

# SPONTANEOUS UNEQUAL EXCHANGE IN THE ROSY REGION OF *DROSOPHILA MELANOGASTER*

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Manuscript received January 30, 1979

## ABSTRACT

A selective system for recovery of exchanges between *trans* mutations at adjacent loci, *l(3)S12* and *rosy*, is described. In addition to the expected cross-over and conversion classes, two exceptional types of offspring were recovered. Triploid offspring arose as 0.01% of all zygotes; the diploid chromosome set was apparently of maternal origin. Nine tandem duplications derived from unequal exchange between nonsister homologues were recovered among  $2.25 \times 10^6$  zygotes screened. From considerations of the proportion of the genome that was assayed in this system, and on the assumption that the rate of unequal exchange observed is typical for the genome as a whole, it appears that one unequal exchange occurs per 500 female meioses in *Drosophila*.

SELECTIVE techniques for recombinational analysis in *Drosophila* have become increasingly popular as a tool for studying gene organization. Common to all these techniques is the generation of a recombinant class that is viable under circumstances in which virtually all other offspring die before reaching adulthood. In this way many more offspring can be screened efficiently than without any selective device. The selective screening of large populations can lead to the observation of unusual meiotic events so infrequent as to escape detection normally.

During the fine-structure mapping of a putative xanthine dehydrogenase regulatory element (CHOVNICK *et al.* 1976), one series of crosses afforded an opportunity to detect some of these unusual events. In particular, triploid and partially trisomic individuals (these latter bearing tandem direct duplications of the rosy region) were recovered at unexpectedly high frequencies. In this report, we describe the nature of the exceptional events in detail.

## MATERIALS AND METHODS

Flies were reared under normal conditions in half-pint milk bottles on cornmeal-sucrose-yeast extract *Drosophila* medium. The mutations used in this study are described in Table 1. All mutations except those marked with an asterisk are described in detail in LINDSLEY and GRELL (1968). Polytene chromosome analysis was performed on larval salivary glands, which were dissected in 45% acetic acid and stained in 2% orcein in lactoacetic acid. All squashes

<sup>1</sup> Supported by Public Health Service research grant GM-23522.

<sup>2</sup> Supported by Public Health Service research grant GM-09886.

TABLE 1

*A brief description of the mutations and rearrangements used in this study*

Mutant designation	Phenotype	Chromosome 3 map position
<i>st</i>	scarlet eye color	44.0
<i>M(3)S34</i>	recessive lethal	44.3
* <i>Ch<sup>v</sup></i>	Chubby body	46.7
<i>Dfd</i>	Deformed eye	47.5
<i>cu</i>	curled wing	50.0
<i>kar</i>	karmoisin—bright red eye color	51.7
<i>kar<sup>2</sup></i>	like <i>kar</i>	
* <i>l(3)S12</i>	recessive lethal	52.0
* <i>ry<sup>402</sup></i>	rosy—brownish eye color	52.0
* <i>ry<sup>405</sup></i>	like <i>ry<sup>402</sup></i>	
* <i>ry<sup>406</sup></i>	like <i>ry<sup>402</sup></i>	
* <i>pic<sup>G231</sup></i>	recessive lethal	52.0
<i>red</i>	red Malpighian tubules, dark red eye color	53.6
<i>Sb</i>	Stubble bristles	58.2
<i>Ubx</i>	Ultrabithorax—enlarged halteres	58.8
<i>e<sup>4</sup></i>	ebony body color	70.7
Rearrangement		Cytological breakpoint
<i>Df(3R)ry<sup>27</sup></i>		Df(3R)87D1–2; 87F1–2
<i>Df(3R)ry<sup>75</sup></i>		Df(3R)87D1–2; 87D14–E1
<i>MKRS, M(3)S34 kar ry<sup>2</sup> Sb</i>		Tp(3)87E8–F1; 92E; 93C; 71B2–C2
new order of MKRS:		
61A–71B/92E–93C/87F–92E/71C–87E/93C–100F		

Those mutations marked with an asterisk (\*) are not listed in LINDSLEY and GRELL (1968).

were temporary and were examined under phase optics on either a Wild M20 or a Zeiss Universal research microscope.

