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# A *Drosophila* model of improving the fitness of translocations for genetic control

## 1. Autosomal translocations with euchromatic breakpoints

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**Summary.** Translocations with euchromatic breakpoints were generated in lethal-free autosomes of *Drosophila melanogaster*. Pairs of initially homozygous-lethal translocations, matched for one breakpoint, were allowed to recombine for ten generations. At the end of the experiment, 10/47 = 21% of crosses (representing 8/26 = 31% of the intial translocations) had at least one line with at least one homokaryotypic third-instar larva, detected among a small sample of salivary gland preparations from each cross. Among these ten crosses, chromosome extractions were performed; 5/10 of the crosses (probably representing 4/8 of the translocations) had at least one chromosome set with relative viability greater than 15%–25%. To a first (and conservative) approximation, 5/47 = 11% of crosses showed improvement of viability of 1 of the translocations in the cross during the controlled recombination regime; overall, 4 of the 26 translocations (15%) showed improvement of viability. Partly because of the conservative criterion of viability used, this figure is less than the 20% of translocations that theoretically should be improvable. Pseudohomokaryotypes (pairs of translocations with both breakpoints nearly matching) did not behave as very fit homokaryotypes. However, some of them generated viable hyperploid assortment products that might be of practical interest to mask deleterious effects at breakpoints of translocations. The improvement of fitness of at least a proportion of low fitness translocation stocks by the use of a controlled recombination procedure should be feasible for many pest species.

**Key words:** Genetic control – Translocations – Fitness – *Drosophila melanogaster*

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### Introduction

In discussing the feasibility of genetic control of pests, Curtis (1968) proposed that the unique population dynamics of translocations could be used to effect the replacement of populations of pests with genetically tailored populations of the same species. There have since been several attempts to develop suitable stocks in various organisms but, despite laboratory indications of the feasibility of population replacement, cage and field experiments have mostly been equivocal or unsuccessful (Laven et al. 1971, 1972; Robinson and Curtis 1973; Lorimer et al. 1976). Only one set of cage experiments has demonstrated a replacement of wild-type chromosomes by chromosomal rearrangement stocks [Feldmann and Sabelis (1981) in *Tetranychus urticae*, the two-spotted spider mite]. One other cage experiment was encouraging: Reid and Wehrhahn (1976) found that translocations in *Drosophila melanogaster*, carefully picked for fitness components, showed initial indications of being able to outcompete a wild-type tester stock when started at a frequency of 90%. Virtually all other laboratory and field experiments have failed. Part of this general failure is undoubtedly due to "laboratory selection," that is, inadvertent selection of a translocation stock to the precise conditions of the laboratory, and away from those of the field, reducing the stock's ability to survive or compete in the wild or even in a competition cage (e.g., Robinson and Herfst 1980). This problem, under the rubric "quality control," has received special attention from biocontrol workers (see, for instance, papers in Hoy and McKelvey 1979). Translocation stocks have other problems, however, that are specific to their biology.

Many workers have found it difficult to generate translocations that are viable and fertile as homozygotes (Seawright et al. 1982), even though Whitten (1971) noted

that "translocations need not involve adverse side effects." Lorimer et al. (1972), for example, generated "more than 40" translocations in *Aedes aegypti*, of which only two were homozygous viable. Deleterious effects of a translocation can be assumed to be due to one or more of the following (Robinson 1977a): (1) background deleterious alleles fixed by the translocation extraction process; (2) deleterious alleles generated at the same time as the translocation and by the same agent of mutation but separable from the translocation; and (3) effects of the translocation itself, whether deletions at the breakpoints, breakage within a gene or its control regions, or position effects of the rearrangement.

The work reported here uses *D. melanogaster* as a model organism to explore the possibility of improving the fitness of the second class of translocations by allowing recombination to occur between the translocation breakpoints and deleterious mutations elsewhere on the chromosomes. The present report contains the results of experiments on translocations with euchromatic breakpoints. Results for translocations with breakpoints in the centric heterochromatin of the autosomes will be presented in a later paper.

## Materials and methods

### *Drosophila melanogaster cultures and stocks*

Flies were kept at 25 °C in 240 ml milk bottles with 40 ml of a standard cornmeal-molasses-agar medium or in 30 ml shell vials with 8 ml of the same medium. The following stocks were used in the experiments (for description of mutants and rearrangements, see Lindsley and Grell 1968):

*(1) pr cn; $e^{11}$.* A stock with chromosomes of standard configuration, carrying the recessive eye-color markers purple (pr) and cinnabar (cn) on chromosome 2, and ebony (e) on chromosome 3. Homozygous purple and cinnabar together produce apricot-colored eyes.

*(2) CyO; TM6/Xa [In(2LR)CyO, Cy $dp^{lv1}$ pr $cn^2$; In(3LR)TM6, $Ubx^{67b}$ $e^s$/T(2, 3)Xa, $ap^{Xa}$].* CyO (Curly of Oster) is a second chromosome balancer marked with the dominant wing mutant Curly (Cy) and the recessive dumpy-lethal vortex ($dp^{lv1}$), which are both homozygous lethal, and pr and $cn^2$. TM6 (Third Multiple Six) is a third chromosome balancer marked with the dominant marker Ultrabithorax ($Ubx^{67b}$), which is homozyous lethal, and $e^s$. Xa (Xasta) is a 2-3 translocation superimposed on inversions in the right arms of chromosomes 2 and 3; it bears the dominant wing marker apterous-Xasta ($ap^{Xa}$) and is homozygous lethal. The only euploid gametes from CyO; TM6/Xa flies are Xa and the double balancer CyO; TM6.

*(3) Wild-type strains.* Flies designated "U" were from isofemale lines collected near Eugene, Oregon, just prior to the experiments. Flies designated "S" were from O72, a line originally from Pope Valley, Napa County, California. Four and a half years prior to the experiments, O72 was established as a doubly homozygous chromosome (2 and 3) line (Seager and Ayala 1982), and has been maintained in the laboratory since then.

### *Experimental procedure*

The overall experimental plan was to generate translocations in a homozygous viable and fertile stock, so that deleterious effects observed would be due either to newly induced mutations or to the translocations themselves, but not to background. Recombination was then to be allowed to occur with another genome in an attempt to eliminate deleterious effects due to new point mutations. Any remaining deleterious linked to its breakpoints under the conditions of recombination used.

There are two major technical difficulties with this design. First, if a translocation is crossed to normal chromosomes and a mixed population perpetuated to allow recombination, the translocation probably will be lost before it can be re-isolated from the mixed population. On the other hand, immediate re-isolation of the translocation does not allow much recombination to occur. To overcome this dilemma, mixed populations were established by crossing pairs of independently derived translocations with at least one cytologically similar breakpoint in common. This procedure minimizes the chance of reconstitution of a normal set of chromosomes by crossing over in the two "differential segments" (i.e., the parts of the chromosomes between the translocation breakpoints in the double heterokaryotype) (Curtis and Robinson 1971; Robinson 1977b), and recovery of at least one of the translocations is assured.

Secondly, total fitness is notoriously difficult to measure. Therefore, a qualitative measure of only one component of fitness, egg-to-adult viability, was used, starting with only homozygous-lethal translocations. Any improvement in this fitness component therefore would be immediately manifested as viable adults. This strategy provided maximum opportunity for, and easy assessment of, improvement of fitness.

