# A Recombinational Hotspot at the *Triplo-lethal* Locus of *Drosophila melanogaster*

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ABSTRACT

In the genome of *Drosophila melanogaster* there is only one locus, *Tpl*, that is triplo-lethal; it is also haplo-lethal. Previous work has identified 3 hypomorphic alleles of *Tpl* which rescue animals carrying a duplication of *Tpl*, but which are not dominant lethals as null mutations or deficiencies would be. We have found that all three hypomorphic alleles act as site-specific hotspots for recombination when heterozygous with a wild-type homolog. Recombination between the flanking markers *ri* and *Ki* is increased 6.5–10.5-fold in the presence of *Tpl* hypomorphic alleles. The increased recombination was found to occur between *Tpl* and *Ki*, while recombination in other adjacent regions is unchanged. The use of isogenic $Tpl^+$ controls, and the use of flanking intervals in the mutant chromosomes allows us to rule out the interchromosomal effect as a cause. We have also observed premeiotic recombination occurring at the *Tpl* hypomorphic alleles in male heterozygotes. We hypothesize that transposons are responsible for both the hypomorphic phenotype and the high frequency of recombination.

THE *Tpl* locus of *Drosophila melanogaster* was identified as uniquely triplo-lethal and haplo-lethal by LINDSLEY *et al.* (1972). While the function of *Tpl* is still a mystery, it has been established that flies bearing 1 or 3 doses of *Tpl* die, either as late embryos or early first instar larvae (DENELL 1976; KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980). *Tpl* is located on the right arm of chromosome three at cytological location 83D4,5-E1,2 (KEPPY and DENELL 1979). The availability of chromosomes carrying a tandem duplication of *Tpl* and others carrying a deficiency has allowed balanced stocks to be established, and selections for new mutations to be carried out. Surprisingly, *Tpl* has been somewhat refractory to mutagenesis. Several deficiencies have been recovered following X- or $\gamma$-irradiation, but chemical mutagens have not increased the mutation rate significantly above spontaneous rates (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980). In addition, most of the mutations recovered after ethyl methanesulfonate (EMS) or formaldehyde treatment have been large, cytologically visible deficiencies, raising the possibility that simple point mutations may not be capable of causing a complete inactivation of *Tpl* (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980). Three alleles were recovered by ROEHRDANZ and LUCCHESI (1980) that are clearly not deficiencies of the locus. These three alleles ($Tpl^{10}$, $Tpl^{17}$, and $Tpl^{38}$) are viable in heterozygous combination with a duplication of *Tpl* and are homozygous lethal, but they are also viable over a wild-type chromosome. These alleles behave, therefore, as hypomorphs. They are cytologically normal and do not complement one another for the recessive lethality (ROEHRDANZ and LUCCHESI 1980). Although they were recovered in an EMS mutagenesis experiment, they were recovered at such low frequencies that they may actually represent spontaneous mutations.

The three hypomorphic alleles were induced on chromosomes carrying the markers *ri* (*radius incompletus*, a recessive wing vein marker), *Ki* (*Kinked*, a dominant bristle marker), and $p^p$ (*pink-peach*, a recessive eye color marker). In a small-scale mapping experiment, ROEHRDANZ and LUCCHESI (1980) found $Tpl^{10}$ to be tightly linked to *Ki*, but did not determine the map order. KAUFMAN (1978) also suggested that his failure to recover $\gamma$-ray-induced revertants of *Ki* was due to its tight linkage to *Tpl*.

In the course of an attempt to separate $Tpl^{10}$ from *Ki* by recombination we discovered that the rate of recombination between $Tpl^{10}$ and *Ki* is abnormally high. In this report we demonstrate that all of the hypomorphic alleles of *Tpl* cause a site-specific increase in premeiotic recombination, and we discuss possible implications for the structure of the *Tpl* locus.

## MATERIALS AND METHODS

**Drosophila media, stocks and culture conditions:** All flies were maintained at 20° on Formula 4-24 Instant Drosophila medium obtained from the Carolina Biological Supply Company, supplemented with live yeast. The hypomorphic *Tpl* alleles used in this study were all kept as balanced stocks of the genotype *C(1)M3*, $y^2$ *bb*/*Y*/$Y^S$*X.*$Y^L$, *In(1)EN*, *y*;; *ri Tpl Ki* $p^p$/*TM2*, *Ubx*. Control experiments relied on the strain in which the *Tpl* mutations were induced.