*The selective system*

**Background:** The fine-structure mating described in this report were designed to localize the site responsible for a two-fold increase in XDH levels detectable in strains bearing the *ry<sup>+</sup>* isoallele (CHOVNICK *et al.* 1976). Preliminary mapping experiments indicated that the site responsible for this increased activity (designated *i409*) resided in the 0.3 map unit interval from karmoisin to rosy, but at or to the left of all markers in the XDH structural element. One possible interpretation of these data was that *i409* marks a control element adjacent to, but to the left of, the XDH structural element. To test this possibility, we constructed *i409N/ i409H* heterozygous females, and from their progeny selected crossovers between *l(3)S12*, the gene just to the left of rosy, and the XDH structural element. The designation *i409H* symbolizes the variant conferring increased XDH levels, and *i409N* represents its standard counterpart. The selected crossovers should include exchanges both to the left and to the right of *i409*. This result obtained, and these data have been described in detail by CHOVNICK *et al.* (1976). However, discussion of the other classes of offspring generated by these matings has been deferred until now.

**The mating protocol:** The mating scheme employed to generate these recombinants is pictured in Figure 1. *M(3)S34, Dfd, l(3)S12, Sb* and *Ubx* all act as recessive lethal mutations. Purine was added to the media in levels sufficient to kill all developing rosy individuals. Thus, in this mating, *ry* represents a recessive lethal lesion as well. Only *l(3)S12+ ry<sup>+</sup>* recombinants should be recovered in individuals derived from fertilization by sperm bearing a *cu kar Df(3R)ry<sup>27</sup> Ubx e<sup>4</sup>*

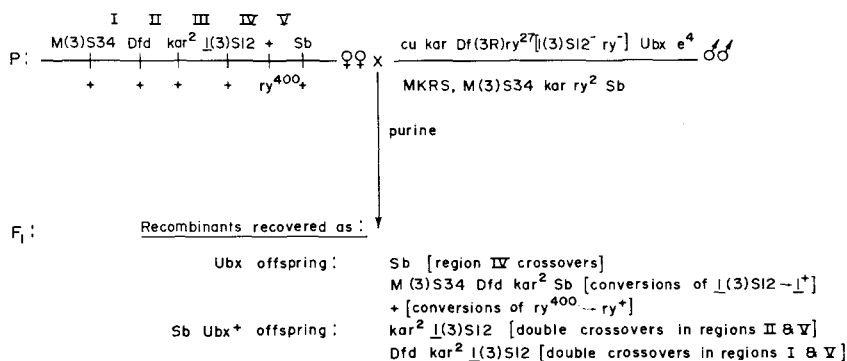


FIGURE 1.—The selective breeding protocol for recovering wild-type recombinants between  $l(3)S12$  and  $ry^{400}$ . Three  $ry^{400}$  alleles— $ry^{402}$ ,  $ry^{405}$  and  $ry^{406}$ —were utilized in separate experiments. The  $ry^{406}$  chromosome also carried  $pic^{G231}$ , a mutation in the locus just distal to *rosy*.  $Df(3R)ry^{27}$  is deleted for the *pic* locus as well as for  $l(3)S12$  and *ry*. The F<sub>1</sub> generation includes the viable offspring expected from symmetric recombination events. *Ubx* offspring carry the *cu kar Df(3R)ry<sup>27</sup> Ubx e<sup>4</sup>* paternal third chromosome, and *Sb Ubx<sup>+</sup>* offspring carry the *MKRS* paternal third chromosome.

chromosome. (Note that the  $ry^{406}$  chromosome also carried  $pic^{G231}$ ; viable offspring from mothers carrying this chromosome also had to be *pic<sup>+</sup>*.) *M(3)S34* and *Sb* were present on the  $l(3)S12$ -bearing chromosome in the females in order to eliminate most of the *MKRS* (and therefore  $l(3)S12^+$ ) bearing individuals. Only double crossovers of the type  $M(3)S34 + l(3)S12^+ ry^+$  *Sb<sup>+</sup>* were recovered as viable progeny when heterozygous with *MKRS*. Total offspring were estimated from control cultures to which no purine was added.