### *Establishment of isochromosomal stocks*

Second and third chromosomes from lines U and S were made homozygous using the extraction scheme shown in Fig. 1, which yields stocks with chromosomes free of lethal and sterile mutations.

### *Generation and extraction of 2–3 translocations*

One fertile line was chosen from each of the U-series and S-series lethal-free stocks, and from each of these 7-day-old males were collected for irradiation. Lightly etherized males were irradiated with 5,000 rads of 50 ke X-rays, filtered through 0.4 mm of aluminum foil and administered at 450 rads/min. Following irradiation, the males were crossed as diagrammed in Fig. 2 to detect and extract chromosomes bearing reciprocal translocations between chromosomes 2 and 3. Translocations were detected by pseudolinkage of pr and cn on the second chromosome with e on the third.

The extraction procedure yielded translocations as double heterozygotes with the balancers CyO and TM6. Balancer-translocation stocks were then established by inbreeding the Cy Ubx $pr^+$ $cn^+$ $e^+$ flies. Stocks were then expanded for at least two generations. If any wild-type flies, lacking the markers Curly and Ultrabithorax, were seen among the several hundred flies generated during this time, the translocation in that stock was judged homozygous-viable and was not used further in experiments. Only translocations in stocks that gave no wild-type flies were scored as lethals (a greater than 95% probability of detecting a viable wild-type with viability $\geq 1\%$, based on binomial expectations, relative to the balancer heterozygotes).

### *Translocation breakpoints and crosses*

Cy Ubx flies from each homozygous-lethal translocation stock were crossed to the pr cn; $e^{11}$ stock. Polytene chromosome preparations were made from the salivary glands of $F_1$ larvae, using a modification of Harshman's (1977) method. The positions of translocation breakpoints were determined with the aid of the

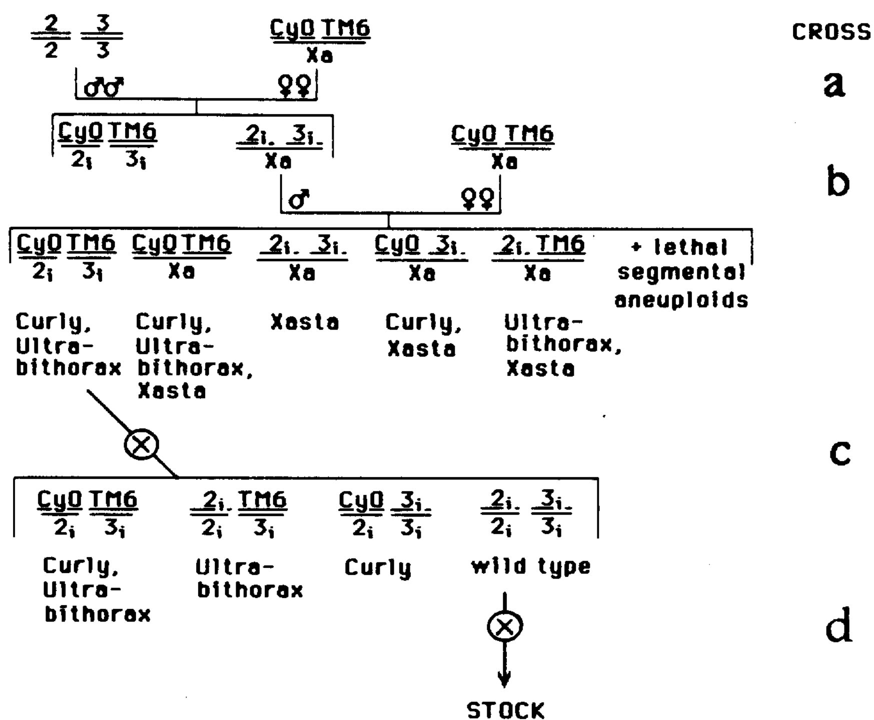


**Fig. 1.** Diagram of the detection and extraction of homozygous viable and fertile second and third chromosomes. Note that the use of a single male in cross *b* picks one each of chromosomes 2 and 3, as sampled by a gamete in cross *a* and carried with Xa. The four possible phenotypes in the progeny of cross *c* provide a test of homozygous viability of the extracted second and third chromosomes separately and together. In crosses where the two chromosomes were found to be homozygous viable in combination, siblings in that progeny class were mated to establish a double homozygous-viable chromosome stock (cross *d*)

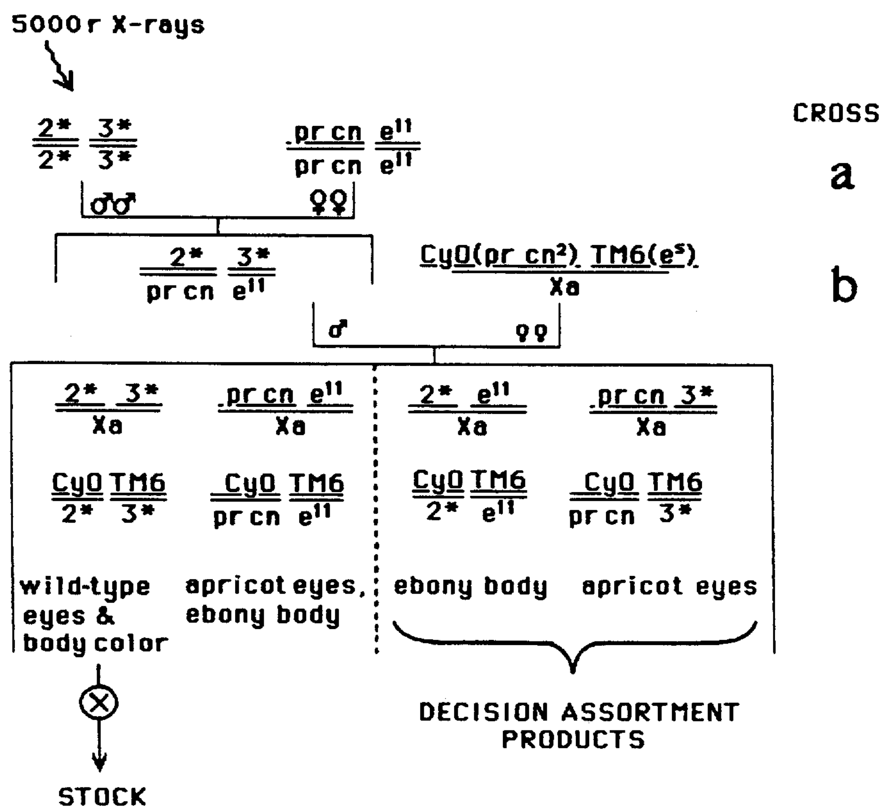


**Fig. 2.** Diagram of the detection and extraction of a 2–3 translocation from irradiated males. The *asterisks* indicate irradiated chromosomes. Note that the use of a single male in cross *b* picks one each of chromosomes 2 and 3, as sampled by a gamete in cross *a*. Among the non-Xasta offspring of cross *b*, the absence of the "decision assortment products" indicates pseudolinkage of e (=ebony body) and pr+cn (=apricot eyes), caused by a 2–3 translocation

photographs and chromosome maps of Lefevre (1976). Only simple reciprocal translocations were saved for further experiments.

