Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5367–5369, September 1982
Genetics

Genetic instability in *Drosophila melanogaster*: Deletion induction by insertion sequences

(chromosome organization/spontaneous deletions/cytogenetic mapping)

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Contributed by M. M. Green, June 7, 1982

ABSTRACT **Females of *Drosophila melanogaster* heteroallelic or homoallelic for X chromosome giant (*gt*) mutants generate deletions involving the wild-type alleles at two X chromosome gene loci: yellow body color (*y*) and white eye color (*w*). The deletions, bidirectional in the case of *y* and with fixed endpoint in the case of *w*, are associated with particular X chromosomes. Distinctive insertion sequences, located proximal to the target loci, are presumed to generate the deletions.**

A substantial body of data is now on hand, stemming from both genetic and biochemical experiments, that confirms the role of insertion sequences (IS) in the production of gene mutations in both prokaryotes and eukaryotes (1–3). Beginning with the pioneering experiments of McClintock in maize, IS are now known to cause both mutationally stable and mutationally unstable mutants. Subsequent to causing a mutation by insertion into the DNA of a gene, an IS may be excised, leading to one of two consequences. The IS may be cleanly excised, resulting in the reversion of the mutant to wild type. Alternatively, the IS, when excised, may remove part or all of the target gene plus adjacent genomic DNA, thereby producing a deletion. Both events are known for maize, *Drosophila melanogaster*, *Escherichia coli*, and yeast.

The >50 families of IS in *D. melanogaster* (4) are present in the genome without any obvious phenotypic effect. Many have been localized cytologically by *in situ* hybridization of cloned IS to specific bands of the *D. melanogaster* polytene chromosomes (5, 6). In principle, an IS can be localized to specific chromosome locations if the IS can be caused to excise and generate deletions of known mapped genes. Here I report the occurrence of high frequencies of deletions involving the yellow body color (*y*) and white eye color (*w*) gene loci on the X chromosome of *D. melanogaster*. Based upon the types of deletions produced, two distinctive IS are inferred to be responsible for the deletions recovered.

MATERIALS AND METHODS

The mutants used in the several crosses are recorded in Table 1 with their phenotypes and polytene chromosome map locations. Standard *Drosophila* culture conditions were used at a room temperature of 22–24°C. Details of specific crosses will be given in the text where appropriate.

RESULTS

For reasons unrelated to the results to be reported, females of the genotype $gt\ w^a/gt^{13z}\ w^+$ were constituted and crossed to males of the genotype $y^2\ w^-\ spl\ sn^3$. Among female progeny scored (Table 2, experiment 1), two unexpected phenotypic classes of females were found. In two separate crosses, clusters of four and two females with *y* mutant phenotype were obtained. In three other crosses, single females with *w* mutant phenotype were obtained. Subsequent progeny tests of the *y* and *w* females established two points. First, all mutant phenotypes are caused by heritable changes linked to the $gt^{13z}\ w^+$ X chromosome and not to the $gt\ w^a$ chromosome. Second, although the *y* mutants were male viable (when the gt^{13z} was rescued by an appropriate duplication), the three *w* mutants were not male viable (when gt^{13z} was rescued).

Each *w* mutant proved to be lethal in compound with a lethal mutant, *sa*, which maps immediately to the left of *w*. When rescued with a duplication, none of the *w* mutants showed the phenotype associated with the *rst* locus that maps immediately to the right of *w*. Therefore, it was concluded that all *w* mutants are, in fact, deletions of both *w* and one or more lethal loci that map to the left of *w*. Polytene chromosome cytology of one *w* mutant confirmed the existence of a deletion: bands 3A9–3C2 are absent. Because of the observed high frequency of mutational events at the *y* and *w* loci, the cross of $gt\ w^a/gt^{13z}\ w^+$ females to $y^2\ w^-\ spl\ sn^3$ males was repeated on a somewhat larger scale. The results are given in Table 2 as experiment 2 and confirm the finding of the first experiment. Again, numerous *y* mutants, some occurring in clusters, were recovered; in addition, seven independent *w* mutants were found. All of the mutants were recovered in females, suggesting that they

Table 1. Synopsis of X chromosome mutants used in text with their polytene chromosome band locations*

Mutant symbol	Band	Phenotype
l^{EC1}	1A4	Recessive lethal
l^{EC2}	1A5	Recessive lethal
l^{EC3}	1A8	Recessive lethal
y	1B1	Yellow body color
y^2		Allele of y
ac	1B2	Achaete—bristles removed
sc	1B4	Scute—bristles removed
svr	1B8	Silver body color
gt	3A2	Giant body size
gt^{13z}		Lethal allele of gt
gt^{Q292}		Lethal allele of gt
sa	3C1	Sparse arista, recessive lethal
w	3C2	White eye color
w^-		♂ viable *w* deletion
w^a		Allele of w
spl	3C7	Split bristles
N	3C7	Notch wings
sn^3	7D1	Singed-3 bristles

* As described by Lefevre (7).

