RADIATION INDUCED TANDEM DUPLICATIONS IN *DROSOPHILA MELANOGASTER*

HARVEY A. BENDER

*Department of Biology and Radiation Laboratory¹, University of Notre Dame,
Notre Dame, Indiana 46556*

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THIS paper reports the successful induction by X rays of two tandem direct duplications for the lozenge region of the X chromosome of *Drosophila*. Numerous tandem direct duplications (ABAB) of spontaneous origin are now known in *Drosophila*: Bar, STURTEVANT (1925), MULLER, PROKOFYEVA-BELGOVSKAYA and KOSSIKOV (1936) and BRIDGES (1936); Hairy wing, DEMEREC and HOOVER (1939); Dp(2:2)Star, LEWIS (1941); Confluens, SCHULTZ (1941); Abruptex, SCHULTZ (in MORGAN, SCHULTZ and CURRY 1941); Beadex, GREEN (1953); and Dp(1:1)white, GREEN (1961), JUDD (1961), RASMUSON (1965), and LEWIS (unpublished). Such duplications are generally believed to arise as the result of nonhomologous exchange between homologous chromosomes. In two cases, Dp(2:2)*S* and Dp(1:1)*w*, the origin of the duplication has indeed been shown to be associated with a crossover between homologous chromosomes.

SLIZYNSKA (1957) showed cytologically that tandem duplications are induced when male larvae are treated with formaldehyde. A tandem duplication for the bithorax region (89D-E) has been deliberately induced by X-raying diploid meiotic stages of the female (LEWIS, personal communication). Recently, THOMAS and ROBERTS (1966) report the induction of a second chromosome (26A-28E) tandem direct duplication in experiments designed to study rearrangements induced in oocytes.

Tandem direct duplications have been detected in a variety of ways: (1) The mutant phenotype associated with the Bar, Hairy wing and the Beadex duplications was the basis for their original detection; (2) SLIZYNSKA, and THOMAS and ROBERTS detected tandem direct duplications cytologically by examining the salivary gland chromosomes of individuals suspected of possessing chromosome aberrations; (3) in the case of the white and bithorax duplications, partial complementation between pseudoallelic mutants was the basis for the detection of the duplications.

The third or complementation method of detecting duplications was the one of choice for the lozenge (*lz*) region. It will be described more fully below. Since duplications provide valuable tools for studying gene dosage relationships, it seemed desirable to develop them for the *lz* pseudoallelic series.

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MATERIALS AND METHODS

GREEN and GREEN (1956) and GREEN (1961) report that lz^{50e} , lz^{49h} , and lz^k complement most of the other known mutants of this series. Thus, lz^{50e}/lz^x (where lz^x represents any of the lz mutants other than 50e, 49h and k) has a nearly wild-type phenotype (near normal eyes, normal tarsal claws and normal female secondary reproductive structures). The lz^{50e} mutant has a very rough eye which is markedly reduced in size, normal tarsal claws and normal female secondary reproductive structures. The noncomplementing lozenge alleles can be grouped into two general phenotypic categories: the first (glossy-like) have rough, homogeneously pigmented, moderately reduced eyes, reduced or missing tarsal claws and pulvilli, and lack secondary reproductive structures. The second (spectacle-like) have a smooth, strongly reduced eye with marked peripheral pigmentation and abnormalities of the tarsi and secondary female reproductive structures. The alleles chosen for use in this study were lz^{50e} and the spectacle-like mutant, lz^{y4} . GREEN and GREEN (1949, 1956) and GREEN (1961) found that 19 lozenge alleles could be assigned to four separate loci or sites within the lz pseudoallelic series. The lz^{50e} allele is in the leftmost sublocus and lz^{y4} is located in the rightmost.

