Spontaneous changes in *Drosophila melanogaster* transposable elements and their effects on fitness

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Twenty-eight spontaneous alterations modifying the hybridization banding pattern of six families of transposable elements (*297*, *Foldback*, *copia*, *jockey*, *P* and *hobo*) have been fixed in a set of mutation-accumulation lines of *Drosophila melanogaster*. Their effect on fitness has been studied by competition with the original pattern. Most alterations affecting transposable elements were shown to be rearrangements with no detectable effect on fitness, showing that spontaneous transposable element mutations mainly generate minor fitness mutations.

Keywords: *Drosophila melanogaster*, fitness, population genetics, quantitative variation, transposable elements.

Introduction

Transposable elements (TEs) are a common feature of eukaryotic genomes and can comprise a substantial fraction of genomic DNA, but their evolutionary significance is not well understood. Transposition is known to be mutagenic, as the insertion of elements may disrupt a structural locus or alter its regulation, and chromosomal rearrangements may be generated by ectopic exchange between nonhomologous TEs. The population dynamics of TEs is one of the main problems of population genetics because of their importance for genomic diversity. The relative contribution of selective forces to the maintenance of TEs in natural populations depends on the rate at which rearrangements affecting TEs (new insertions and excisions, recombination between elements, deletions and insertions within elements) occur and the consequences of such events for the genome of the host.

Some theoretical models dealing with the consequences on viability of changes affecting TEs have been elaborated (for a discussion see Biémont *et al.*, 1997; Brookfield & Badge, 1997; Charlesworth *et al.*, 1997), but empirical determinations of the significance of the different parameters are partial and scarce. Direct estimations of variation affecting *Drosophila melano-*

gaster TEs have been made for the euchromatic section of chromosome X, II or the three major chromosomes (by, respectively, Eggleston *et al.*, 1988; Harada *et al.*, 1990; Nuzhdin & Mackay, 1994, 1995), by *in situ* hybridization, and for the whole genome using the Southern blot method (Di Franco *et al.*, 1992; Domínguez & Albornoz, 1996). Rates of movement vary widely depending on the elements tested and the stock analysed. Direct determinations of the effects of TEs on fitness in *D. melanogaster* are limited to the effects of hybrid dysgenesis (Mackay, 1986; Lyman *et al.*, 1996) and show that new *P*-element insertions predominantly induce lethal or detrimental mutations.

In this paper, we study the nature, and effects on fitness, of spontaneous changes affecting six TE families (*297*, *Foldback*, *copia*, *jockey*, *P* and *hobo*) that have been fixed in a set of mutation-accumulation lines of *D. melanogaster* derived from an isogenic population. The homogeneous origin of the lines allowed us to estimate the effects that TE mutations have on fitness by competition between the original and the modified insertion patterns. Most of the observed hybridization pattern changes were simultaneous band gains and losses that were consistent with rearrangements (Domínguez & Albornoz, 1996). The study of the segregation of the different hybridization patterns allowed us to confirm this hypothesis.

Materials and methods

Lines were obtained from a base population which had been made isogenic for all chromosomes following a

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scheme of crosses with balancer chromosomes. The base population carried the recessive eye-colour marker *sepia* (*se*) as an indicator of possible contamination. Starting from the base population, two sets (B and C) of 100 inbred lines each were established (Santiago *et al.*, 1992), either by single brother–sister mating (lines B, denoted B1–B100) or by double-first-cousin mating (lines C, denoted C1–C100). From generation 47, all C lines were also maintained by brother–sister mating. The marker phenotype was verified at each generation. Flies were reared on the standard medium formula of this laboratory (brewer's yeast–agar–sucrose) at $24 \pm 1^\circ\text{C}$. In a previous study (generation 80, Domínguez & Albornoz, 1996), lines were tested for changes in the insertion pattern of nine TE families (*P*, *hobo*, *copia*, *gypsy*, *blood*, *Foldback*, *412*, *297* and *jockey*). At generation 120, there were 171 surviving lines. The lines that showed alterations in the TE pattern at generation 80 were re-tested by means of Southern blots from pools of flies. One line (C66) that showed the original insertion pattern was selected as a control.

The possibility that TE movements have an effect on fitness was tested by competition between the modified insertion pattern and the original one. Each line was crossed with the control to obtain the two reciprocal F_1 s. Two independent replicates, with a frequency of 0.5 of

the altered pattern, were made. Each replicate was founded with 20 pairs, 10 males and 10 virgin females from each of the two reciprocal F_1 offspring. The resultant F_2 and successive descendants were kept in one 250 mL glass bottle under crowded conditions for 10 generations. The entire offspring from each generation was transferred to a fresh bottle. In order to avoid generation overlapping, parents were allowed to lay for 7 days and then discarded. After twelve generations (F_{12}), a sample of females from each replicate was tested for their pattern of TEs by means of Southern blots from individual flies. One fly from the tested line and another from the C66 were included as controls. Frequencies of the modified hybridization pattern were compared with 0.5, the expected value if movements have no effect on fitness.