**Progeny tests:** All *Ubx* offspring were testcrossed to  $kar^2 Df(3R)ry^{75}/MKRS$  mates. Balanced stocks of all recombinants were constructed for further analysis. All recombinant stocks were analyzed for polytene chromosome rearrangements. Other matings are described in the text.

## RESULTS

Three crosses were performed using the protocol described in Figure 1. The results are summarized in Table 2. The  $l(3)S12$ -*ry* crossovers and conversions recovered in experiments A and B were described previously (CHOVNICK *et al.* 1976). Experiment C, which has not been previously reported, utilized a third *rosy* null mutation ( $ry^{405}$ ) induced on the  $ry^{+4}$  isoallele. The data pertaining to the mapping of *i409* derived from experiment C are consistent with the previously reported experiments A and B (GELBART and McCARRON, unpublished). As will be elaborated below, the survivors of this selective cross were of diverse origins. The original purpose of this cross was to generate crossovers and conversions within the  $l(3)S12$ -*ry* interval. It was clear that most of the *Sb Ubx<sup>+</sup>* offspring were not of the desired crossover or conversion classes and so were not analyzed further. Rather, as described in CHOVNICK *et al.* (1976), attention was focused on the *Ubx* offspring.

Of the estimated 2,250,000 offspring screened in this cross, 1793 survived to adulthood (0.1%). In addition to the  $l(3)S12$ -*ry* crossovers and conversions, the double crossovers in the  $M(3)S34$ -*ry* and *ry*-*Sb* intervals were recovered as expected. Because there is known to be high positive interference in adjacent

TABLE 2

*A summary of the classes of offspring that survived the selective system outlined in Figure 1*

Rosy allele tested		Viable offspring recovered						
Experiment number	Rosy allele	Cross-overs*	Conversions* <i>l(3)S12</i> → +	<i>ry</i> <sup>400</sup> → +	Independent tandem duplications	Triploids	Double crossovers	Zygotes screened
A	406†	11	0	—†	3	139	382	720,000‡
B	402	4	1	0	2	10	639	810,000
C	405	9	1	5§	4¶	56	525	720,000

Three independent experiments, involving three different *ry*<sup>400</sup> alleles, are described.

\* A detailed description of the crossover and conversion classes is in CHOVNICK *et al.* 1976.

† The *ry*<sup>406</sup> chromosome also carried *pic*<sup>G231</sup>. Because of the presence of this recessive lethal mutation and the deletion of this locus in *Df(3R)ry*<sup>27</sup>, the *ry*<sup>400</sup> → *ry*<sup>+</sup> conversion could not have been recovered.

‡ The previously reported sample size for cross A was 360,000 (CHOVNICK *et al.* 1976). However, this estimate neglected to take into account the inviability of *ry*<sup>406</sup> *pic*<sup>G231</sup>/*Df(3R)ry*<sup>27</sup> individuals, even in cultures to which no purine was added. This led to a 50% underestimate of sample size in this previous report.

§ This includes one cluster of two identical conversions arising from the same culture. They are counted as one event.

¶ This includes one cluster of three identical duplications arising from the same culture. They are counted as one event.

intervals on one chromosome arm, but no interference across the centromere (STEVENS 1936), we presume that most of the double crossovers in these three experiments actually involved one exchange in 3*L* proximal to *M(3)S34* and one exchange in 3*R* between *ry* and *Sb*. The numbers of double crossovers in the three experiments are proportional to the three estimates of sample size.

Two types of exceptional offspring were also encountered. Triploids were obtained at a frequency of one per 10<sup>4</sup> progeny. Included as triploids are true triploid females (3*X*, 3*A*), intersexes (2*X*, 3*A*) and metamales (1*X*, 3*A*). The intersexes and metamales were sterile, but some of the triploid females were weakly fertile. These females, upon testcrossing, generated offspring consistent with the presence of three third chromosomes, one of which was *kar ry*<sup>+</sup>, one *kar*<sup>+</sup> *ry*, and one *kar ry*. Thus, at least for the *kar-ry* region, these individuals contained two maternally derived nonsister homologues and one paternal third chromosome. The phenotypes and postulated genotypes of the two types of recovered triploids are presented in Figure 2. As noted in this figure, these two classes were distinguished by their *Sb* and *Ubx* phenotypes.