*Experimental crosses*

Translocations were grouped by breakpoints. Those for which a breakpoint was not matched by a similar breakpoint (i.e., in the same or adjacent numbered salivary map region) in at least one other translocation were discarded.

Experimental crosses were established between euchromatic translocations with a matching breakpoint. For each pair, males of one translocation and virgin females of the other were placed together in a vial. Three lines were established by transferring the parents to fresh vials at 4 day intervals until they were discarded at day 12.

Adult $F_1$ flies were scored within 12 h of emergence as balancer-translocation heterozygotes (Cy/T) bearing Cy and Ubx as markers, or translocation-translocation heterozygotes (T/T), which were wild-type. The two classes of $F_1$ flies were separated and used to start two series of lines for each of the three initial replicate lines. The two series from each of the first two repetition groups (T/T1 and Cy/T1, and T/T2 and Cy/T2) were kept in vials, while those from the third (T/T3 and Cy/T3) were put into bottles. A discrete generation regime was established in which adults were put in a new container, allowed to oviposit for 1 week, then removed, and after 1 more week their progeny was transferred to a new container to repeat the cycle. The culture temperature was 22°–25 °C. These regimes were continued for 10 generations.

The numbers of Cy; Ubx and wild-type flies in the Cy/T lines were counted each generation. Flies in the T/T lines were simply transferred each generation.

After 10 generations, lines that showed evidence of possible improvement of translocation viability were examined cytologically and genetically as described in "Results."

## Results

*Lethality in U- and S-series flies before extraction of isochromosome lines*

The incidence of lethal chromosomes in the original U and S lines is summarized in Table 1. Counting the combination lethal "lethal 2+3," 11% and 21% of second and third chromosomes, respectively, were lethal overall. The frequency of lethals in line S did not differ significantly from that in line U ($X_1^2=0.095$, $P\approx0.35$).

*Generation of translocations*

Table 2 shows the results of irradiation and screening for 2–3 translocations. The rate of induction of 2–3 translocations and the proportion of homozygous lethal translocations among these (overall 68%) did not differ significantly between strains ($X_1^2=1.34$, $P\approx0.25$, and $X_1^2=0.59$, $P\approx0.45$, respectively). Fifteen U-series and 11 S-series lethal translocations were identified as showing a matching breakpoint with at least 1 other translocation. These translocations and their breakpoints are listed in Table 3. Hereafter, names of translocations are abbreviated to indicate only the identity of the matched breakpoint. For

**Table 1.** Numbers of homozygous viable and lethal (< 10% of expected wild-type offspring from cross c of Fig. 1) chromosomes 2 and 3 detected in 50 attempted extractions each from U and S lines; a "combination lethal" is a set of second and third chromosomes each of which is homozygous viable when the other is heterozygous, but which are lethal when both are homozygous (Wallace et al. 1966)

| | Viable 2+3 | Lethal 2 | Lethal 3 | Lethal 2+3 | Combination lethal |
|---|---|---|---|---|---|
| U-series | 27 | 3 | 7 | 0 | 1 |
| S-series | 23 | 6 | 4 | 1 | 0 |

**Table 2.** Results of irradiation of sperm with 5,000 rads of X-rays and screening for translocations. In addition to the translocations shown, eight and five T (Y; 2; 3)s were detected in lines U and S, respectively. These could not be tested for viability as they are inherited only through males

| | Sperm tested | T (2–3)'s | Lethal T (2–3)'s |
|---|---|---|---|
| U-series | 409 | 53 | 38 |
| S-series | 568 | 60 | 39 |

**Table 3.** Translocations used in experiments. Translocations are named according to the original wild-type strain and their breakpoints, which are designated using the numbering system of Bridges as interpreted by Lefevre (1976)

| Name | Breakpoints |
|---|---|
| U30 ; U100 | 2L (29F1–29F2) ; 3R (100 (E?)) |
| U38 ; U99 | 2L (38D–E) ; 3R (99A–B) |
| U2R-1 ; U62-1 | 2R (base) ; 3L (62F–63A) |
| U2R-2 ; U62-2 | 2R (base) ; 3L (64B) |
| U2R-3 ; U75-2 | 2R (base) ; 3L (75C2–C3) |
| U2R-4 ; U88-1 | 2R (base) ; 3R (88C–D) |
| U2R-5 ; U88-2 | 2R (base) ; 3R (88F–89A) |
| U41-1 ; U71-2 | 2R (41C–D) ; 3L (72D–E) |
| U41-2 ; U67-1 | 2R (41D) ; 3L (67D–E) |
| U49-1 ; U85 | 2R (49B2–B3) ; 3R (86C–D) |
| U49-2 ; U67-1 | 2R (49F–50A) ; 3L (66E–F) |
| U58 ; U71-1 | 2R (59E–F) ; 3L (71(E?)) |
| U2L ; U64 | 2L (base) ; 3L (64B) |
| U34 ; U75-1 | 2L (34F–35A) ; 3L (78B) |
| U45 ; U90 | 2R (45D–E) ; 3R (90A–B) |
| S30-1 ; S94 | 2L (29F–30A) ; 3R (94B–C) |
| S30-1 ; S67-1 | 2L (30A) ; 3L (67D–E) |
| S38-1 ; S75 | 2L (38C–D) ; 3L (75B–C) |
| S38-2 ; S84 | 2L (38D–E) ; 3R (84B–C) |
| S2R-1 ; S62 | 2R (base) ; 3L (62(A1–F?)) |
| S2R-1 ; S67-3 | 2R (base) ; 3L (67F–68A) |
| S2R-3 ; S71 | 2R (base) ; 3L (71(B–E?)) |
| S2R-4 ; S85 | 2R (base) ; 3R (85D–E) |
| S41 ; S67-2 | 2R (41E) ; 3L (67E–F) |
| S58-1 ; S64 | 2R (58E) ; 3L (64F–65A) |
| S58-2 ; S88 | 2R (58E–F) ; 3R (89B) |

