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Differential regulation of *P* and *hobo* mobile elements by two laboratory strains of *Drosophila melanogaster*

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Summary

Analysis of the transposition behaviour of the *P* and *hobo* elements borne by the *31·1/CyL⁴ MRF* (*P*), *23·5Δ/CyL⁴ MRF* (*hobo*) and *23·5*/Cy MRF* (*hobo*) strains in the progeny of dysgenic crosses with two ME laboratory strains (*Berlin-k* and *dp b cn bw*) at 25 °C revealed that: (*a*) the two ME laboratory strains affect differently the transposition rates of *P* and *hobo* elements. More precisely, *P* element transposition is higher in heterozygotes with *dp b cn bw* than in those with *Berlin-k*. In contrast, the transposition rate of *hobo* elements is higher in *Berlin-k* than in *dp b cn bw* heterozygotes. (*b*) Like *P*, *hobo* has the potential to transpose at high frequencies and to non-homologous chromosomes. (*c*) The dysgenically inactive *hobo* elements of the *31·1 MRF* strain transpose more frequently than the dysgenically active *hobo* elements of the *23·5 MRF* strains in certain crosses. (*d*) There are insertion hot spots for *P* and *hobo* elements. For the *P* elements there are enough data to suggest that the insertion hot spots are different in the two EM strains. The data are discussed on the basis of the involvement of putative host factors in transposition regulation of the *P* and *hobo* elements.

1. Introduction

The genomes of most, if not all, organisms contain moderately repetitive DNA sequences that are able to move. These are known as transposable elements. In *Drosophila melanogaster* more than fifty families of transposable elements have been identified. Among them the best studied ones, are *P*, *hobo* and *I*, the reason being that their mobilization is associated with genetic syndromes of hybrid dysgenesis (Kidwell *et al.* 1977). These syndromes are characterized by a series of genetic abnormalities, such as gonadal dystrophy (GD) sterility for *P* and *hobo* and embryo lethality for *I*, male recombination, insertion mutations, chromosome breakage, chromosome rearrangements, non-disjunction etc. among the progeny of crosses involving inducers of dysgenesis. The *P* and *hobo* elements share some dysgenic and structural similarities (for review see Louis & Yannopoulos, 1988; Engels, 1989; Blackman & Gelbart, 1989) and belong to the general family of elements with short inverted repeats that includes the *Ac* elements of maize and the *Tam3* elements of *Antirrhinum majus* (Calvi *et al.* 1991). A characteristic feature of these elements is that they code for a transposase, an enzyme that catalyses their own movement (O'Hare & Rubin, 1983; Karess & Rubin, 1984; Blackman *et al.* 1989). The *I* element, in contrast, is a retroposon (LINE-like) and thus, it transposes via an RNA intermediate (Fawcett *et al.* 1986; Finnegan, 1989).

It has been estimated, that under non-dysgenic conditions *P* elements transpose at a rate of about 0·29/X chromosome/generation, while a much higher rate can be observed in outcrosses involving M strains (Bingham, Kidwell & Rubin, 1983; Eggleston *et al.* 1988). This rate can be as high as 3·45 when specific *P* strains are used (Ajioka & Eanes, 1989; Robertson *et al.* 1988). On the other hand, nothing is known about the transposition rate of the *hobo* elements under dysgenic conditions. The only available data concern those obtained by Harada *et al.* (1990) who estimated, indirectly, transposition rates of 0·02 and 0·05 per second chromosome per generation, for two lethal second chromosome lines of different origin.

The available data support the view that the transposition rates of *P* elements depend on the structure of the elements themselves, their integration sites in the host genome and most probably on the genetic background of the host individuals (Spradling & Rubin, 1983; Levis *et al.* 1985; Robertson *et al.*

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1988; Eeken *et al.* 1991). *P* element insertion and excision occur only in the presence of *P* transposase (O'Hare & Rubin, 1983). However, the finding that *P* transposase recognizes internal sites on *P* element DNA (Kaufman *et al.* 1989), while another protein interacts with the terminal inverted repeats (Rio & Rubin, 1988), suggests that non-*P* element-encoded proteins may be required for efficient transposition. This notion is further substantiated by genetic data showing that various ME laboratory strains respond differently to the dysgenic activities of the same *P* and *hobo* inducer strain (Stamatis *et al.* 1981; Yannopoulos *et al.* 1987; Daniels *et al.* 1988).