The other unexpected class of offspring was heterozygous for *Ubx* and was fully fertile and clearly diploid; however, assays of the XDH levels of the *crossover/kar*<sup>2</sup> *Df(3R)ry*<sup>75</sup> heterozygotes often produced values intermediate to the normal and increased activity strains. In addition, some individuals in this class had the opposite configuration of outside markers to those expected if they were standard *l(3)S12-ry* recombinants.

These conflicting data were resolved by cytological examination of polytene chromosomes of crossover/+ heterozygous larvae. In every case in which doubt was raised about the nature of a surviving chromosome, it turned out to possess a tandem direct repeat having arisen *via* maternal unequal exchange between

	M(3)S34	Dfd	kar <sup>2</sup>	l(3)S12	+	Sb	+
A.	+	+	+	+	ry <sup>400</sup>	+	+
	+	+	kar	-	-	+	Ubx
	M(3)S34	Dfd	kar <sup>2</sup>	l(3)S12	+	Sb	+
B.	+	+	+	+	ry <sup>400</sup>	+	+
	M(3)S34	+	kar	+	ry <sup>2</sup>	Sb	+

FIGURE 2.—The two basic types of triploids recovered among the F<sub>1</sub> of the cross outlined in Figure 1. Type A is characterized by weak expression of the Stubble and Ultrabithorax phenotypes, while type B has an extreme Stubble phenotype.

nonsister homologues. A total of nine independent tandem duplications were recovered in these experiments, and two others were found among progeny derived from small pilot experiments. The origins and outside markers carried by these duplicated chromosomes are listed in Table 3. One of these tandem duplications arose as a cluster of three individuals in one culture; in this case, the duplications are cytologically indistinguishable and are presumed to have arisen from one gonial event.

Six of the eleven duplications have been maintained in stock, and photomicrographs of *Dp(3;3)/+* polytene chromosome preparations of each are presented

TABLE 3  
*The origin, phenotype and inferred orientation of the tandem duplications recovered in these experiments*

Duplication designation	ry allele	Phenotype	Unequal exchange orientation*
121.3†	402	+	?
148.4	402	<i>Sb</i>	B
148.8	402	<i>Sb</i>	B
122.3‡	406	<i>Dfd</i>	A
124.3	406	<i>Dfd</i>	A
124.5	406	<i>Dfd</i>	A
124.6	406	+	?
149.4	405	<i>Dfd</i>	A
149.9	405	<i>Sb</i>	B
149.12	405	<i>Sb</i>	B
149.14‡	405	<i>Sb</i>	B

\* Orientation A would be *Dfd* [*kar*<sup>2</sup> *l(3)S12 ry*<sup>+</sup>] [*kar*<sup>+</sup> *l*<sup>+</sup> *ry*<sup>400</sup>] *Sb*<sup>+</sup>, orientation B would be *Dfd*<sup>+</sup> [*kar*<sup>+</sup> *l*<sup>+</sup> *ry*<sup>400</sup>] [*kar*<sup>2</sup> *l(3)S12 ry*<sup>+</sup>] *Sb*. Those individuals with a + phenotype are presumably unequal exchanges that simultaneously possessed a second exchange, either between *Dfd* and the duplication, or between the duplication and *Sb*.

† Recovered from pilot experiments in which sample size was not estimated.

‡ Recovered as a cluster of three individuals from one culture with identical phenotypes.

