a number of P_2 females were tested in each case.

In case the detached X was of type *y In49 sn*^{x2} the Bar is, as previously explained, a poor marker but scute-S1 remains a valid marker. Hence in these cases the females of the stock having the detached X should preferably be crossed to males having both *sc* and the marker "*r*". When these are unavailable the non-Bar F_1 females should be bred individually and attention paid only to the offspring of those F_1 females which failed to produce scute (*y sc*^{s1}) sons, since these must have contained the detached X. Unfortunately this was not always carried out but the absence of scute sons was verified. It will be seen in the Discussion that from the results of these crosses and backcrosses it is possible to detect cappings or capturings involving chromosome IV and cappings by chromosomes II and III, provided one or more of the loci tested were involved in the half-translocation.

A series of additional crosses were made to detect, by means of linkage studies, cases of capturing of an acentric detached X by autosomes II or III.

All work was carried out at 25 ± 1°C.

RESULTS

Four different detachments known to result from intra-arm deficiency were used in the "control" tests for half-translocations. Three of these were maintained in the female and one in the male. Of the 20 detachments originally detected in the male and the 78 detachments originally detected in the female (HERSKOWITZ 1954) which gave y or sc phenotypes and hence possibly arose as half-translocations, 19 stocks maintained in the male and 27 stocks maintained in the female (of which only 2 were male-viable) were put through testcrosses. For 7 additional detachments the data from one or another cross are insufficient and are not presented. However, for most of the cases of detachment from which fertile stocks had been obtained fairly adequate tests were carried through.

The data for the 4 "control" and 46 "experimental" detachments, concerned with whether or not an autosomal genetic marker is included in a half-translocation in which a detached arm of "snoc" was capped or captured, are summarized in table 1. Since it was neither possible nor feasible to test all cases of detachment for all the autosomal markers used, the number of cases actually tested for a given marker is given in parenthesis under that marker in all rows in which data from more than one case are presented. The "controls" show that in a total of 30 tests for the presence of autosomal markers, including all of the 9 factors studied, there were no (false) positive cases.

Among the 46 other detachments studied 24 were proven by these tests to be half-

TABLE 1

Autosomal marker is included (+) or is not (−) in a half-translocation in which a detached arm of "snoc" was capped or captured

No. of detachments tested	L II R				L III R		IVR			Interpretation
	net$^+$	*al*$^+$	*sp*$^+$	*M33a*$^+$	*ru*$^+$	*b*$^+$	*gvl*$^+$	*sv*$^+$	*Cat*$^+$	
"Control" 4	−(1)	−(4)	−(4)	−(4)	−(4)	−(4)	−(1)	−(4)	−(4)	Gross deletion
"Experimental"*										
1	+	+	−	−	−	−	−	−	−	Capping
1		−	−	+	−			−	(−)	Capping
1	−	−	+	+		−	−	−	−	Capping
1		−	−	−	−	−	−	−	+	Capping
1	−	−	−	−	−	−	−	+	+	Capping
1	−	−	−	−	−	−	+	−	−	Capturing
18	−(2)	−(15)	−(15)	−(16)	−(15)	−(6)	+(6)	+(14)	+(17)	Capping or Capturing
22	−(11)	−(17)	−(17)	−(19)	−(16)	−(17)	−(14)	−(18)	−(20)	Unknown
— 46**										

* The numbers of *In49*-bearing detachments maintained as stocks in the female were in the different experimental rows: 1 (row 2), 11 (row 7), 6 (row 8).

** For 7 additional detachments the data are insufficient.

translocations. One involved a cap from IIL which included the loci *net*$^+$ and *al*$^+$. Two were cappings by IIR: one of these cases included the locus *M33a*$^+$ but not the more proximal *sp*$^+$, the other included both loci. Two cases of capping by IVR were demonstrated: in one the cap included *Cat*$^+$ but not the presumably more proximal *sv*$^+$, while in the other both of these factors but not the still more proximal *gvl*$^+$ factor were included. One case of capturing by IV was identified by *gvl*$^+$ being linked to the X chromosome detachment while *Cat*$^+$ and *sv*$^+$ were not. Figure 3B, C shows why this case is regarded as capturing, the preceding ones as capping. Eighteen other cases were found of X-IV half-translocations which could have been either of capping or capturing type since all the loci tested for were found present on the detachments. Here the alternatives of a capping in which IVR was broken proximally to *gvl*$^+$ or a capturing following breakage of the heterochromatic IVL arm were equally possible, as shown in figure 3D.