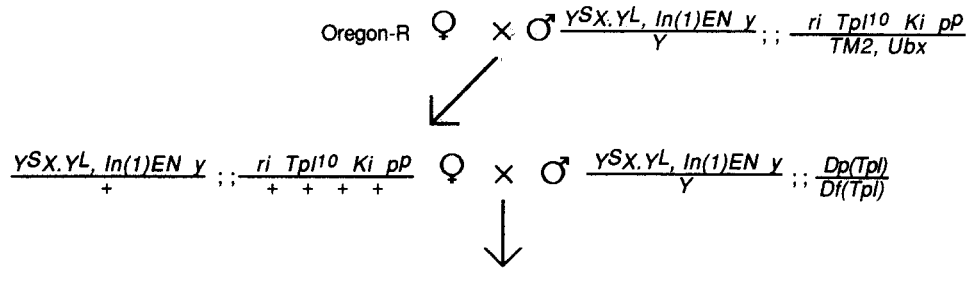


FIGURE 1.—Genetic cross performed to obtain $Tpl^{10}$ $Ki^+$ recombinants. Wild-type (Oregon-R strain) virgin females were crossed to males of the genotype $Y^SX.Y^L$, *In(1)EN, y/Y;; ri* $Tpl^{10}$ *Ki* $p^p$*/TM2, Ubx.* Virgin female progeny which were Ki and not Ubx were selected and mated to males of the genotype $Y^SX.Y^L$, *In(1)EN, y/Y;;* $Dp(3R)21l73$ $p^p/Df(3R)18i77$ $p^p$. The $Y^SX.Y^L$, *In(1)EN, y* chromosome is a compound of the *X* and *Y* chromosomes, carrying the recessive cuticle marker *yellow*. Progeny from this cross will only survive to adulthood if they carry the duplication chromosome and the $Tpl^{10}$ mutation; all other combinations die due to too many or too few doses of *Tpl*. These flies were scored for Kinked bristles and any $Ki^+$ progeny were kept and analyzed further. The second and fourth chromosomes are all wild type and are not shown.

This strain is of the genotype *C(1)RM, y v f/*$Y^SX.Y^L$*, In(1)EN, y; bw; ri Ki* $p^p$. These strains, as well as the Oregon-R wild-type strain, and *l(3)DTS-2/TM3, Ser were kindly provided by J.* LUCCHESI (University of North Carolina). The *Tpl* duplication over deficiency stock used in these studies was *C(1)M3,* $y^2$ *bb/Y/*$Y^SX.Y^L$*, In(1)EN, y;;* $Dp(3R)21l73$ $p^p$*/*$Df(3R)18i77$ $p^p$. This stock was selected from a stock kindly provided by R. DENELL (Kansas State University), in which the $p^p$ marker was heterozygous. The *l(3)DTS-2* mutation is described in HOLDEN and SUZUKI (1973), the *Tpl* alleles are described in ROEHRDANZ and LUCCHESI (1980), and KEPPY and DENELL (1979), and all others in LINDSLEY and GRELL (1968).

**Genetic crosses:** Crosses were carried out at 25°. Except where noted, crosses were carried out in vials using 3 or 4 virgin females and 3 or 4 young males per vial. After 4 days, the adults were transferred to fresh vials, and after an additional 6 days they were discarded.

## RESULTS

**Frequency of recombination between $Tpl^{10}$ and *Ki* is higher than expected:** The published map positions of *ri*, *Ki*, and $p^p$ are 47.0, 47.6 and 48.0, respectively (LINDSLEY and GRELL 1968). Cytological and genetic data had suggested that *Tpl* is very close to *Ki*, and certainly between *ri* and $p^p$. Consequently, when we set up the cross shown in Figure 1 in order to recover $Tpl^{10}$ $Ki^+$ recombinant chromosomes, we anticipated screening large numbers of $Dp(Tpl)/Tpl^{10}$ survivors in order to find $Ki^+$ recombinants. In fact, of the first 114 progeny scored, 5 were $Ki^+$. This would imply a map distance of 4 centimorgans, a distance greater than that expected between *ri* and $p^p$. Test crossing the recombinants showed that they were all *ri* $Tpl^{10}$ $Ki^+$ $p^+$, placing *Tpl* to the left of *Ki*.