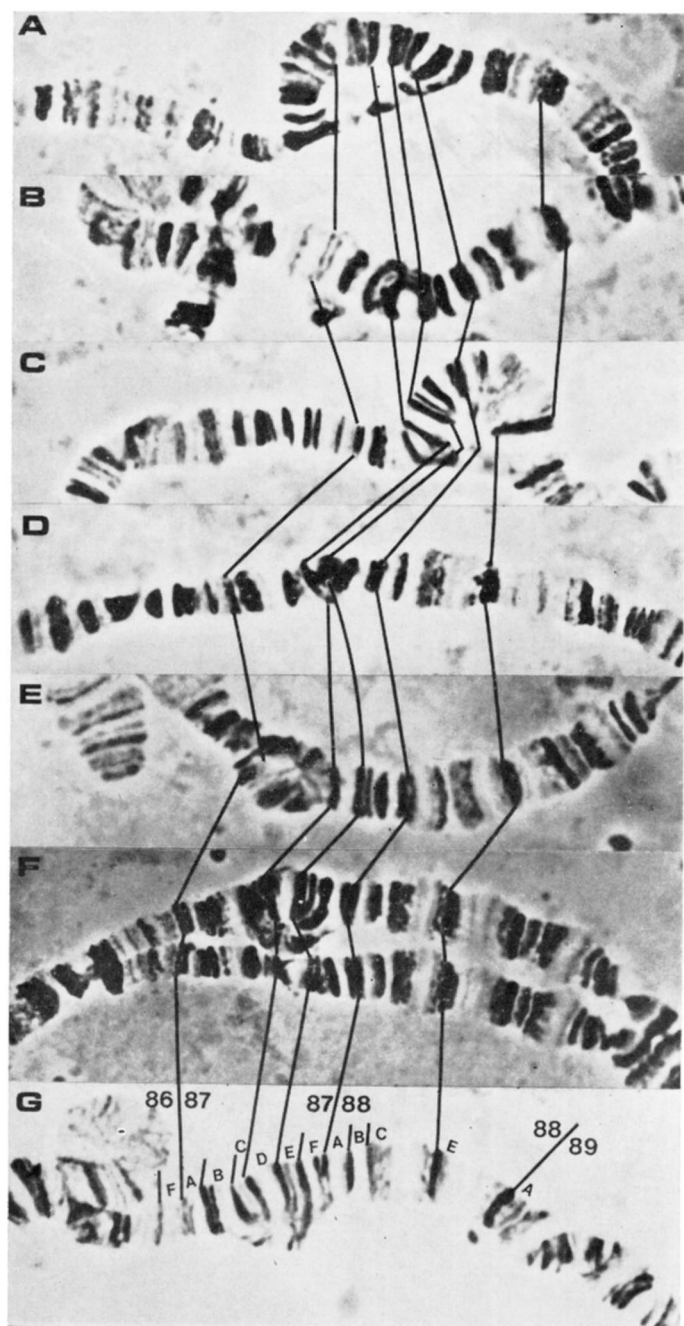


FIGURE 3.—Photomicrographs of polytene chromosome squashes of six of the tandem duplications described in the text and in Figure 4. These chromosome squashes were made from larvae heterozygous for one of the duplications and a normal third chromosome. The duplications are: (A) *Dp(3;3)124.3*, (B) *Dp(3;3)148.8*, (C) *Dp(3;3)124.5*, (D) *Dp(3;3)149.14*, (E) *Dp(3;3)121.3*, (F) and (G) *Dp(3;3)149.4* [homologues are asynapsed in (F) and synapsed in (G)].

in Figure 3. As is typical of tandem duplications, the duplicated segment tends to loop back on itself. Thus, short duplications (one division or less in length) tend to look like knots, making accurate breakpoint analysis difficult. Our best estimate of the breakpoints of these duplications is diagrammed in Figure 4. Figure 3F exemplifies the appearance of  $Dp(3;3)/+$  heterozygotes in nuclei with asynapsed homologues. One homologue appears normal while the other displays the "knot" characteristic of the duplicated segment in synapsed homologues (compare with Figure 3G). Such asynapsed nuclei have been noted for all the duplications, and clearly demonstrate that none of these rearrangements are simply minute inversions.