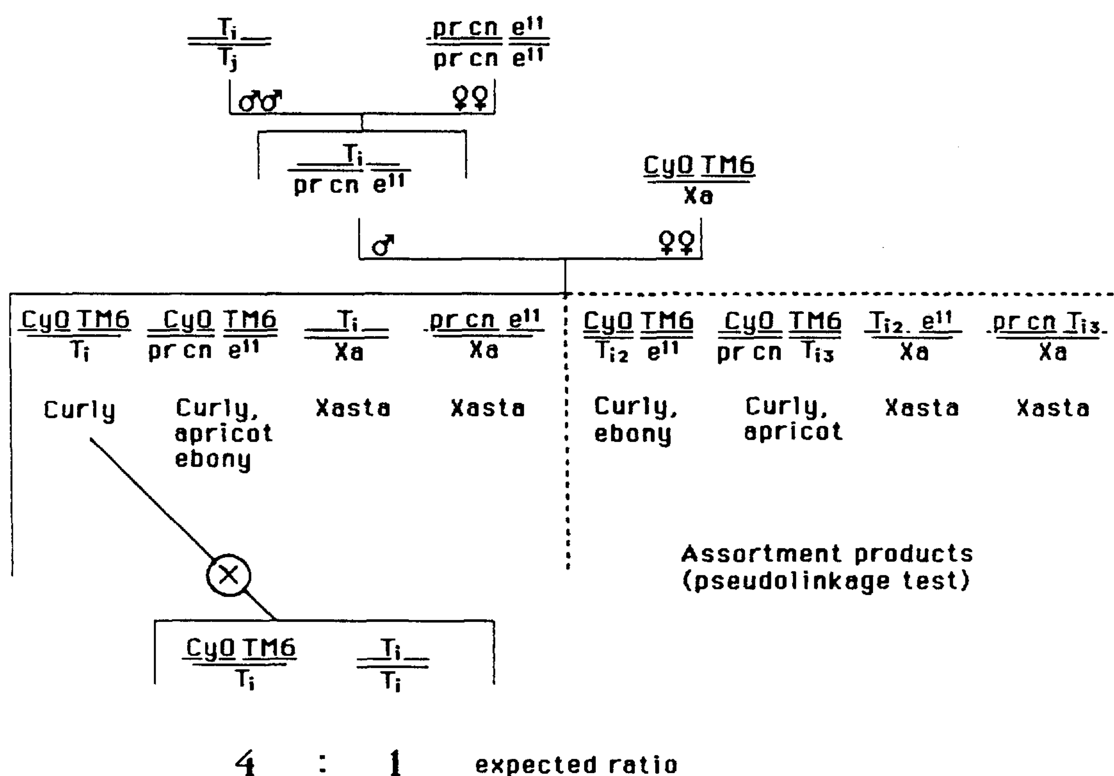


**Fig. 3.** Diagram of crosses to extract an individual translocation ($T_i$) and test its homozygous viability. $T_{i2}$ and $T_{i3}$ represent the second and third chromosomes of the extracted translocation. The absence of the *Cye* and *Cy* "apricot" assortment products confirms that the extracted chromosomes are indeed translocations. The 4:1 ratio in the final progeny is the expectation given translocations whose homozygous viability is equal to the viability of balancer heterozygotes and a 1:1 ratio of alternate to adjacent 1 assortment

example, cross U41-1; U71-2 × S41; S67-3 is between translocations with paired breaks near the centromere of chromosome 2 (at about map division 41), and the cross is called U41-1 × S41. Breaks in the centric heterochromatin are identified by the arm of the chromosome at the base of which they occur, e.g., U2R-1 is broken at the base of the right arm of chromosome 2.

*Relative viabilitites of U × U, S × S and U × S hybrids*

The expected proportion of translocation-translocation heterozygotes emerging from the initial crosses to set up the recombination experiments is 1/3, assuming equal egg-to-adult viabilities of the two balancer-translocation heterozygotes and the translocation-translocation heterozygote. Combining data from all three replicates, the mean proportions of wild-type offspring from the U × U, S × S and U × S initial crosses were 0.259 ($n$ = number of $F_1$ individuals scored = 5,504), 0.250 ($n = 6{,}252$) and 0.303 ($n = 18{,}310$), respectively. The first two proportions are not significantly different ($X^2_1 = 1.28$, $P \approx 0.25$), but the U × S viability differs significantly from the combined value for the other two ($X^2_1 = 20.75$, $P < 0.001$). U × S egg-to-adult viability averaged about 20% higher than U × U and S × S viability (0.303/0.254 = 1.19). This difference is presumably due to complementation in the hybrids of slightly deleterious genes fixed in the two original homozygous stocks U and S.

*Cy/T experiments – frequencies of wild-type flies and of homokaryotypic larvae*

The proportions of wild-type flies emerging over 10 generations in representative Cy/T lines are summarized in Fig. 4. Table 4 gives the number of lines that were in each of four frequency categories in the tenth generation. The frequencies of polymorphism and of fixation of wild-type (i.e., classes other than "lost" and "$f = 0, \approx 0$") were significantly higher among inter-series crosses (U × S) than among intra-series (U × U and S × S) crosses. The category "$f = 0, \approx 0$" probably represents lines fixed for one balancer-translocation heterozygote that produces a low percentage of weak homokaryotypes each generation. As such, these lines may represent cases of improvement of viability of a translocation (from lethal to occasionally viable), but are considered here as having remained lethal.

The numbers of lines in the different frequency categories from replicates 1 and 2 (vials) are not significantly different ($X^2_4 = 8.58$, $0.05 < P < 0.10$), and have been pooled. However,

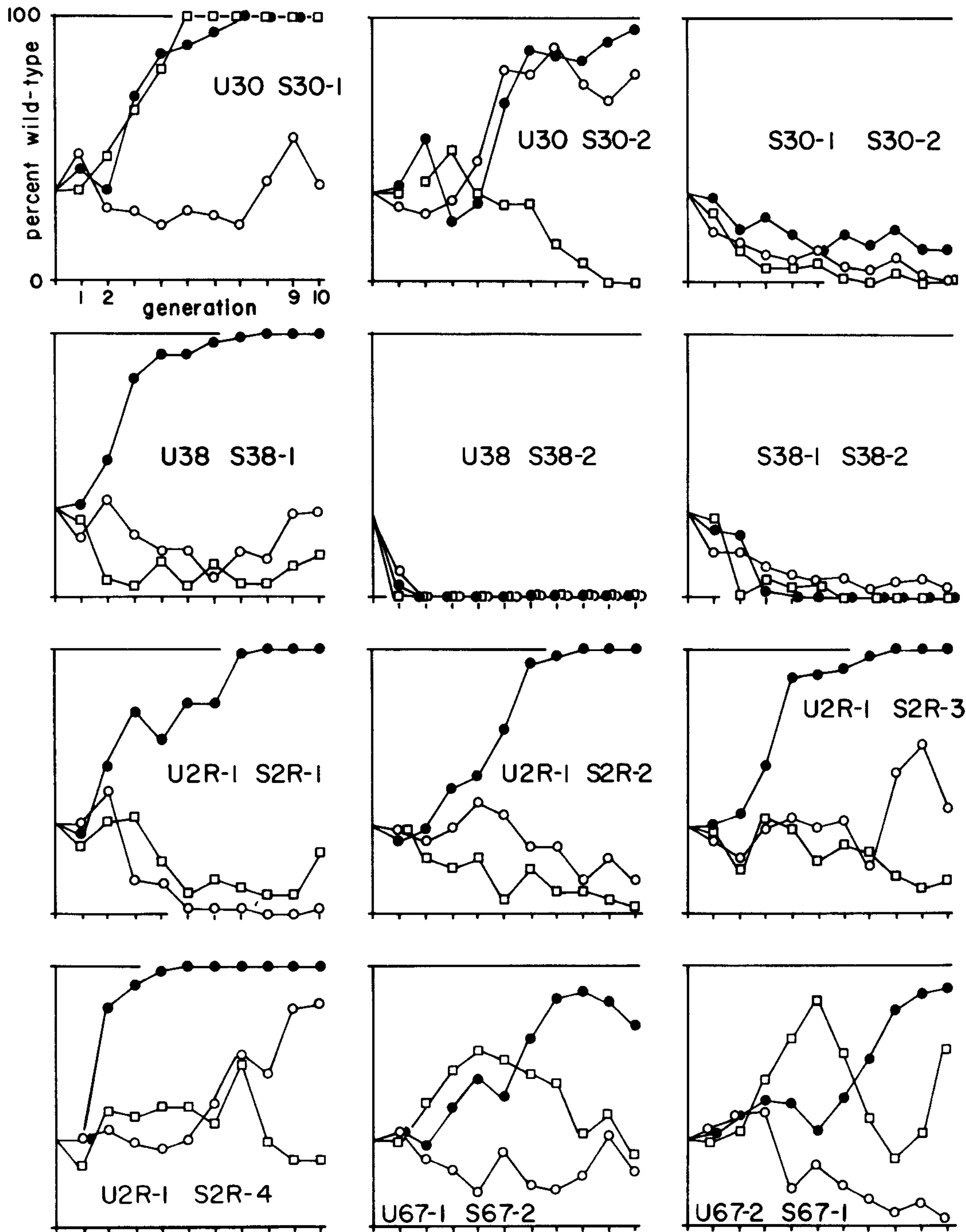


**Fig. 4.** Frequencies of wild-type flies in each generation in representative crosses of the Cy/T experiments. Each cross was established as three replicates. The ordinate in each case shows frequency between 0% and 100%; the abscissa is marked in generations from 0 to 10. Lines 1 (indicated by *open circles*) and 2 (*open squares*) were kept in vials; lines 3 (*dark circles*) were kept in bottles