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Abbreviation: IS, insertion sequence(s).

Table 2. Deletions associated with the *y* and *w* loci recovered among the female progeny of parental females of given genotypes

Exp.	Genotype ♀♀	Number of deletions*		Σ ♀ progeny scored
		y	*w*	
1	$gt\ w^a/gt^{13z}\ w^+$	1X2 + 1X4	3X1	33,667
2	$gt\ w^a/gt^{13z}\ w^+$	2X1 + 1X2 2X3 + 1X6 + 1X8 + 1X11	7X1	89,418
3	$y^{+13z}\ gt\ w^a/y^{+13z}$ gt	3X1 + 1X2	2X1	24,021
4	$y^{13z}\ gt\ w^a$ (homozygous)	1X1 + 2x2	0	34,258
5	$gt^+\ w^a/gt^{13z}\ w^+$	0	0	62,326
6	$gt\ w^a/y\ gt^{Q292}\ w^+$	—	6	82,129

* 2X1, two independent deletions recovered once; 1X2, the same deletion recovered twice from one cross, etc.

occurred only on the $gt^{13z}\ w^+$ X chromosome and not on the $gt\ w^a$ chromosome.

Progeny tests as outlined above confirmed this conclusion and established two additional facts. First, not all changes at the *y* locus were male viable when gt^{13z} was rescued with an appropriate duplication. This was interpreted to mean that the *y* mutants included the loss of *y* and one or more lethal loci known to map adjacent and to the left of the *y* locus. Second, all members of a cluster appeared to be the same—i.e., when one was lethal, all were lethal. As will be described shortly, further testing established the lethal *y* mutants to be deletions because they were lethal in compound with known recessive lethal mutants. Balanced stocks were obtained for each of nine *y* mutants, and all were mapped by determining the loci that they uncovered. The genetic and cytological extent of the *y* mutants is shown in Fig. 1. It will be noted that the longest deletion included the loss of a minimum of 13 salivary gland chromosome bands. (No attempt was made to map to the left of 1^{EC1} and to the right of *svr*.) By extrapolation, the smallest deletion involves only the loss of the *y* locus. Individual members of a cluster were mapped separately and proved to be inseparable from one another.

The *w* deletions were only approximately mapped. Five proved to be *w* deletions equivalent to those described above—viz., they begin at *w* and extend to the left; one proved to be a *w* deletion beginning at *w* and extending to the right to include *N*, which maps to band 3C7; and one proved to be a deletion of only the *w* locus.

To explain the foregoing results, it was assumed that adjacent to the y^+ and w^+ loci on the gt^{13z} X chromosome there occur IS sequences that, under the influence of the *gt* mutants, are perturbed, causing the excision of the IS plus adjacent gene loci and thereby generating the several deletions recovered. (Clusters of *y* deletions mean that perturbation occurs in the germ line before meiosis. The deletion, once produced, is replicated mitotically several times. As a consequence, identical deletions appear in more than one ovum, resulting in a cluster.) This explanation can be tested in two ways. First, no *y* deletions occurred on the $gt\ w^a$ chromosome presumably because the specific IS does not occur adjacent to the y^+ locus on this chromosome. If the y^+ locus of the gt^{13z} chromosome, henceforth designated y^{+13z}, is transferred by crossing over to the $gt\ w^a$ chromosome, the specific IS is simultaneously transferred, and it should now be possible to generate *y* deletions on the $gt\ w^a$ X chromosome. Such a $y^{+13z}\ gt\ w^a$ chromosome was generated by selecting $y^{+13z}\ gt\ w^a$ sons from mothers of the genotype $y\ gt\ w^a/y^{+13z}\ gt^{13z}$. Females of the genotype $y^{+13z}\ gt\ w^a/y^{+13z}\ gt^{13z}$ were obtained and crossed to $y^2\ w^-\ spl\ sn^3$ males. The results of this experiment are given as experiment 3 in Table 1. Four *y* deletions were recovered in this experiment—three on the $gt\ w^a$ X chromosome and one on the gt^{13z} chromosome. All proved to be bidirectional deletions equivalent to the longest described in Fig. 1. Finally, in a subsequent experiment, *y* deletions were recovered among the female progeny of homozygous $y^{+13z}\ gt\ w^a$ females (Table 2, experiment 4), obviating the need for the gt^{13z} mutant.

A second test of the aforementioned hypothesis of IS-generated deletions states that perturbation is in some way associated with mutation at the *gt* locus. Because *gt* mutants are recessive, it follows that no deletions are expected among the progeny of females of the genotype $gt^+\ w^a/gt^{13z}\ w^+$. The results of such an experiment (Table 2, experiment 5) are consistent with expectation: no deletions were recovered among the progeny of heterozygous gt^{13z} females.