The cytology of the longer duplications is unambiguous and demonstrates that they are tandem direct duplications. However, the shorter rearrangements exhibit such tight pairing within themselves that a clear duplication loop is not displayed. Instead, one sees a tight "knot" in the appropriate regions (polytene chromosome region of 87). The fact that asynapsed homologues display the same "knot" argues strongly that it represents an intrachromosomal duplication. If

#### Duplicated Polytene Chromosome Region

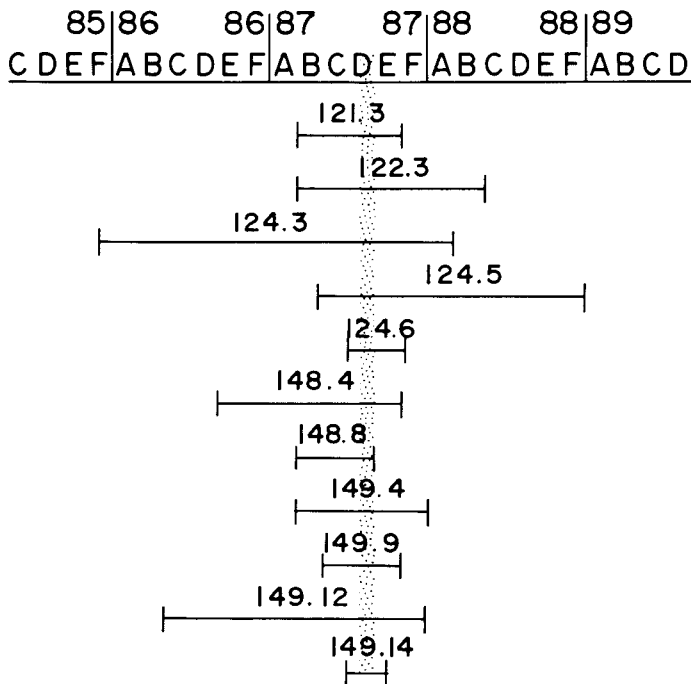


FIGURE 4.—A diagram depicting the extents of the repeated segments in each of the 11 tandem duplications recovered among the  $F_1$  of the cross outlined in Figure 1.  $Dp(3;3)121.3$  and  $Dp(3;3)122.3$  were recovered from pilot experiments in which sample sizes were not estimated. Thus, these two duplications are not included in frequency estimations. The shaded area represents the  $1(3)S12-ry$  interval, which, because of the selection, had to be duplicated in each of these chromosomes. Note that no two of the duplications are identical in extent.

they are indeed tandem direct duplications, they should be either  $[l(3)S12\text{ }ry^+]$   $[l(3)S12^+\text{ }ry]$  or  $[l(3)S12^+\text{ }ry]\text{ }[l(3)S12\text{ }ry^+]$ . Duplication/+ heterozygous females should generate some crossover progeny in which one segment of the duplication has been separated from the other. Some of the small duplications derived from experiments B and C were tested to see if the  $[l(3)S12^+\text{ }ry]$  half of each duplication could be isolated by virtue of the rosy eye-color mutation it contains. Two different protocols were employed, depending upon the outside markers contained on the duplication-bearing chromosome. The protocols were designed to provide heterozygous outside markers clearly not within the duplicated region, and to mate the heterozygous females to tester rosy males such that any crossover separating the *ry* segment of the duplication would be detected. The mating schemes and the results of these crosses are summarized in Table 4.

In each of the three cases, the rosy crossovers were associated with the proximal part of the duplication-bearing homologue and the distal portion of the tester *3R* chromosome. This is consistent with the idea that the rosy locus is present as part of a tandem duplication, with one *ry*<sup>+</sup> and one *ry* mutant allele. Moreover, the directions of the  $l(3)S12^+\text{ }ry$  crossovers indicate that the *ry* mutant must be present in the proximal half of the duplication in 148.8, 149.9 and 149.14.