Because we wanted to see if the reciprocal recombination product could be recovered and because we wanted to see if the other hypomorphic alleles, $Tpl^{17}$ and $Tpl^{38}$, would also show aberrant recombination behavior, we set up the cross shown in Figure 2 with all three alleles. In this cross, all recombinants between *ri* and *Ki*, and between *Ki* and $p^p$ were recovered, and then tested for the hypomorphic allele of *Tpl* by crossing them to the $Dp(Tpl)$ $p^p/Df(Tpl)$ $p^p$ stock. A control was also done using the same *ri Ki* $p^p$ stock that ROEHRDANZ and LUCCHESI (1980) used to recover the hypomorphic alleles. The data are shown in Table 1. Our control map distances for the *ri–Ki* interval and the *Ki–*$p^p$ interval are well within the expected range, but when a hypomorphic allele of *Tpl* intervenes between *ri* and *Ki* the recombination frequency is elevated 6.5–10.5-fold. $\chi^2$ analysis of the data shows that the recombination frequency between *ri* and *Ki* is highly significantly different from the control frequency for all three hypomorphic alleles ($P < 0.001$), while the recombination frequency between *Ki* and $p^p$ is not significantly different in any of the crosses. The biases in recovery seen in Table 1 are apparently due to a maternal effect of the *Tpl* hypomorphic alleles. The viability of *Tpl/+* heterozygotes is reduced compared to +/+ siblings when the mother is *Tpl/+*, but not when the mother is +/+ (our unpublished data). Since all flies carrying a hypomorphic *Tpl* allele are affected, whether recombinant or not, this does not affect our conclusions on recombination.

The recombinants between *ri* and *Ki* were tested to see whether the recombination event occurred between *ri* and *Tpl* or between *Tpl* and *Ki*. Of the 22 ri $Ki^+$ $p^+$ recombinant progeny from all three experimental crosses, 18 were fertile, and all 18 carried the *Tpl* hypomorphic mutant. Similarly of the 53 $ri^+$ Ki $p^p$ recombinants, 41 were fertile, and all 41 were $Tpl^+$. These results show once again that *Tpl* maps to the left of *Ki*, and also that the excess of recombination seen when the females were heterozygous for a hypomorphic *Tpl* mutation occurs to the right of *Tpl* and the left of *Ki*. The few progeny which were recombinant in the *Ki* to $p^p$ interval were also tested and found to be nonrecombinant in the *ri* to *Ki* interval; no double crossovers were seen.