their combined counts differ substantially from those of replicate 3 ($X_5^2 = 74.96$, $P < 0.001$). In bottles, wild-type flies had a relative advantage over Cy, Ubx flies. This does not seem to be due to an egg-to-adult viability component of differential fitness, since such an effect would have been manifested as differential emergences in vials and bottles of wild-type adults from the initial crosses. Among the progenies of the initial cross the total wild-type emergence from vials was $28.17 \pm 0.68\%$ (mean $\pm$ s.e., $n = 140$) and from bottles was $28.69 \pm 0.99\%$ ($n = 69$). These proportions are not significantly different ($t = 0.44$, $P \gg 0.5$), so that egg-to-adult viability does not seem to differ between bottles and vials. However, it is known that Curly-winged adult flies "tend to get mired more easily than wild type flies" (Erk 1955, p 338). The bottles were provided with a piece of tissue paper inserted

**Table 4.** Numbers of lines in different frequency categories in the tenth generation of the Cy/T lines. The lines are grouped by type of original cross; the two vial replicate crosses are pooled. "$f$" is the percent of wild-type flies in the progeny of a line. The frequency class "$f=0, \approx 0$" includes those lines with fewer than 5 wild-type individuals (among more than 50 total) in generations 9 and 10 combined. The "lost" category represents lines that died out during the course of the experiments

| | | lost | $f=0$, $\approx 0$ | $0<f<50$ | $50 \leq f<100$ | $f=100$ |
|---|---|---|---|---|---|---|
| Reps 1 & 2 | U×U | 0 | 30 | 2 | 0 | 0 |
| (vials) | S×S | 0 | 11 | 2 | 0 | 0 |
| | U×S | 0 | 17 | 47 | 15 | 5 |
| Rep 3 | U×U | 9 | 6 | 0 | 0 | 1 |
| (bottles) | S×S | 2 | 7 | 3 | 0 | 0 |
| | U×S | 5 | 4 | 0 | 7 | 26 |

**Table 5.** Fractions of chromosome extractions that had more than 5% viable homozygous adults from the last cross of Fig. 3; this is interpreted as ≥10% viability relative to the balancer-translocation heterozygotes. Data for lines are grouped by cross. When sufficient viable homozygotes were present within an extraction males and females were crossed; five extractions, in two lines, were fertile in such crosses [indicated by *asterisks* (*)]. The translocation they contained is identified in *parentheses*

| Line | | ≥10% viable |
|---|---|---|
| (1) U30 | S30-1 | 0/10 |
| (2) U30 | S30-1 | 0/10 |
| (1) U2R-1 | S2R-1 | 3/10 |
| (2) U2R-1 | S2R-1 | 4/10 |
| (3) U2R-1 | S2R-1 | 2/10* (U2R-1) |
| (3) Cy/U2R-1 | Cy/S2R-1 | 1/8 |
| (1) Cy/U2R-1 | Cy/S2R-3 | 0/10 |
| (1) Cy/U2R-1 | Cy/S2R-4 | 2/10 |
| (3) U2R-2 | S2R-3 | 0/9 |
| (3) U2R-3 | S2R-1 | 6/9 |
| (3) Cy/U2R-3 | Cy/S2R-1 | 7/11 |
| (3) Cy/U41-1 | Cy/S41 | 0/10 |
| (1) U67-2 | S67-2 | 0/10 |
| (2) S67-1 | S67-2 | 5/9**** (all S67-1) |
| (2) U71-2 | S71 | 0/5 |
| (3) U71-2 | S71 | 1/10 |

into the medium, and thus had a very large, convoluted, moist interior surface compared to the vials, which did not have a paper insert. Therefore the apparent selective advantage of wild-type flies in bottles may be ascribable to differential selection against Curly-winged adult flies in the bottle environment.

Among 51 lines (representing 32 crosses) that showed tenth generation fixation or a high frequency (>50%) of wild-type flies, larval salivary gland squashes showed five different crosses with at least one homokaryotype among two (or more) slides made from each of the lines. One line (replicate 3 of Cy/U2R-1 × Cy/S2R-1) of these five was apparently fixed for a hyperploid assortment product of the two translocations, since 10/10 larvae sampled carried it (see below under "Pseudohomokaryotypes"). In two other crosses U2R-1 was detected as homokaryotypes. The remaining two crosses of the five showed heterokaryotypes for the two translocations with which the crosses were made, as well as one of the two possible homokaryotypes in each case (U2R-3 in one case, U41-1 in the other). No lines showed homokaryotypes for both translocations, nor were different translocations detected as homokaryotypes in different lines from the same cross. Thus, 3 of the initial 26 translocations were detected as homokaryotypes at least once in the Cy/T experiments.

*T/T experiments – homokaryotype larvae*

For the T/T replicates that survived 10 generations, 2 or more larval salivary gland preparations were made from 105 lines (representing 43 crosses), and a single preparation was made from an additional 17 lines (4 more crosses). Among these, 13 lines (from 8 crosses) were found to have at least 1 homokaryotypic larva. Three of these were from crosses that also had homokaryotypes in the Cy/T experiments (U2R-1, U2R-3, U41-1), and one line (2) (U2R-1 × S2R-1) was apparently fixed, since 10/10 larvae sampled were U2R-1. Eight of the 26 initial translocations were thus detected as homokaryotypes in the T/T experiments, including the 3 that had been detected in the Cy/T experiments.

For the Cy/T and T/T experiments combined, the mean number of larvae scored per cross was $10.8 \pm 1.0$ (mean ± s.e., $n=47$). Overall, 10/47 (21%) of the original crosses yielded at least one homokaryotypic larva; these represent 8/26 (31%) of the initial translocations.