DISCUSSION

The selective system utilized in the  $l(3)S12\text{-}ry$  mapping differs in one important respect from the standard purine selection system that we have employed elsewhere (see for example, GELBART *et al.* 1976). The standard purine screen requires that an intragenic exchange occurs between two mutant rosy alleles in *trans*, generating a *ry*<sup>+</sup> recombinant, which can survive the rigors of the purine-containing medium. This direct requirement for XDH activity of a wild-type recombinant negates the possibility of recovering unequal exchanges. On the other hand, for flies to survive the  $l(3)S12\text{-}ry$  selective screen, they had to contain at least one copy of each of the following segregating alleles:  $M(3)S34^+$ ,  $l(3)S12^+$ , *ry*<sup>+</sup>, *pic*<sup>+</sup> and *Sb*<sup>+</sup>; additionally, these flies had to possess a reasonably euploid genome to be viable. This selection is clearly more flexible and has allowed us to detect a number of aberrant genetic events.

These events occur with surprising frequencies. Triploid offspring account for 0.01% of all zygotes. In this selection, at least for the rosy region of chromosome

TABLE 4  
*The results of crosses designed to separate ry<sup>400</sup> from four of the tandem duplications*

Female genotype	Number of progeny	Rosy bearing crossovers
<i>Dp(3;3)148.8 Sb/ Ch<sup>v</sup> e<sup>s</sup></i>	2,127	1 <i>Ch<sup>+</sup> ry Sb<sup>+</sup> e</i>
<i>Dp(3;3)149.4/ st sbd</i>	4,322	0
<i>Dp(3;3)149.9/ st sbd</i>	5,028	2 <i>st<sup>+</sup> ry sbd</i>
<i>Dp(3;3)149.14/ st sbd</i>	11,268	10 <i>st<sup>+</sup> ry sbd</i>

Females were crossed to *kar ry* tester males.



3, the diploid gamete was matroclinous, and maintained heterozygosity for the input alleles in the *l(3)S12* to *Sb* region. Since triploids were always extreme *Sb*, *Ubx*<sup>+</sup> or weak *Sb*, weak *Ubx* in phenotype, we infer that the diploid complement (at least for chromosome 3) was maternally contributed. These triploids may represent the results of abortive meioses in which one division simply did not occur.

Even more striking is the occurrence of tandem duplications arising at a rate of four per million zygotes. While this rate appears quite low, it is indicative of a high frequency of such events in the genome as a whole. The longest duplications we recovered are roughly two polytene chromosome divisions in length, or approximately equal to duplication of 2% of the genome. We assume that hyperploids for larger portions of the genome (at least in this region) are reduced in viability and/or fertility, and thus would not be recovered as fertile duplication-bearing offspring. Taking the estimates we have available, and assuming that there is nothing special about the rate of duplication production in the rosy region, we can estimate the frequency of unequal exchanges in the genome. Our selective system allowed us to recover nine independent tandem duplications in 2,250,000 offspring. To take into account (1) that these events must produce reciprocal deficiencies which would not have survived, and (2) that only half of the zygotes received the proper paternal third chromosome (*cu kar Df(3R)ry<sup>27</sup> Ubx e<sup>+</sup>*) to have warranted further analysis, the true frequency of unequal exchange in the region must be  $[9/2,250,000] [2] [2]$  or  $1.6 \times 10^{-5}$ . As we were probably screening only 2% of the genome for duplications because of our size constraints on viability and fertility, and because of our positional constraints that the duplications include the *l(3)S12* and *ry* loci, the frequency of maternal unequal exchanges between nonsister homologues must be 50-fold greater, or 0.001. In other words, an unequal exchange event occurs once in every 500 female meioses!

It is not clear whether all crosses of the type just mentioned generate unequal exchanges. In particular, it is curious that a cross reported by McCARRON *et al.* (1979) involving a similar sort of selection and screening five-million zygotes did not generate any tandem duplications. There are several possible reasons for the difference between our results. Possibly, genetic background contributes strongly to the occurrence of these exchanges. As a more trivial explanation, delays in eclosion of tandem duplication-bearing offspring in McCARRON *et al.*'s cross might have caused them to be overlooked as "leakers" (see McCARRON *et al.* 1979, Table 6 and related text). In addition, our screen involved selection of these duplications as heterozygotes with *Df(3R)ry<sup>27</sup>*, a deletion of 87D and 87E. McCARRON *et al.* (1979) screened recombinants with two tester chromosomes: one deleted for 87D only and the other not deleted at all. Thus, their unequal recombinants would have been more aneuploid than ours, and thus less viable and/or fertile. Indeed, less than 10% of their survivors from their screen proved to be fertile equal recombinants. Among the others may well have been some viable, but infertile tandem duplications.