In no case among the "experimentals" or "controls" were there seemingly false positives that indicated the presence of two different autosomal segments on the detached X.

Most of the 22 detachments not demonstrable as half-translocations by hyperploidy of the autosomal markers used were tested for capturing by means of linkage tests between the X and the two major autosomes (table 2). None of these had appeared Minute and therefore, if cases of capturing, must have involved breaks so near the end as not to remove the locus of an *M*$^+$ gene. One of 21 stocks tested for X-II linkage gave a positive result, while none of 16 tested for X-III linkage gave a positive result.

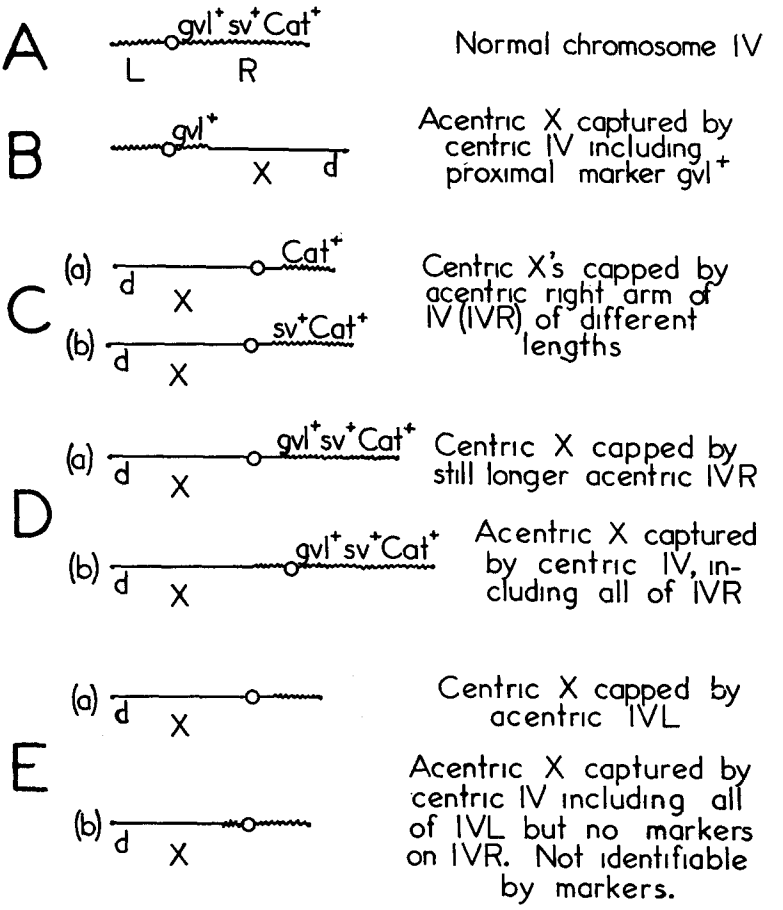

FIGURE 3.—Cappings and capturings of centric and acentric detachments of an attached-X chromosome by chromosome IV. Symbols are the same as in legend to figure 1. The major portion of the detached X is marked by "X" and its distal tip by "d".

A. Normal gene arrangement in chromosome IV (see text for evidence of order for *Cat*$^+$ and *sv*$^+$). B. Acentric arm of X captured by centric part of IV, proved because *gvl*$^+$ is linked to the X while *Cat*$^+$ and *sv*$^+$ are not. C. Centromere-bearing X capped by accentric fragments of IV carrying, as in C(a), *Cat*$^+$ but neither *sv*$^+$ nor *gvl*$^+$, or, as in C(b), *Cat*$^+$ and *sv*$^+$ but not *gvl*$^+$. D(a) Cappings and D(b) capturings which are not distinguishable from one another by the markers for IV since all of these or all tested for were found linked to the X. E(a) Cappings and E(b) capturings which are not identifiable as half-translocations by means of markers since only unmarked portions of IV are joined to the X.

TABLE 2

Results of tests for linkage between X and long autosomes

Autosome tested	Positive tests	Negative tests
II	1*	20
III	0	16

* Interpretation of this case (No. 58, included among the 22 "unknowns" of table 1) is that the detached X was captured by II.

