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Possible influence of *hobo* mobile element on the frequency of somatic mutations in the wing cells of *Drosophila melanogaster*.

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The classic experiments of B. McClintock have demonstrated the induction of somatic mutagenesis by *Ac*-type mobile elements. Molecular analysis of *P*-element transcripts (belonging to *Ac*-type transposons) in somatic and generative tissues demonstrates that *P* element transpositions are tissue-specific and occurring only in generative tissues due to a tissue-specific mRNA splicing (Engels *et al.*, 1987). However, a body of facts is inconsistent with this hypothesis: for example, *P* element transpositions occur premeiotically in the cells dividing by mitosis. Gonadal sterility depends on the temperature of raising of larvae and the period of sensibility begins very early at an embryonic stage when the generative tissue is still being formed.

The *hobo* element is closely related to the *P* element in its characteristics. In this work we study the influence of a full-size *hobo* element in the genome of flies on the frequency of *mwh* somatic mosaics in the wing cells of *Drosophila melanogaster* estimated by somatic mutation and recombination test. We revealed a two-fold increase in frequency of *mwh* mutant spots in fly stocks containing an unstable *hobo* element in the genome.

Materials and Methods

Drosophila melanogaster stocks: *mwh* (provided by U. Graf); *flr^3/TM3,Ser* (provided by U. Graf); *Oregon R* (contains only deleted *hobo* copies (Streck *et al.*, 1986); *Canton S* (lacking *hobo* copies) (Periquet *et al.*, 1994); *C(1)DX, y f;* π_2, (*P*-cytotype stock); *Harwich* (contains *P* elements and is a strong inductor stock); *Uc-1*, contains a full-size *hobo* element in an unstable *Notch* gene, stock provided by J. Lim (Eggleston *et al.*, 1996); *y^+N^+* - recombinant between *$y^{2\text{-}743+}$pn cv v f c B* (strain containing full-size *hobo* copy and unstable for *yellow* gene) and *Uc-1* strain; The presence of *hobo* element in *mwh* and *flr^3/TM3,Ser* stocks has not been studied. Presumably, these stocks do not contain *hobo* (at least, its full-size copy) as most of laboratory stocks.

Crosses: We crossed virgin *mwh* females to the males of studied lines and analyzed the wings of F1 progeny under light microscope at 400×, as described previously (Graf *et al.*, 1992). Only typical *mwh*-type mutations were scored while the single cells with two wing hairs were not counted to rule out morphoses. The results were analyzed in two ways. In Table 1, the mutation frequency is calculated by dividing the total number of mutant spots by the number of wings analyzed. In Table 2, the mutation frequency is calculated by dividing the number of wings carrying the mutant spots by the total number of wings analyzed. Statistical validity of data was calculated by the _ ^{2} method (Graf *et al.*, 1992).

Results and Discussion

In *mwh/y*$^+$*N*$^+$ *a*nd *mwh/Uc-1* heterozygotes containing full-size and deleted copies of *hobo*, the frequency of mutant spots in the wing cells is increased two-fold (Table 1). When calculating the number of cells with mutant spots (Table 2), a statistically valid increase in the number of mutations is detected only in *mwh/y*$^+$*N*$^+$. When scoring only the spots comprised by more than a single cell (to avoid the possibility of scoring a morphosis cell as a mutation), the *mwh/y*$^+$*N*$^+$ and *mwh/Uc-1* heterozygotes demonstrate a multifold increase in the number of such spots, as compared to control stocks lacking *hobo* element.

Thus, the somatic mosaicism frequency in the progeny of crosses between the stocks containing a full-size *hobo* copy and *mwh* stock is increased as compared to the crosses between the stocks lacking *hobo* elements (or its full-size copy) and *mwh*. These results can, possibly, be explained not only by the presence of *hobo* in the genome but also by a genetic background inducing its transpositions. A genetic instability in *Notch* locus detected in *Uc-1* line and in *Notch* and *yellow* loci in *y*$^+$*N*$^+$ line is associated with the presence of the *hobo* element in these lines and induced by dysgenic crosses (Eggleston *et al*., 1996; Zakharenko *et al*., 2000 a, b). An increase in the frequency of *singed* somatic mosaics in males has been shown by Zakharov and colleagues (Zakharov *et al*., 1983). Instability in the *singed-49* allele was later reported to be associated with *hobo* (O'Hare *et al*., 1998). In the presence of the *P* element, a two-fold increase of somatic mutations in the wing of *Drosophila melanogaster* females has been shown when shifting the larvae from 25 to 29^{o}C degrees (Getz and Schaik, 1991). Golubovsky and colleagues have shown that in several fly lines the increase in the proportion of chromosomes carrying *P* elements reduces the mutation frequency in the *singed* gene both in generative and somatic tissues. Whatever the heterogeneity of the effects observed, the mutation events occurring both in generative and somatic tissue should be noted. We believe that *Ac*-type mobile elements can influence the mutation frequency in

Table 1. Frequency of *mwh* mutant spots in wing cells of *Drosophila melanogaster* in heterozygotes (numbers of spots comprised by more than a single cell are given in parentheses)

Line genotype	Number of wings	Number of mutant cells comprised by			Number of mutant spots for a wing x 10^{-2}
		1 cell	2 cells	more than 2 cells	
Crosses with *hobo* (-) strains					
mwh/OregonR	100	4		2	6 (2)
mwh/Canton S	100	7		1	8 (1)
mwh/ flr^3	100	6	3		9 (3)
mwh/Harwich	100	7	1		8 (1)
mwh/C(1)DX,y f; π$_2$	100	6	1		7 (1)
Crosses with *hobo* (+) strains					
mwh/y$^+$*N*$^+$	100	7	4	5	16 (9)
mwh/Uc-1	100	6	3	4	13 (7)

Table 2. Number of wings with 1, 2 or more mutant spots in *mwh* heterozygotes. Number of wings with large spots (comprised by more than a single cell) and/or the number of wings with more than one mutation per wing

Line genotype	Number of wings without spots	Number of wings with a single spot		Number of wings with two or more spots		Proportion of mutant wings x 10^{-2}
		Spot of a single cell	Spots comprised by more than single cell	Spot of a single cell	Spots comprised by more than single cell	
Crosses with *hobo* (-) strains						
mwh/Oregon R	93	5	2			7 (2)
mwh/_anton S	92	7	1			8 (1)
mwh/ flr^3	92	5	3			8 (3)
mwh/Harwich	93	5	1	1		7 (2)
mwh/C(1)DX,y f; π$_2$	93	6	1			7 (1)
Crosses with *hobo* (+) strains						
mwh/y$^+$*N*$^+$	86	5	8		1	14 (9)
mwh/Uc-1	91	2	4	2	1	9 (7)

unstable alleles not only in the germ cell line but also in somatic tissues of *Drosophila melanogaster*.

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