While little data on other regions exist concerning the frequency of unequal

exchange, clearly the rosy region is not unique in generating such spontaneous events. GREEN (1959a, 1961) reported unequal exchanges in the zeste-white region of the X chromosome, and JUDD (1961) and GREEN (1959b) also reported intragenic unequal exchanges within the white locus. FINNERTY (1968) has noted similar events in the vicinity of the maroon-like locus of the X chromosome. SOFER (personal communication) has recovered unequal exchanges among the progeny of females heterozygous for complementing alcohol dehydrogenase (*Adh*) mutations. The frequencies of unequal exchange that they observed is somewhat lower than in the present report, but their selective systems probably did not allow quantitative recovery of tandem duplication-bearing offspring.

The phenomenon of unequal exchange bears on two areas. First, it is often postulated that duplication of genetic information, followed by an accumulation of mutations in one or both segments of the duplication, leads ultimately to genetic divergence and speciation (see for example, OHNO 1970). Knowing the frequency with which such duplications are generated is important in constructing models for divergence. It would be interesting to know if our estimate of unequal exchange in the genome represents a typical rate for such events in eukaryotes. In any case, several questions are raised. Are these events controlled by the same mechanisms as regulate symmetric exchanges? Do unequal exchanges involve illegitimate recombination or do they occur only between interspersed middle repetitive sequences in the genome (MANNING, SCHMID and DAVIDSON 1975)? With respect to these questions, we can point out that in a prokaryotic system, *Salmonella typhimurium*, ANDERSON and ROTH (1977, 1978a,b) have presented data suggesting that many unequal exchanges involve recombination between regions of the genome containing homologous sequences.

Unequal exchange forces us to be cautious in our analysis of fine-structure recombination data. The frequencies of intragenic recombination, for example, are often of the same order of magnitude as the frequency of unequal exchange which we have observed. Therefore, when selective systems are employed that permit survival of tandem duplication-bearing offspring, they will have significant effects on the estimation of recombination distances. In fact, when two markers are very close together one potentially can be misled in the order of the two markers, particularly if very small numbers of recombinants are recovered. When systems vulnerable to unequal exchange are used, recombinants must be scrutinized to be sure of their nature. The general scheme that must be viewed with caution is of the type:

$$\frac{LA +}{+ IB} \times \frac{LA IB}{BALANCER, LA^+ IB^+}$$

in which only non-BALANCER flies are recovered or scored. The mutations *LA* and *IB* can be nonallelic recessive lethal mutations, or they can be allelic mutations that exhibit interallelic complementation. Any tandem duplication of the type  $[LA +] [+ IB]$  or  $[+ IB] [LA +]$  generated will survive in combination with the *LA IB* chromosome and, if care is not taken, will be considered a sym-

metric crossover. Thus, misleading maps for tightly linked loci might be generated. Cytological analysis will detect all but the smallest duplications.

As noted above, unequal exchanges occur with surprising frequency in the female germ line of *Drosophila*. Their presence can be inferred genetically and confirmed cytologically, as well as by the demonstration of intrachromosomal heterozygosity for recessive visible mutations. In addition, at least four of the duplications (124.5, 148.8, 149.4 and 149.14) clearly include the acetylcholinesterase structural gene (RIBOLINI and GELBART, unpublished), known to reside in 87E1-5 and known to be dosage sensitive (HALL and KANKEL 1976). All nine independent duplications appear to be of varying extents, indicating that a large number of asymmetric pairing configurations are possible. A more detailed description of the nature of these events must await further work.

We deeply appreciate the detailed review of this manuscript by MARGARET MCCARRON, and thank her for her many constructive suggestions.

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