*Chromosome extractions*

Between 8–10 chromosome sets were extracted (Fig. 3) from each of the 16 lines that produced homokaryotypic larvae (representing all 10 such crosses). This procedure provided a test of homozygous viability to adulthood of the extracted translocations. The results are summarized in Table 5. Lines are categorized as "≥10% viable" if the frequency of wild-type adult offspring was equal to or greater than 5% among the total (generally more than 200) scored. Depending on the ratio of alternate to adjacent 1 disjunction and other processes not measured in the experiments, this criterion represents an actual viability relative to the balancer-translocation heterozygotes of probably 10%–25% (see "Discussion"). The proportions of such lines were not significantly different between the Cy/T and T/T experiments (3/5 for Cy/T; 6/11 for T/T; $X_1^2=0.05$, $p \approx 0.5$). Of the ten crosses that yielded homokaryotypic larvae, five gave at least one extraction with

≥10% viability among the offspring; these probably represent four of the eight translocations detected, namely U2R-1, U2R-3, S67-1 and U71-2. [This could only be checked in one extraction from (3) U2R-1 S2R-1 and four from (2) S67-1 S67-2, as only these extractions were sufficiently viable and fertile to give larvae for analysis. The former was U40-1, the latter four were all S67-1. These are the same translocations found as homokaryotypes in the screening prior to extractions]. Thus, of the 47 crosses tested, 5 (11%) gave at least 1 extraction with a translocation of ≥10% viability at the end of the 10 generations of the experiment; this represents improvement in viability of 4 (15%) of the 26 initial translocations.

The original shelf stocks of U40-1 and S67-1, in which the initially homozygous lethal translocations were carried with the balancers CyO and TM6, were scrutinized several months after the experimental period was over. At that time, among more than 200 balancer heterozygotes emerging, no wild-type flies were detected in either stock, indicating that the translocations that had not undergone the recombination regime were still homozygous lethal.

## Discussion

### *Lethality of translocations*

Thirty-eight of the 53 U-series and 39 of the 60 S-series translocations were homozygous lethal, giving a mean lethality of 68% among 2–3 translocations generated by 5,000 rads of X-rays. The criterion used here for homozygous lethality was that no wild-type flies emerge among several hundred adults during the expansion of the balancer-translocation stock (i.e., <1% viability relative to the balancer-translocation heterozygote with 95% confidence, binomial expectation). This is a more rigorous criterion of lethality than that used in many other studies; typically, <10% viability relative to the balancer heterozygote is scored as lethality. Extrapolation from available data generated at lower radiation dosages (Ytterborn 1970; Ives 1972; Sobels 1972; Reid and Wehrhahn 1976; B. Leigh, personal communication) suggests that at 5,000 rads more than 80% of 2–3 translocations should be homozygous lethal by a <10% relative viability criterion (Boussy 1982), which is probably consistent with my figure of 68% by a <1% criterion.

### *Apportioning lethality to causes and estimating improvability*

What proportion of the lethality of the translocations is due to the translocations themselves, and what proportion is due to point mutations or small deletions not at the translocation breakpoints? There are few published data on rates of generation of recessive lethal mutations for autosomes, but reasonable extrapolation can be made from data for sex-linked (X-chromosome) recessive lethals. From the data of Timofeeff-Ressovsky, Lea (1956) derived the relationship

$$P_x = 1 - e^{-2.89 \times 10^{-5} D},$$

where D is the dose of (X- or gamma-) radiation in rads and $P_x$ is the probability of a sex-linked lethal mutation. The data of King (1947), Edington (1956, 1958) and Sobels (1969) agree well with this model.

In *D. melanogaster*, each of the four arms of the two autosomes is roughly equivalent in euchromatic length to the X chromosome. If recessive lethals are generated at random and at the same rate per length of chromosome in the autosomes as in the X, the rate of generation of lethals for chromosomes 2 and 3 combined ($P_{2+3}$) should be

$$P_{2+3} = 1 - e^{4(-2.89 \times 10^{-5} D)} = 1 - e^{-11.6 \times 10^{-5} D} \quad (1)$$

The predictions of this equation are in reasonable agreement with the data of Ives (1972), who measured the frequencies of lethals among chromosome sets that had been irradiated but in which a translocation had not been generated. This can be interpreted as a direct estimate of the rate of generation of autosomal recessive lethals. Furthermore, a similar extrapolation from sex-linked lethals for the second chromosome alone is in reasonable agreement with the data of Wallace (1951). From Eq. (1), at 5,000 rads the expected frequency of at least one lethal among a 2–3 autosome set is 44%.

If L is the total lethality among translocations, and we suppose that lethality due to the translocations themselves and lethality due to recessive lethals are independent, then

$$L = l + t - l \times t \quad (2)$$

where l = frequence of recessive lethals, t = frequency of translocations with lethal breakpoint effects, and l × t = frequency of co-occurrence of the two sources of lethality. From Eq. (2), rearrangement gives

$$t = \frac{L - l}{1 - l} \quad (3)$$

The proportion of translocations that are lethal due solely to recessive lethals not associated with the breakpoints is

$$I = \frac{L - t}{L} \quad (4)$$

which is a measure of "improvability", the proportion of translocations that should be amenable to improvement by recombination with other chromosomes. Substitution of Eq. (3) into Eq. (4) and rearrangement gives

$$I = \frac{l(1 - L)}{L(1 - l)} \quad (5)$$

If L = 80% and l = 44% [from Eq. (1)], then from Eq. (5), I = 20% is the expected frequency of translocations generated at 5,000 rads that are amenable to improvement by recombination.

Lefevre (1974) measured the lethality of individual rearrangement breakpoints in *Drosophila melanogaster* subjected to 2,000–3,000 rads of X-rays. Among 37 euchromatic breakpoints of inversions that were originally detected in lethal chromosomes, 24 (65%) breakpoints could be shown to be non-lethal. Among 51 translocation breakpoints tested, 17 (33%) were non-lethal. Without resolving the discrepancy between inversion and translocation breakpoint lethalities, he concluded that, on average, about 50% of breakpoints generated with 2,000–3,000 rads of X-rays would be non-lethal. Then $0.5^2 = 0.25$ of lethal translocations generated with 2,000–3,000 rads of X-rays would be expected to be amenable to improvement of viability, since their lethality would not be at the breakpoints and thus inextricably linked to the translocations. It is not clear how to extrapolate the proportion of non-lethal breakpoints from 2,000–3,000 to 5,000 rads, but the improvability of translocations generated at the higher dose might be expected to be somewhat lower than the 25% expected at 2,000–3,000 rads, and thus Lefevre's data seem consistent with the estimated improvability of 20% calculated above.

From this theoretical basis we can interpret the data presented here. The larval salivary gland analysis reported here indicated that 21% of crosses, representing 15% of the initial translocations, had some survivorship to late third instar of homozygous translocations. However, the initial criterion of viability was survival through metamorphosis to adulthood, and the incidence of homokaryotypic larvae was not determined prior to the 10 generation recombination experiment. The larval survival results thus provide only an upper limit to the fitness improvement of the translocations. They also provided a preliminary screening for the next step in the analysis – assaying homozygous viability of adults by chromosome extraction.

In the absence of viability effects and if assortment is 1 : 1 for alternate and adjacent 1 disjunction, it is expected that 20% of the adults resulting from the final cross of the extraction procedure will be T/T (see Fig. 3). Since there is a preponderance of alternate disjunction from many translocation heterozygotes (Burnham 1962), the expected proportion of T/T offspring may approach 50%, but is usually lower (and is bounded by 20%). Therefore crosses with > 5% wild-type adults among the offspring represent an actual level of viability of the homozygous translocations of 10%–25% (depending on the above considerations) relative to the balancer-translocation heterozygotes, and this is indicated as "≥ 10%" in Table 5. This is a conservative estimate of the proportion of crosses in which the viability of a translocation was significantly improved over the original homozygous lethal state.