Virgin females, $sn^3 lz^{y4} v/lz^{50e}$, were irradiated with approximately 4000r of X rays (220kv, 14ma, at a dose rate of approximately 200r per minute) and mated to unirradiated wild-type males. The bristle mutant *singed-3* (sn^3) and eye color mutant *vermilion* (v) are markers located at 21.0 and 33.0 respectively in the X chromosome (BRIDGES and BREHME 1944). Cytological studies of presumptive duplications were carried out on acetolactic orcein squashes of the salivary gland chromosomes.

RESULTS AND ANALYSES

The induction of nonhomologous exchange in the neighborhood of the lz region in $lz^{50e}/sn^3 lz^{y4} v$ females might be expected to yield sons whose eye phenotype was wild-type or nearly so. This is illustrated in Figure 1. Diagrams A and B show nonhomologous pairing and exchange resulting in the formation of lz duplications. A duplication containing lz^{50e} in one section and lz^{y4} in the other

FIGURE 1.—A diagram of the lozenge region. A and B propose configurations which could result in tandem direct duplications. C diagrams a conventional crossover between the lz^{50e} and lz^{y4} heteroalleles and both resultant chromosome products. It was anticipated that a chromosome recovered from an exchange as diagrammed in B would be indistinguishable from the sn^3 marked chromosome exchange product (C). Thus only individuals carrying the vermilion marker and having wild-type eyes (A) were sought and tested.

section of the duplication ($\dots lz^{50e} + \dots$)($\dots + lz^{y4} \dots$) might be expected to have the phenotype of $lz^{50e} +/+ lz^{y4}$; that is, to have a nearly normal eye.

The double mutant (C-2) is known to have a lozenge phenotype and the reciprocal product (C-1) has wild-type eyes and is expected to carry the sn^3 marker. The latter might be difficult to distinguish phenotypically from the product shown in 1B. Therefore, only F_1 males that were vermilion in eye color and non-lozenge in eye morphology (1A) were saved for analysis.

Irradiation of $sn^3 lz^{y4} v/lz^{50e}$ females yielded three non-lozenge, vermilion sons among approximately 40,000 F_1 males scored. Such males, which arose independently of one another, were placed individually with virgin $y f$ attached-X and *Ext/FM6* females. From these double matings, attached-X and balanced (*FM6*) stocks were established for each of the X chromosomes derived from the three vermilion males.

Tandem direct duplications involving the *lz* region were observed in two of the three established lines. The third appeared cytologically normal and has not been analyzed further. The two duplications are given the symbols, $Dp(1:1)lz-1$ and $Dp(1:1)lz-2$, abbreviated here as Dp-1 and Dp-2. Virgin Dp-1/*FM6* were mated to sn^3 males. The F_1 mated *inter se* and the F_2 males were scored. In the case of the remaining duplication, virgins Dp-2/*FM6* were mated to $tan-3$ (t^3 , locus 27.5) or to sn^3 , miniature (m , locus 36.1) males. Again, the F_1 were mated *inter se* and the F_2 males were scored.

Dp-1 males have slightly roughened eyes, thin or missing posterior postalar bristles, normal tarsal claws and good fertility. Females homozygous for this duplication have considerably more roughened eyes, abnormalities of the posterior postalar bristles similar to those of the male, normal tarsal claws and markedly reduced fertility. However, the secondary reproductive structures are normal. The tandem duplication is rather large, extending from the 8D through 8F region of the salivary gland chromosome map.

Dp-2 males have very slightly roughened eyes, normal posterior postalar bristles, normal tarsal claws and good fertility. Females homozygous for the duplication have moderately rough eyes, thin or missing posterior postalar bristles, normal tarsal claws, good fertility and normal secondary reproductive structures. The tandem duplication is quite small and includes the 8D1 band of the salivary gland chromosome map. The cytological limits of both duplications are consistent with the conclusion (GREEN and GREEN 1961) that *lz* is located in the 8D region.