We saw some evidence that the recombinant prog-

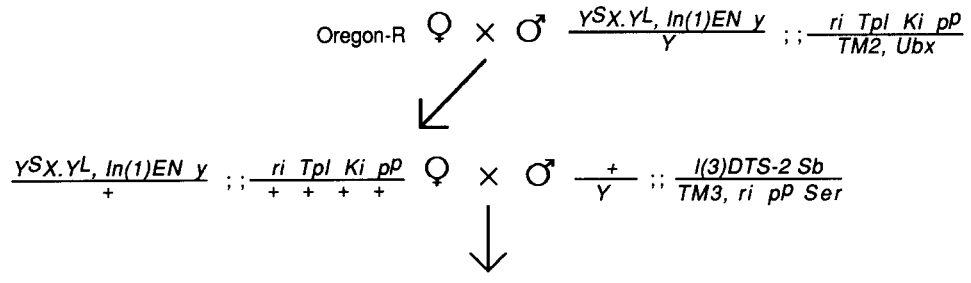


FIGURE 2.—Genetic cross to measure recombination frequencies in the vicinity of *Tpl*. The first cross is the same as that in Figure 1, except that the *Tpl* allele was varied. These experiments were done with $Tpl^{+}$, $Tpl^{10}$, $Tpl^{17}$, and $Tpl^{38}$. All other markers were constant. Females of the genotype $Y^{S}X.Y^{L}$, *In(1)EN*, *y*/+;; *ri Tpl Ki* $p^{p}$/++++ (where *Tpl* represents either $Tpl^{+}$, $Tpl^{10}$, $Tpl^{17}$, or $Tpl^{38}$) were mated to males of the genotype +/*Y*;; *l(3)DTS-2 Sb/TM3, ri* $p^{p}$ *Ser*. At 25° flies bearing the *l(3)DTS-2 Sb* chromosome die (the few escapers can be recognized by the Stubble bristle marker) and the *TM3* chromosome carries the recessive mutations *ri* and $p^{p}$ so recombinants in the *ri–Ki* interval and the *Ki–*$p^{p}$ interval can be scored immediately, and the recombinant chromosome saved as a balanced stock. The second and fourth chromosomes are all wild type and are not shown.

**TABLE 1**

**Recombination in *ri Ki* $p^{p}$/+++ females carrying $Tpl^{+}$, $Tpl^{10}$, $Tpl^{17}$, or $Tpl^{38}$ on the *ri Ki* $p^{p}$ chromosome**

| | Summary of progeny | | | |
|---|---|---|---|---|
| | | Hypomorphic alleles | | |
| Phenotype | Control $Tpl^{+}$ | $Tpl^{10}$ | $Tpl^{17}$ | $Tpl^{38}$ |
| ri Ki $p^{p}$ | 1137 | 1082 | 362 | 446 |
| + + + | 1117 | 1420 | 626 | 626 |
| ri + + | 3 | 15 | 1 | 6 |
| + Ki $p^{p}$ | 2 | 19 | 20 | 14 |
| ri Ki + | 3 | 4 | 0 | 1 |
| + + $p^{p}$ | 1 | 1 | 2 | 5 |
| Total progeny | 2263 | 2541 | 1011 | 1098 |
| | Recombination frequencies ± SD (cM) | | | |
| | | Hypomorphic alleles | | |
| Interval | Control $Tpl^{+}$ | $Tpl^{10}$ | $Tpl^{17}$ | $Tpl^{38}$ |
| *ri–Ki* | 0.2 ± 0.1 | 1.3 ± 0.23* | 2.1 ± 0.45* | 1.8 ± 0.41* |
| *Ki–*$p^{p}$ | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.5 ± 0.2 |

Crosses were carried out as described in Figure 2.
* Significantly different from the control value at $P < 0.001$.

eny might be appearing in clusters. While the majority of the females (70–80%) produced either zero or one recombinant among their progeny, a few vials containing three females produced up to nine recombinants. Clustering would indicate that at least part of the recombination occurring between *Tpl* and *Ki* is premeiotic (mitotic recombination in the germline). The small number of total progeny these females produce (40 on average), creates a problem of distinguishing meiotic from premeiotic recombination under these circumstances. Accordingly, we decided to look at this question in males, because of the absence of meiotic recombination in *Drosophila melanogaster* males.

**Recombination at hypomorphic alleles of *Tpl* occurs premeiotically in males:** Some mass mating experiments showed that heterozygous males of all three hypomorphic alleles of *Tpl* produced small numbers of recombinants, with the frequency of recombination varying from 0 to 3% in any given experiment (data not shown). To examine this further, single male crosses were set up as shown in Figure 3. For *ri* $Tpl^{10}$ *Ki* $p^{p}$/+ + + +, one such male produced 7 *ri* $Tpl^{10}$ + + recombinants and 10 + + *Ki* $p^{p}$ recombinants out of 155 total progeny (11% recombinants), while 9 sibling males produced 0 recombinants out of 846 total progeny. Similarly, one *ri* $Tpl^{17}$ *Ki* $p^{p}$/+ + + + male produced 8 *ri* $Tpl^{17}$ + + recombinants and 8 + + *Ki* $p^{p}$ recombinants out of 132 total progeny (12% recombinants), while 7 sibling males produced 0 recombinants out of 1019 total progeny. These clusters of recombinant progeny indicate that the recombination event is a reciprocal one and occurs in spermatogonial cells a few cell divisions prior to meiosis. Balanced stocks were made from the four recombinant chromosomes described. Using these stocks, the recombinant chromosomes were reunited to form viable *ri* $Tpl^{10}$ + +/+ + *Ki* $p^{p}$ and *ri* $Tpl^{17}$ + +/+ + *Ki* $p^{p}$ flies, indicating that the recombination event did not produce a lethal lesion in both homologs, and that the phenotype of the *Tpl* mutation on both homologs is unchanged.