Individual adult flies were homogenized in 100 μL of extraction buffer (0.1 M NaCl; 0.1 M Tris-HCl, pH 8.4; 20 mM EDTA). Proteinase K and SDS were added to a final concentration of 100 $\mu\text{g mL}^{-1}$ and 1%, respectively, and the mixture was incubated at 65°C for 1 h. After cooling at room temperature for a few minutes, 20 μL of potassium acetate were added. After 30 min on ice, the samples were spun for 5 min in an Eppendorf centrifuge. Ethanol (200 μL) was added to the supernatant, and after 1 h at -70°C , the samples were spun again for 15 min.

Table 1 Changes in banding pattern of transposable elements in *Drosophila melanogaster* at generation 120

Line	TE families					
	297	<i>Foldback</i>	<i>copia</i>	<i>jockey</i>	<i>P</i>	<i>hobo</i>
B1	+					
B37		+, –				
B39	+, –					
B41		+, –				
B42	+, –					
B49		+, –				
B53		+, –				
B59		+, –				
B63		+, –				
B64	+, –				+, –	
B78	+, +, +, –					
B80	–					
B82	–					
B96		+, +, –	+, –			
C20				+		
C23	+, –					
C31		+, –				
C46		+, –				
C65		+, –				+, +, –, –
C67	–	+, –				
C72		+, +, –				

Symbols + and – denote band gain and loss, respectively.

The pellet was washed twice in 70% ethanol, dried under vacuum and resuspended in TE buffer (Sambrook *et al.*, 1989). The DNA extraction from pools of flies, together with the enzymes used to digest genomic DNA and probes for *hobo*, *copia*, *jockey*, *297*, *P* and *Foldback (FB)* transposable elements have been previously described in

Domínguez & Albornoz (1996). Digested DNA was separated by electrophoresis in 30 cm–0.5% or 0.7% agarose gels and TBE buffer (Sambrook *et al.*, 1989). Gels were run at 30 or 65 V for 48 or 24 h, respectively. DNA was transferred to a charged nylon membrane under saline conditions, following the manufacturer's

Fig. 1 Details of the *FB* hybridization pattern. Band gains and losses are indicated by arrowheads.

Fig. 2 (a) Details of the *FB* hybridization pattern showing the segregation of the *FB* rearrangement (*r*) from line B42 in a sample from the F_{12} offspring. (b) Details of the *297* hybridization pattern showing the segregation of the *297* insertion (*i*) and rearrangement (*r*) from line B78 in a sample from the F_{12} offspring.

recommendations. Probe DNA was ^{32}P -labelled by random priming and hybridized to membranes in $6 \times \text{SSC}$, 0.5% SDS, $100 \mu\text{g mL}^{-1}$ denatured herring sperm DNA overnight at 65°C . Membranes were washed twice in $2 \times \text{SSC}$ and 0.1% SDS, once in $0.1 \times \text{SSC}$ and 0.1% SDS at 65°C for 30 min, briefly washed in $0.1 \times \text{SSC}$ at room temperature and autoradiographed for 1–4 days at -70°C with one intensifying screen. Filters were stripped with boiling 0.5% SDS.

Results

Observed differences from the original hybridization pattern are presented in Table 1. Every line conserved the pattern alterations detected at generation 80 and two of them have fixed new changes (lines B78 and C67; compare with table 1 from Domínguez & Albornoz, 1996). The most frequent change was a band gain and a loss (Fig. 1). This kind of pattern alteration may be the consequence of two transposition events, one insertion plus one excision, but the same would be expected from rearrangements leading to the gain or loss of the TE genetic material. These changes were previously interpreted as rearrangements of the element on the basis of their statistical nonindependence (Domínguez & Albornoz, 1996). Analysis of individual hybridization patterns in the competition tests proved this hypothesis: band gains and losses did not segregate; all the homozygotes for a band loss also showed the band gain, as can be seen in the example of Fig. 2a. Thus, they were closely linked or, more probably, they were a consequence of a unique event. Rearrangements affecting the element size or their restriction map will be reflected as band shifts. Therefore, all the changes affecting more than one band that did not show segregation at the F_{12} were classified as rearrangements in Table 2. Most rearrangements were reflected as a band shift, but lines B78 and B96 cosegregated for two gains plus a loss for elements 297 and *FB*, respectively. Single band gains or losses were classified as insertions or excisions, respectively, but some additional work would be necessary to confirm this.