Five out of 47 crosses (11%) provided evidence of improvement in viability (from zero to ≥ 10%) during the course of the experiments. Of the 26 translocations with which the experiments were started, 4 (15%) were actually represented among the homokaryotypes found at the end of the experiments. This is probably an underestimate of improvability, due to small sample sizes in the screening procedures and the conservative criterion of viability used. It seems likely that 20% improvability (as argued on theoretical grounds above) is a reasonable expection for autosomal translocations generated in *D. melanogaster* at 5,000 rads.

It should be noted that improvability here means an increase from lethal to non-lethal. The ultimate goal of a practical project with real pest species is improvement from non-competitive translocations to competitive ones. The above results and calculations for lethals, then, represent a model for how non-lethal effects might also be apportioned between intrinsic effects of the translocation and independent mutations for any traits. The experiments reported here constitute a direct demonstration that the theory for apportioning lethal effects is correct to a reasonable approximation.

*Pseudohomokaryotypes*

McDonald and Asman (1982) defined "pseudohomozygotes" as heterokaryotypes between translocations with nearly identical breakpoints. They hypothesized that such heterokaryotypes would produce segmentally aneuploid gametes with such small aneuploid regions that they would function normally, and that the benefits of hybridization between two strains would be conferred on the "pseudohomozygote" strain. In their attempts to derive such stocks in *Culex tarsalis*, however, they succeeded only in establishing a balanced polymorphism, with weak homokaryotypes kept together in the stock by the genic advantage of the heterokaryotype, in spite of its meiotic disadvantages.

Among my crosses are six with both breakpoints in one translocation nearly matching those in the other (differential segments of size less than three of Bridges's numbered regions). I call these "pseudohomokaryotypes," which is more explicit than "pseudohomozygotes" and does not imply genic homozygosity. Table 6 lists them and the results of the experiments described above.

Chromosome slides were made from five of the six pseudohomokaryotype crosses. Four of the crosses showed some homokaryotypes, and one had lines that were apparently fixed for one translocation (U2R-1). Extractions were performed on lines from three of the six crosses, and two of these had one or more extractions with > 10% viable adults. These numbers are too small to show significant differences from the proportions among other translocations. In the Cy/T series, if the pseudohomokaryotypes were strongly favored, they should have been able to out-perform the balancer heterokaryotypes and the proportion of wild type flies should have been high. It was high in only one line [(3) Cy/U2R-1 Cy/S2R-1], indicating that the other five crosses, and the other two replicates of Cy/U2R-1 Cy/S2R-1, did not behave as the arguments of McDonald and Asman (1982) suggest they should have.

A careful re-examination of the chromosomes of (3) Cy/U2R-1 Cy/S2R-1 showed 10/10 slides to be carrying a hyperploid assortment product of the two translocations U2R-1 and S2R-1 [duplicated region uncertain; the hyperploid homozygote apparently eliminates a possible small deletion in S2R-1 (62A1–62F?)]. Hyperploids were also detected as heterozygotes (segmental trisomics) in lines (2) U67-2 S67-2 and (3) U67-2 S67-2, and in (2) U71-2 S71 and (3) U71-2 S71. In (3) U71-2 S71, both hyperploid combinations of the two translocations were found [both combinations are trisomic for regions 40–41C and 71(B–E?)–72D].

Hyperploid assortment products were also seen as heterozygotes (trisomics) in the nearly pseudohomokaryotypic crosses

**Table 6.** Pseudohomokaryotype crosses, their breakpoints, and the results of the experiments for their replicates. "$f$" is the frequency of wild-type flies in generation ten of the Cy/T experiments; "Ho", "Het" and "Hyper" are homokaryotypic, heterokaryotypic and hyperploid larvae, respectively. The last column shows the proportions of extractions with more than 5% homozygous viable wild-type flies in the last cross of Fig. 3, which is interpreted as ≥10% viability relative to balancer-translocation heterozygotes

| Cross | Breakpoints | Line | | $f$ | slides | ≥10% viable |
|---|---|---|---|---|---|---|
| U2R-1 | 2R (base); 3L (62F–63A) | T/T | rep 1 | | 9 Ho (U2R-1)<br>1 Het | 3/10 |
| × | | | | | | |
| S2R-1 | 2R (base); 3L (62A1–62F) | | rep 2 | | 10 Ho (U2R-1) | 4/10 |
| | | | rep 3 | | 1 Ho (U2R-1) | 2/10 |
| | | Cy/T | rep 1 | 0.02 | | |
| | | | rep 2 | 0.23 | | |
| | | | rep 3 | 1.00 | 10 Hyper | 1/8 |
| U2R-4 | 2R (base); 3R (88C–D) | Cy/T | rep 1 | 0 | | |
| × | | | rep 2 | 0 | | |
| U2R-5 | 2R (base); 3R (88F–89A) | | | | | |
| U67-2 | 3L (67D–E); 2R (41D) | T/T | rep 1 | | 1 Ho (U67-2)<br>8 Het<br>2 Hyper | 0/10 |
| × | | | | | | |
| S67-2 | 3L (67E–F); 2R (41E) | | rep 2 | | 2 Het<br>1 Hyper | |
| | | | rep 3 | | 2 Het | |
| | | Cy/T | rep 1 | 0.17 | | |
| | | | rep 2 | 0 | | |
| U67-2 | 3L (67D–E); 2R (41D) | Cy/T | rep 1 | 0 | | |
| × | | | rep 2 | 0 | | |
| S67-3 | 3L (67F–68A); 2R (base) | | rep 3 | 0 | | |
| S67-2 | 3L (67E–F); 2R (41E) | T/T | rep 3 | | 3 Het | |
| × | | Cy/T | rep 1 | 0.05 | | |
| S67-3 | 3L (67F–68A); 2R (base) | | rep 2 | 0 | | |
| | | | rep 3 | 0 | | |
| U71-2 | 3L (72D–E); 2R (41C–D) | T/T | rep 1 | | 4 Het | |
| × | | | | | | |
| S71 | 3L (71B–E); 2R (base) | | rep 2 | | 1 Ho (U71-2)<br>8 Het<br>5 Hyper | 0/5 |
| | | | rep 3 | | 2 Ho (U71-2)<br>6 Het<br>3 Hyper | 1/10 |
| | | Cy/T | rep 1 | 0.16 | | |
| | | | rep 2 | 0.14 | | |

U2R-1 S2R-2, U2R-2 S2R-3, and S2R-2 S2R-3 [in which the differential segments are three to eight chromosomal map units (Lefevre 1976)]. For all the above lines, the complementary hypoploid assortment products (segmental monosomics) were not seen among the small samples of chromosomes examined.

The hyperploid combinations of chromosomes did not seem to be particularly fit relative to balancer heterozygotes (as demonstrated by their not performing better than did other translocation pairs in the Cy/T tests). While not performing exactly as predicted for pseudohomokaryotypes, the above lines showing hyperploid chromosome sets do demonstrate that certain kinds of chromosome manipulations might be feasible in the quest for vigorous translocation stocks. In the cases cited in which hyperploid assortment products were present, it seems likely that deleterious or lethal effects of a breakpoint of one translocation were complemented by the unbroken segment of the paired chromosome, yielding an advantage relative to either homozygous translocation. The construction and use of such stocks might be a way to ameliorate deleterious effects at the breakpoints of translocations that could not be removed by recombination. However, a hyperploid heterokaryotype stock will always have an "assortment load" (analogous to segregational load at a single locus), due to the assortment of the paired translocated chromosomes. The assortment load might reduce the fitness of a stock enough that an unstable equilibrium point, above which a mixed population with wild chromosomes would go to fixation of the translocation, would not exist or would be unattainable in the field (e.g., >95%). This would have to be independently evaluated in each case.