## DISCUSSION

Recombination in the proximal region of the third chromosome has long been known to be slightly peculiar. MORGAN, BRIDGES and STURTEVANT (1925) first observed that although map distances in the vicinity of the centromere are small, the coefficient of coincidence is quite high. Several investigators have repeated that observation and suggested explanations for the abnormally high number of double recombinants observed (GREEN 1975; SINCLAIR 1975; DENELL and KEPPY 1979). Our observations relate to a site-specific increase in the recombination rate which is a different phenomenon. An interchromosomal effect

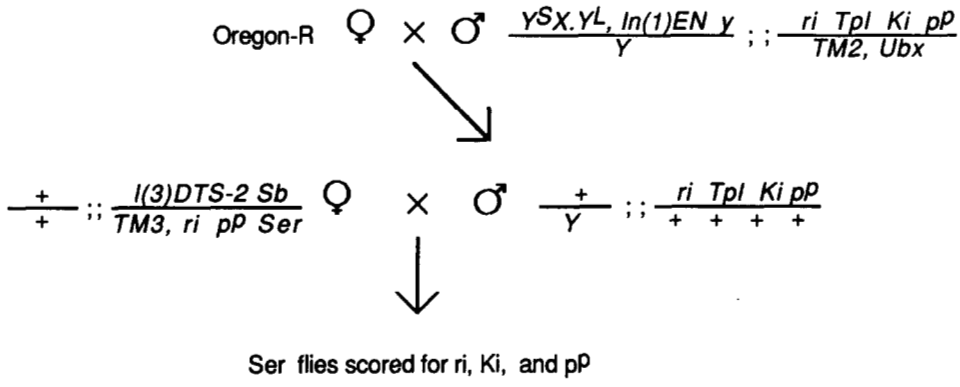


FIGURE 3.—Genetic cross to detect recombination in males. These crosses were done as in Figure 2 except that heterozygous males were crossed to +/+;; *l(3)DTS-2 Sb/TM3, ri p$^p$ Ser* females, and their progeny were scored for recombination. Most individuals in our *ri Tpl Ki p$^p$/TM2, Ubx* stocks have a free *Y* chromosome as indicated, thus ensuring the fertility of the heterozygous male progeny. The second and fourth chromosomes are all wild type and are not shown.

on recombination has been observed in this region (SINCLAIR 1975; DENELL and KEPPY 1979), but several factors allow us to rule this out as an explanation for our data. The interchromosomal effect is a phenomenon observed in Drosophila in which heterozygosity for an inversion on one pair of homologous chromosomes causes an increase in meiotic recombination in all other chromosomes (LUCCHESI 1976). Any such effects in this experiment due to heterozygosity for the $Y^SX.Y^L$, *In(1)EN* chromosome would also have been seen in the $Tpl^+$ controls. The magnitude of the interchromosomal effect is only 2–4-fold, which is noticeably less than the increase we see here. The interchromosomal effect is also a general effect on recombination and would have been expected to increase recombination in the adjacent intervals *ri–Tpl* and *Ki–p$^p$*, which show no increase in recombination frequency. Finally, the interchromosomal effect is a meiotic phenomenon, and what we are seeing here is at least partly premeiotic.

Very little is yet known about *Tpl*. The extreme dosage sensitivity of the locus has made it uniquely challenging to work with, and very little information on the function of the locus is available. It was suggested by DENELL (1976) and LUCCHESI (1977) that *Tpl* might play a role in counting *X* chromosomes and directing the pathways of sex determination and dosage compensation. While this idea had some merit [see BAKER and BELOTE (1983) for a discussion], it did not explain all of the data, and a direct experimental test of the hypothesis found it to be flawed (CHRISTENSEN and LUCCHESI 1988). The molecular basis of the hypomorphic phenotype is unknown. The hypomorphic alleles behave as if they are about half of a dose, but whether this is due to a missense or nonsense mutation affecting the gene product, a decrease in the amount of the gene product, a deletion of half of the copies from an array of reiterated genes, or something more baroque is not known.