Three lines carried alterations of the hybridization pattern that could be interpreted as a consequence of more than one change in the same TE family. Line B78 has fixed one 297 insertion and the above-mentioned rearrangement that causes two band gains and one loss (Fig. 2b). In line C72 one *FB* insertion and one rearrangement (band gain and loss) could be distinguished, and line C65 bore two *hobo* rearrangements, both reflected as one band shift.

Table 2 also shows the results of competition tests (frequencies attained at the F_{12}) for all TE movements. When only one band gain or loss was segregating, homozygotes were distinguished from heterozygotes by

comparing the relative band intensities, determined with the application GEL READER from NCSA (1991). As Southern blots were made with single flies, a band in a heterozygote will be half as intense as the same band in a homozygote.

Transposable element mutations remained segregating at intermediate frequencies with a general mean of 0.48 ± 0.03 , a value remarkably close to the 0.5 expected for neutrality of TE movements. The distribution (Fig. 3) was slightly negatively skewed ($g_1 = -0.16 \pm 0.44$) and slightly leptokurtic ($g_2 = 1.07 \pm 0.86$). The variance between replicates within lines (0.0195) resulted from the joint effects of 10 generations of genetic drift and the sampling error. The variance between line mean frequencies (0.0344), that include effects of TE movements on viability, was compared with the variance within lines by an analysis of variance. The *F*-value was significant ($F_{27,28} = 5.35$, $P = 0.0007$), denoting that some TE changes affect viability. Individual line frequencies were compared with the 0.5-value. Significance is based on Student's *t*-test, after applying the Bonferroni correction (Rice, 1989). Only two *FB* rearrangements departed from neutrality, one with a positive effect on fitness and the other with a negative effect. Previous tests were based on the normal distribution, which may have some problems because of the small sample sizes. Thus, frequencies were also compared by the nonparametric Kruskal–Wallis test, which gave a *P*-value of 0.0460, again indicating that some TE changes are not neutral.

Discussion

The most frequent alterations affecting TEs (21 out of the 28 movements studied) were rearrangements that were reflected in the hybridization pattern as nonindependent band gains and losses. Only seven changes could be classified as insertion or excision events, but further additional work is necessary to confirm this, because some deletions or amplifications may also be reflected as single changes, depending on the restriction enzyme and the probe used.

For elements *P* and *hobo*, these observations could be related to transposition events, as nonreplicative transposition to a nearby site (Tower *et al.*, 1993). Alternatively, the same pattern differences could be the result of tandem duplications or internal deletions promoted by the element itself, as a consequence of aberrant transposition (Gray *et al.*, 1996; Preston *et al.*, 1996). A prevalence of this kind of rearrangement rather than transposition has been found to be induced by *P*-element transposase (Delattre *et al.*, 1995). The same events have been described in the phenomenon of hybrid dysgenesis associated with the *hobo* element, and there is

Table 2 Nature of the changes experienced by the transposable elements and frequencies attained at F_{12} , after competition tests in *Drosophila melanogaster*

Element	Line	Change	Replicates		Mean frequency
			Frequency	Number	
297	B1	Insertion	A 0.22	9	0.22
			B 0.21	14	
	B39	Rearrangement	A 0.40	10	0.53
			B 0.65	10	
	B42	Rearrangement	A 0.40	15	0.52
			B 0.63	19	
	B64	Rearrangement	A 0.78	18	0.82
			B 0.87	19	
	B78	Rearrangement	A 0.56	8	0.53
			B 0.50	10	
	B78	Insertion	A 0.44	8	0.47
			B 0.50	10	
	B80	Excision	A 0.45	11	0.41
			B 0.37	15	
	B82	Excision	A 0.70	10	0.63
			B 0.55	10	
Foldback	C23	Rearrangement	A 0.35	10	0.48
			B 0.60	10	
	C67	Excision	A 0.38	13	0.42
			B 0.45	11	
	B37	Rearrangement	A 0.72	9	0.44
			B 0.17	9	
	B41	Rearrangement	A 0.70	10	0.57
			B 0.44	16	
	B49	Rearrangement	A 0.72	9	0.75
			B 0.78	9	
	B53	Rearrangement	A 0.39	9	0.37
			B 0.35	10	
	B59	Rearrangement	A 0.83	9	0.89; $P = 0.0133$
			B 0.94	9	
	B63	Rearrangement	A 0.70	5	0.63
			B 0.56	8	
copia	B96	Rearrangement	A 0	9	0; $P = 0.0006$
			B 0	9	
	C31	Rearrangement	A 0.44	9	0.58
			B 0.71	7	
	C46	Rearrangement	A 0.39	9	0.39
			B 0.39	9	
	C65	Rearrangement	A 0.29	7	0.31
			B 0.33	6	
	C67	Rearrangement	A 0.28	9	0.22
			B 0.17	6	
	C72	Insertion	A 0.39	8	0.44
			B 0.50	8	
	C72	Rearrangement	A 0.69	8	0.63
			B 0.56	8	
jockey	B96	Rearrangement	A 0.33	9	0.31
			B 0.28	9	
jockey	C20	Insertion	A 0.50	10	0.36
			B 0.22	9	