Genetic confirmation that the tandem duplication contained the two original *lz* alleles was obtained in the case of both duplications. From Dp-1/ sn^3 females there were 23 lz^{50e} and 2 $lz^{y4} v$ males among approximately 15,000 F_1 males scored. In pooled data from two separate experiments, females heterozygous for Dp-2 produced 4 lz^{50e} and 15 lz^{y4} males in about 9,000 observed F_1 males. The frequency of lz^{50e} males, when multiplied by two, is a measure of the recombination frequency in the region between the *lz* locus in the left section of the duplication and the breakpoint of the duplication (0.3%) in Dp-1 and 0.08% in Dp-2) while the frequency of the lz^{y4} males, when multiplied by two is a measure

of the frequency of recombination in the region between the *lz* locus in the right section and the duplication breakpoint (0.03% and 0.3% in Dp-1 and Dp-2 respectively). Since the complementary crossovers to these unequal crossover types are expected to be indistinguishable phenotypically from wild type, and therefore not detectable in these experiments, the recombination frequency for the two respective regions has been corrected by multiplying by two the frequency of the detectable recovered crossovers.

All recovered *lz*^{50e} and *lz*^{y4} males were mated to attached-X virgin females. In all instances they were fertile and in all ways appeared identical to the original *lz*^{50e} and *lz*^{y4} alleles.

DISCUSSION

Figure 2 is a model which diagrams the proposed pairing relationships of the *lz* region of the duplications with that of a normal nonduplication chromosome. The crossover which would lead to an F₁ male with a *lz* phenotype is heavily outlined in each instance. Figure 2A and B diagrams a crossover in the left and right section of Dp-1 and Figure 2C and D structure crossovers in the left and right sections of Dp-2.

Indirect evidence suggests that the *tan* locus may be included within the right

FIGURE 2.—A model of the proposed pairing relationships of the *lz* region of the two induced tandem duplications with a normal, nonduplicated, chromosome. A and B diagram crossovers leading to the recovery of the *lz*^{50e} and *lz*^{y4} alleles, respectively, in Dp-1. C and D diagram crossovers leading to the recovery of the *lz*^{50e} and *lz*^{y4} alleles in Dp-2. The calculated frequency of recombination between each *lz* allele and the breakpoint of the duplication is shown in the appropriate region of the diagram.

section of Dp-2 (Figure 2C,D). The tan locus is 0.2 crossover units to the left of *lz* (BRIDGES and BREHME 1944) and the calculated distance between *lz*^{u4} and the breakpoint in this study is 0.3 units. Further, one non-tan, *lz*^{y4}, *v* male was recovered from Dp-2/*t*^s females; the simplest explanation for the occurrence of such an individual is that the normal wild-type tan allele is in the duplication and a crossover between tan and the breakpoint took place. Attempts to introduce the *t*^s allele by crossing over into the chromosome with Dp-2 have failed (over 10,000 F₁ flies from Dp-2/*t*^s females have been observed). Again the simplest explanation is that *t*^s lies within the duplication; that is, the crossover (*t*^s *lz*^{50e}) (+*lz*^{y4}) would be expected to have a normal body color instead of tan, and therefore this product would be undetectable.

A comparative analysis of the two duplications is instructive. Dp-2 demonstrates no obvious position effects in that the eye and tarsal claw morphology closely approximates that of the *lz*^{50e}/*lz*^{y4} individual. However, the eye morphology of the Dp-1 male is markedly rougher. Why this is so is not clear; it may represent a position effect phenomenon or perhaps the inclusion in this longer duplication of loci which roughen the eye morphology when present in duplicate. The eye morphology of females homozygous for either duplication is clearly more extreme than for the respective hemizygous males. The modification of the posterior postalar bristles in Dp-1 and Dp-2 females and Dp-1 males is likely due to increased dosage for one of the genes in the region common to both duplications.

The effect upon development of varying dosages of mutant and normal *lz* alleles is being studied, and a more precise cytological localization is being made of both tan and lozenge.

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SUMMARY

Tandem direct duplications of the lozenge (*lz*) region have been induced by X-irradiation of the diploid meiotic stages of the female. Detection was based upon partial complementation between two of the *lz* pseudoallelic mutants, and the duplications were confirmed by cytological and genetic analysis.

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