Even though we do not know the function of *Tpl*, we believe that the site-specificity of the recombination data presented here argues that the increase in recombination is not related to the *Tpl* gene or gene products directly. It seems more likely that the same genetic lesion causes both the hypomorphic phenotype and the increase in recombination. Two possibilities come to mind. Either the hypomorphic mutations are point mutations that affect expression of *Tpl* somehow, and the single-base change also creates an initiation site for recombination, or the hypomorphic alleles are due to a transposon insertion which modifies expression of *Tpl* and the recombination we see is due to the activity of the transposon. Single-base changes that dramatically alter recombination at the site of the change are well-known in some systems. The Chi site of phage lambda is a particularly well-understood example of this (SMITH *et al.* 1981). Another example is the *ade6-M26* mutation of *Schizosaccharomyces pombe* (PONTICELLI, SENA and SMITH 1988). However, several lines of evidence lead us to believe that the transposon model is the more likely one.

The hypomorphic alleles resulted from an EMS mutagenesis selection, but they actually represent a very rare phenotype. KEPPY and DENELL (1979) screened $4.3 \times 10^5$ chromosomes mutagenized with EMS and did not recover any hypomorphic mutations (they did recover a number of deficiencies). ROEHRDANZ and LUCCHESI (1980) screened $1.3 \times 10^5$ chromosomes and recovered the three hypomorphic alleles. This gives an overall mutation rate of $5 \times 10^{-6}$. This rate is low enough that ROEHRDANZ and LUCCHESI questioned "whether EMS acted as a mutagenic agent in producing the mutations or whether they could have been spontaneous." If these are spontaneous mutations due to transposon insertions, both the ineffectiveness of EMS in producing hypomorphic alleles and the sporadic recovery of such alleles could be explained. Recently we have done a hybrid dysgenesis screen for mutations at *Tpl* which would presumably be due to *P* element insertions. That we have recovered 15 new hypomorphic alleles, and no nulls, out of approximately $7 \times 10^4$ chromosomes (our unpublished data) supports the notion that transposons may be responsible for ROEHRDANZ and LUCCHESI'S hypomorphic alleles. While we do not yet know the molecular nature of these new hypomorphic alleles,

some of them are unstable, reverting at high frequencies, and at least one of them appears to be a hotspot for recombination (our unpublished data).

Several investigators (SINCLAIR and GRIGLIATTI 1985; ISACKSON, JOHNSON and DENELL, 1981) have proposed a model for the male recombination seen in hybrid dysgenesis, in which nicks or double-strand breaks are left behind at the site of a *P* element following excision or a replicative transposition. These lesions then lead to recombination as envisaged in the model of SZOSTAK *et al.* (1983). Although the three hypomorphic alleles of *Tpl* examined herein are not due to *P* element insertions (nor to several other transposons we have screened by *in situ* hybridization) (our unpublished data), we suggest that something very similar is happening here. Since we do not see revertants among the recombinant progeny, the events we see here could not be caused by a precise excision event that reverts the hypomorphic phenotype, nor to an imprecise excision that produces a more extreme phenotype. Although we did not see such events, a precise excision which reverted the mutation and then led to a recombination event would have been very difficult to distinguish from gene conversion. Previous studies on transposon-induced recombination in Drosophila have utilized widely spaced markers in order to detect crossing over in a large region of a chromosome; this is the first time that a specific lesion at a specific locus has been implicated. In addition, due to the nature of the male recombination cross we have done, both reciprocal products of the recombination event are available as balanced stocks. As the molecular tools become available we will compare the DNA sequences of the *Tpl* mutations and the recombinant chromosomes which will offer information about the details of the process. If it actually turns out that the hypomorphic alleles are point mutations, then it will be very interesting to try to understand why the recombination event always occurs to the right of the nucleotide change that causes the mutation, and would be the first example of a specific sequence acting to initiate recombination in a higher organism. Alternatively, if transposons are responsible for the hypomorphic alleles, then analysis of the recombinant chromosomes may be very informative about the transposition process and the initiation of recombination.

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