Table 2 (Continued)

Element	Line	Change	Replicates		Mean frequency
			Frequency	Number	
<i>P</i>	B64	Rearrangement	A 0.56	9	0.50
			B 0.44	9	
<i>hobo</i>	C65	Rearrangement	A 0.67	9	0.53
			B 0.39	9	
	C65	Rearrangement	A 0.78	9	0.58
			B 0.37	8	
				Mean	

Fig. 3 Distribution of mean frequencies of transposable element (TE) movements at the end of the experiment of competence (F_{12}).

also evidence that they can be promoted by the *FB* family (Bingham & Zachar, 1989). Hybridization pattern alterations for retrotransposons *copia* and 297 were not congruent with transposition-related events. These elements transpose via an RNA intermediate and, consequently, in a replicative way. Therefore, our observations are better interpreted as a consequence of internal rearrangements. Assuming that all the band shifts detected in generation 80 were a consequence of rearrangements (three lines showing this kind of change were lost), the mean rate of rearrangements that results from the data of Domínguez & Albornoz (1996) is 8.5×10^{-6} (23/2 693 600) per element per generation.

DNA loss (Petrov *et al.*, 1996; Petrov & Hartl, 1998) may be a factor, common to all the families studied, leading to the observed hybridization pattern alterations. The reported rate of deletion was approximately 0.16 times the nucleotide substitution rate. This rate in *Drosophila* has been estimated as 8.5×10^{-9} per base per generation (Drake *et al.*, 1998). The mean size of the nine

families tested by Domínguez & Albornoz (1996) was 5.3 kb. Thus, the rate of deletion would be of 7.21×10^{-6} , a value very close to the above-estimated rate of rearrangement. Unequal crossing-over may be another factor involved in rearrangements. Ectopic exchange between copies of retrotransposons in heterozygosis have been shown to produce rearrangements (Goldberg *et al.*, 1983; Davis *et al.*, 1987), but this can be excluded because the starting population was homozygous.

It has been suggested (Junakovic *et al.*, 1997) that host factors play a significant role as regulators of the activity of TEs. Our experimental material probably reflects a stable genotype where transposition of the different TE families is repressed. There was no *P* or *hobo* hybrid dysgenesis during the construction of the base population, and the frequency of movements that could be classified as 'true' insertions or excisions was very low: 8.7×10^{-7} and 1.3×10^{-6} , respectively (Domínguez & Albornoz, 1996). Consequently, it can be deduced that, in the absence of genomic instability, the main source of spontaneous variation for TEs will be small, probably internal, rearrangements, either promoted by the element itself or by general mechanisms (DNA loss or unequal recombination).

The competition experiment between the original and modified hybridization patterns showed that TE mutations mainly have minor fitness effects. Only two out of the 28 TE mutations analysed have effects on fitness, one negative and the other positive. These two detected effects may not be associated with the TE rearrangements *per se*, because either the control or the tested lines may have accumulated other mutations. The apparent effect of a particular TE mutation could be caused by a cis-linked mutation with the same effect or a trans-linked one with the opposite effect. Attention must be paid primarily to the distribution of frequencies, which was symmetrical and with a mean very close to 0.5, showing that the contribution of the overall variation of TEs to fitness was small or negligible. As TE movements are a common class of spontaneous

mutation event in *Drosophila* (Green, 1988), our results are consistent with recent observations of small changes in fitness traits in long-term mutation-accumulation experiments using the same set of lines (Fernández & López-Fanjul, 1996, 1997) and other experiments with a similar design (Keightley & Caballero, 1997).

The distribution of spontaneous mutant effects on fitness is highly dependent on the technique used to isolate them. Mutations accumulating against a balancer chromosome are effectively sheltered from natural selection. In inbred lines, however, the fixation probability of unconditionally deleterious mutations will be small and most fixed mutations will be neutral or mildly detrimental, that is, those having a major role on population survival (Lande, 1995). Both methods result in partially overlapping descriptions: accumulation against a balancer chromosome gives a better representation of the original distribution of mutant effects, whereas the distribution obtained from inbreeding is more relevant to the genetic variation in natural populations after purging selection has acted.

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