Lindsley et al. (1972) showed that in *D. melanogaster* about one-half of tested segmental hypoploidies were viable when the heterozyous deficiency was 1/100th or less of the euchromatic genome. Among hyperploidies, however, evaluated as trisomics for chromosomally terminal segments of different sizes, trisomy for about 500 polytene chromosome bands (about 1/10th of the euchromatic genome) resulted in about 50% viability. The observed hyperploids in the present experiments were trisomic for from <1% to about 7% of Bridges's numbered regions; as noted above, no hypoploids were observed. Together with the results of Lindsley et al. (1972), these results suggest that, for a

pseudohomokaryotype to have a good chance of being hyperploid viable, the differential segments between its breakpoints must total less than about 5% of the euchromatic genome.

*General considerations*

If one generates a translocation from a random stock and then makes it homozygous, the resulting line has a high probability of being homozygous for deleterious and lethal alleles. The mean frequency of lethal autosomes in *D. melanogaster* found among extractions from wild populations in a large number of studies is about 25% [summarized in Simmons and Crow 1977; more recent estimates are in agreement (Seager and Ayala 1982)]. Thus, at least a quarter of second and of third chromosomes can be expected to be lethal in wild *D. melanogaster*, and $(1-0.25)^2=56\%$ is the maximum expected proportion of viable autosome sets (seconds + thirds) that can be expected to be extracted from a population. For some populations this is an optimistic expectation: Wallace et al. (1966) found that 79.8% of the autosomal genomes they extracted from populations of *D. melanogaster* from Bogotá, Colombia, were homozygous drastic (lethal and semilethal). This means that translocations generated in genomes derived from natural populations without screening will be, on average, of low homozygous fitness, due to naturally occurring lethal and deleterious alleles.

The accumulation of deleterious and lethal mutations in a line can also be a problem. The 18% lethality per chromosome of initial extractions from the S-stocks is of interest as it represents lethals that have accumulated in four and a half years (probably some 120 generations) in an initially lethal-free stock. The line O72 (from which the S-series was extracted) had not been kept at large population size during the approximately 120 generations since its origin, so there should have been some inbreeding loss of lethals and reduced opportunity for mutation, relative to the expectation for a large population. Wallace (1950, 1951, 1956) and Wallace and King (1951) reported a similar level of accumulation of lethals on the second chromosome of *D. melanogaster* in a laboratory cage population started with lethal-free chromosomes. After 60 generations the frequency of lethals in their experiment reached an approximate equilibrium at around 28%. The expectation is thus that lines will accumulate lethals over time.

In most species it is not possible to establish lethal-free lines, although inbreeding procedures can select for lines with reduced homozygous lethality. However, inbreeding also reduces useful variability and can decrease fitness due to "inbreeding depression" on many traits (Robinson 1977a; see below). If the level of background lethality in *D. melanogaster*, whether accumulated in the lab or from a natural population, is typical of other species [as it seems to be: Sakai and Baker (1971) found 31.2% lethality among single chromosomes extracted from a wild population of *Culex tritaeniorhynchus*], then the probability of deriving a viable translocation from chromosomes chosen at random will be generally low, depending on their sizes. It is not surprising that the only successful population replacement experiments with chromosomal rearrangements were with *Tetranychus urticae* (Feldmann and Sabelis 1981), since in spider mites males are haploid, and much of the genome is subject to hemizygous selection against deleterious and lethal alleles every generation. However, the experiments described here show that 11% or more of newly generated lethality in a diploid species is amenable to improvement by allowing recombination to introduce viable alleles into the translocation stock. The same technique should be effective on background lethality.

It is worth noting that crosses between translocations generated in the two different backgrounds (U and S) generally fared much better than did crosses within U or S. Competitive abilities of translocation-translocation heterozygotes compared to balancer-translocation heterozygotes, shown in Table 4, are dramatically higher for the U × S crosses than for the U × U and S × S crosses, which corroborates the 20% higher egg-to-adult viability found in wild-type offspring of the initial U × S crosses, compared with the U × U and S × S crosses. This result is expected, as complementation between slightly deleterious alleles in the two isogenic stocks should improve fitness in the heterozygotes (heterosis). It underscores the intuitive notion that variability in a stock can be important, and justifies efforts to introduce variability into stocks raised for genetic control purposes.

Contrary to this notion, Robinson (1977a) has argued that, at least for some species, inbreeding may not be grossly deleterious. However, his own data for fitness traits of strains of *Hylemya antiqua*, the onion fly, show significant increases in egg, larval and adult mortalities with inbreeding. He calculated k values for the three life stages in eight sibmated sublines (k value $=\log N_1-\log N_2$, where $N_1$ = number of individuals entering a life stage and $N_2$ = number surviving that stage; Varley and Gradwell 1960). The overall increase in k value from the control (parental) population to the mean of the third generation of sibmating was from 0.43 to 0.89; this corresponds to a drop in overall survivorship from egg to adult from 37% before inbreeding to only 13% afterward. All else being equal, such a decrement in fitness could strongly affect the outcome of field releases.

The procedures essential to the success of the process of controlled recombination used here are: (1) the establishment of crosses between translocations, rather than to wild-type chromosomes; and (2) the use of translocations with nearly matching breakpoints for the crosses. The first allows extended opportunity for recombination without loss of one or the other translocation. The second prevents the reconstruction by recombination of a wild-type chromosome in the mixed population. Despite observations by Roberts (1970, 1972) that translocations in *D. melanogaster* can strongly impede recombination, de-

pending upon the locations of the breakpoints in the chromosome arms, a significant proportion (11%) of the crosses described here showed improvement of viability of one of the translocations involved in the cross in only 10 generations. Overall, of the 26 translocations with which crosses were started, four (15%) showed improvement in viability in at least one cross. This improvement was most clearly demonstrated by two translocations (U2R-1 and S67-1) that were both homozygous viable and fertile at the end of the experiments, while the same translocations kept as balanced stocks were still completely inviable. I have demonstrated here improvements in egg-to-adult viability; there is no a priori reason why other components of fitness should not be as amenable to improvement by recombination.

Much of the work described here was only possible through the choice of *Drosophila melanogaster* as the experimental organism (Fitz-Earle and Holm 1983). However, the critical technique of matching of translocation breakpoints (and the possible identification of pseudohomokaryotypes) should be applicable to any species that has easily observed polytene chromosomes (this includes many Dipteran pests). Many pest species have more chromosomes than *D. melanogaster*, and other differences, such as recombination in males as well as females. In such cases, assortment of the additional chromosomes not involved in the translocations and the increased total incidence of recombination will assist the transfer of favorable alleles into a translocation stock, and the smaller proportion of the genome that each chromosome comprises will lessen the likelihood of its bearing lethal and deleterious mutations. The approach outlined above may thus be useful in improving the fitness of translocation stocks in many pest species.

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