DISCUSSION

The results contain genetic proof, in support of the strong indirect evidence from X-ray frequency-dosage experiments (HERSKOWITZ 1954), that detachment of attached-X chromosomes results, in a high proportion of the cases, from chromosomal rearrangements of half-translocation type. For of the 46 detachments studied adequately 25, or about half, were demonstrably X-autosome half-translocations. The four of these with chromosome II involved 3 cappings and 1 capturing. The 21 other proved cases were interchanges with IV, among which it was possible to identify 2 cases as consisting of capping and 1 of capturing.

The identification of such a high proportion of detachments as half-translocations makes it extremely likely that the 21 unidentified detachment cases arose either as capturings by IVR broken proximally to *gvl*$^{+}$, or as cappings or intra-X deletions in which the breakage that produced the cap was distal to any of the loci tested for. In this connection three points should be especially noted. (1) The genetic markers for III are not nearly so satisfactory as those for II, being much further from the distal ends of the map; this makes it particularly likely that some cappings with III were unidentified. (2) The absence of markers for IVL, all of which is heterochromatic and probably highly breakable, would prevent detection of capping by this arm. (3) It is possible that some detached X's, especially of the *y In49 sn*^{x2} type kept in the female, failed to be identified as half-translocations because they had lost, by crossing over prior to the testcrosses, sex-linked markers used to distinguish them. It is also possible that the capping or capturing by a relatively long segment of IV would have so complicated the disjunction of free IV chromosomes that the tests to prove half-translocation were not clearly positive or negative, and thus led to the exclusion of such detachments from the data. Hence it is clear that while the half-translocations must be at least as numerous as inferred, and of the types designated, the methods used would have permitted some of those originally present to escape detection.

The present experiments, by giving direct evidence of the abundance of half-translocations, demonstrate that the exponential frequency-dosage relationship found for detachment represents multi-breakage events, as had been presumed. Without this direct evidence it might have been postulated instead that single breakages had caused the detachments but that these breaks required one or more additional "hits" for their "healing". Thus the results make less tenable the view that in Drosophila single breakages can become healed.

As expected because of the generally lower viability of hypoploids than of hyperploids, fewer cases of capturing than of capping were found. Another datum worthy of note is the relatively large number of half-translocations with IV, also found by LINDSLEY and NOVITSKI (1953) and by PARKER (1954). Several factors may operate to favor this. There is almost certainly a greater amount of breakable material, or at least a higher effective breakability, in IV than in the subtermini of other chromosomes, especially in view of the fact that viable half-translocations can result from breakage anywhere in IV, including the heterochromatic regions near the centromere. Moreover, IV in oocyte stages probably lies closer to the proximal heterochromatin of the X than do the subtermini of other chromosomes—proximity of breaks favoring interchange, especially in oocytes. Finally, for cases of capturing, the possibility exists that the size of the tip of X, II or III which is viable in hypoploid condition

may be smaller than either arm of IV, which is viable (as is the whole of IV) in haploid condition. This argument acquires cogency when we consider not total inviability but the relative inviability associated with Minute deficiencies, inasmuch as breaks very near the termini of II and III would result in Minute hypoploids, whereas no break in IVL and only breaks in a relatively small proximal portion of IVR would give a Minute hypoploid. The corresponding argument relating to viability in cases of cappings is probably invalid, at least for some of the distal pieces in question, since some viable hyperploids for tips of II and III are known with more extra material than in IV.

The case of capping by IVR in which the cap contained Cat^+ but not sv^+ nor gvl^+ is evidence that Cat^+ is more distally located on IVR than these other two factors.

SUMMARY

It has been possible to prove genetically that at least one half of the tested cases of detachment of attached-X chromosomes by X-rays consisted of half-translocations with autosomes which involved sections of chromosome long enough to contain an allele of a known genetic marker, yet short enough to allow survival. Both cases of capping and of capturing were identified but (by the conditions of the testing) more of the former.

While it is known that recoverable cases of detachment ordinarily require more than one event for their occurrence (HERSKOWITZ and MULLER 1953; HERSKOWITZ 1954; PARKER 1953; KUTSCHERA 1954), it is proved here that these events involve breakages in different chromosome regions, which then undergo interchange of pieces. The alternative postulate, that there are two events at a single chromosome site, namely, breakage and then healing, each requiring a separate hit (or at least the combination requiring two hits) is thereby rendered improbable.

An excess of X-IV half-translocations was found. Possible explanations suggested for this excess are differences in distribution of chromatin, especially heterochromatin, in viability effects of aneuploidy, and in proximity of breakages.

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