It is relevant to emphasize here that the production of deletions is not confined to the action of the *gt* and gt^{13z} mutants but occurs when other mutant *gt* alleles are used in heteroallelic or homoallelic condition. One example suffices to make this point. Thus, gt^{Q292}, which is a male lethal allele induced on an X chromosome carrying a *y* mutant and w^+, was combined in females with $gt\ w^a$. Females of the genotype $y\ gt^{Q292}\ w^+/gt\ w^a$ generate deletions at the *w* locus (Table 2, experiment 6), all occurring on the $y\ gt^{Q292}\ w^+$ X chromosome. Genetic mapping of these deletions established five to involve only the *w* locus and one to involve both *w* and loci to the left.

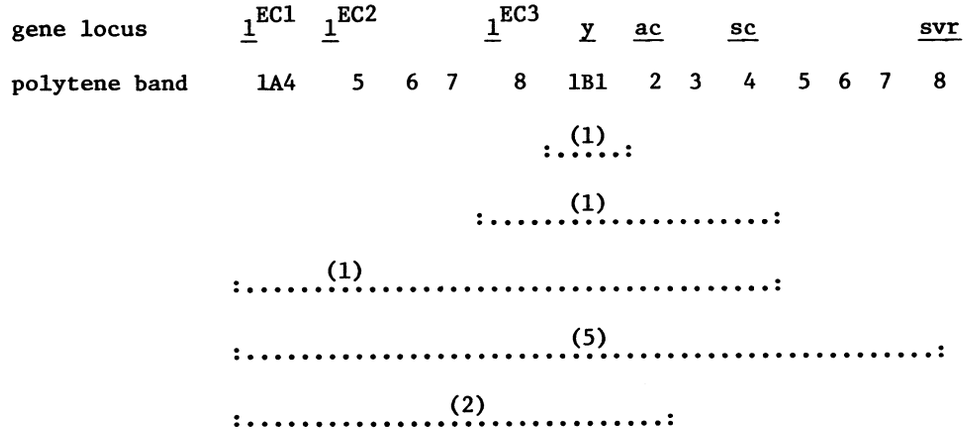

FIG. 1. Cytogenetic definition of deletions involving the *y* locus. Gene localizations are as described by Lefevre (7). The frequency of each deletion is in parentheses.

DISCUSSION

Several conclusions are warranted by the results described.

(*i*) The deletions generated at the *y* and *w* loci are associated with the excision of an IS located proximal to these gene loci. This conclusion is consistent with the already known deletion-generating property of IS in both *E. coli* and *D. melanogaster*.

(*ii*) Because the deletions involving *w* are fixed, endpoint deletions comparable to those generated in IS1 in *E. coli* (8) and those involving *y* are bidirectional, a feature of IS4-generated deletions in *E. coli* (9), it seems reasonable to conclude that two distinctive IS are also involved in *D. melanogaster*.

(*iii*) That some X chromosomes harbor the IS at the sites involved and some do not is consistent with the known results of mapping studies of cloned IS in different genetic stocks of *D. melanogaster*. An IS which maps to one chromosomal site in one stock may or may not map to the same site in another stock.

(*iv*) The high frequency of deletions generated at both loci appears to be a function of perturbations in chromosome replication caused by mutants at the *gt* locus. Although the precise nature of the perturbation remains unclear at this time, DNA replication seems to be disturbed in homoallelic or heteroallelic *gt* flies because, on the average, their larval developmental time is almost doubled; their polytene chromosomes are significantly increased in breadth, implying extra chromosome replications; and the size of the adult flies is giant by virtue of increased cell size rather than cell number. Not all IS respond to perturbation by *gt* mutants. The w^a mutant included in all of the experiments reported is associated with the *D. melanogaster* IS designated copia (10). None of the deletions described involved w^a, indicating that copia is refractory to excision under the influence of *gt* mutants. This supports the suggestion that different IS are associated with the *y* and *w* loci.

(*v*) Some insight into the cause of spontaneous chromosome deletions can be gleaned from the experiments described. If perturbations cause a high frequency of IS-mediated deletions, it can be reasoned that, in the absence of both extrinsic or intrinsic perturbations, spontaneous IS excision will be infrequent but will occur. Stated another way, spontaneous deletions can be mediated by IS sequence excision. There is good reason to believe that other types of spontaneous chromosomal aberrations, translocations, inversions, etc., that occur infrequently also may be intimately associated with IS. The numerical abundance of IS in the *Drosophila* genome argues for their role in spontaneous genetic events.

I thank George Lefevre, Jr., for the cytological determinations reported. The study reported herein was supported by National Institutes of Health Grant GM22221.

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