The *P-M* and *hobo* systems of hybrid dysgenesis are independent of each other (Stamatis *et al.* 1989) and available data support the view that *P* transposase does not promote the movement of other families of transposable elements, including *hobo* (Woodruff *et al.* 1987; Eggleston *et al.* 1988). Little is known about the putative *hobo* transposase as it has not yet been identified. However, Blackman *et al.* (1989) described an autonomous *hobo* element that is able to mediate its own transposition as well as the mobilization of other non-autonomous elements in germ line transformation experiments.

In the present study we analysed by *in situ* hybridization the transposition behaviour of *P* and *hobo* elements borne by the strains *31·1/CyL⁴ MRF* (*P*) and *23·5Δ/CyL⁴* (*hobo*) and its derivative *23·5*/Cy MRF* (*hobo*) in dysgenic crosses with two laboratory strains *Berlin-k* and *dp b cn bw* that completely lack both *P* and *hobo* elements (ME strains). The analysis revealed that on average the *P* transposition rate was higher in the *dp b cn bw* heterozygotes while the *hobo* transposition rate was higher in the *Berlin-k* heterozygotes. Moreover, for the *P* elements for which there are enough data, were shown to insert at different hot spots in the two EM strains.

2. Materials and methods

The following strains of *Drosophila melanogaster*, maintained at 25 °C were used (for description of mutants and laboratory strains see Lindsley & Grell, 1968):

(i) *31·1/CyL⁴ MRF*, a genuine *P* strain established from a wild second chromosome isolated in 1972 form a Greek natural population. It also contains *hobo* elements (complete and defective) without any noticeable *hobo* dysgenic induction potential, but it possesses strong *hobo* repressor abilities (Yannopoulos & Pelecanos, 1977; Yannopoulos *et al.* 1987; Stamatis *et al.* 1989).

(ii) *23·5Δ/CyL⁴ MRF*, a *hobo* strain established from a wild second chromosome isolated from the same natural population with *31·1/CyL⁴ MRF* in 1978. (Stamatis *et al.* 1981).

(iii) *23·5*/Cy MRF*, a *hobo* strain established from *23·5Δ/CyL⁴ MRF* through the replacement of all chromosomes, except the *23·5 MRF* second chromosome, from those *Cy/Sp* ME laboratory strain (Yannopoulos *et al.* 1987). The *23·5 MRF* (*hobo*) strains also contain complete and defective *P* elements, possess *P* cytotype, but low if any *P* induction potential (Q strains in the *P-M* system) (Yannopoulos *et al.* 1986, 1987). *31·1/CyL⁴ MRF* (*P*) strain expresses its higher dysgenic activities at 29 °C while *23·5Δ/CyL⁴ MRF & 23·5*/Cy MRF* (*hobo*) strains at 25 °C (Stamatis *et al.* 1981; 1989).

(iv) *Berlin-k* a wild type laboratory strain lacking both *P* and *hobo* elements (ME strain) (Eeken, 1982).

(v) *dp b cn bw*, a multimarker old laboratory strain also devoid of *P* and *hobo* elements (ME strain).

(vi) *C(1)D y w f* an attached-*X* chromosome strain devoid of both *P* and *hobo* sequences.

The mating scheme used for the estimation of *P* and *hobo* transposition rates on the X chromosomes of *Berlin-k* and *dp b cn bw* laboratory strains is shown in Figure 1. More precisely, virgin *Berlin-k* and *dp b cn bw* females were mass-crossed to *31·1/CyL⁴ MRF* (*P*), *23·5Δ/CyL⁴ MRF* (*hobo*) and *23·5*/Cy MRF* (*hobo*) males at 25 °C (6 vials for each cross). F1 males of the genotypes given in Fig. 1, were then mass-backcrossed separately with *Berlin-k* or *dp b cn bw* virgin females at 19 °C depending on the ME strain used in the original cross (A) (10 vials for each cross). B1 third instar female larvae (taken at random from the 10 vials) from the last crosses were then used for salivary gland squashes. Furthermore, F1 *CyL⁴/Berlin-k* sons of the cross ♀♀ *Berlin-k* X *31·1/CyL⁴* ♂♂ were mass crossed to *C(1)D y w f* virgins at 25 °C (B) (6 vials). Heterozygotes for the *CyL⁴* male progeny of the genotype *CyL⁴/+* (+ refers to the second chromosome of the *C(1)D y w f* strain) of this cross were then mass crossed to *Berlin-k* virgins at 19 °C (10 vials). Third instar female larvae 'B2' of this cross were then used for salivary gland squashes. All the strains used were raised in a normal corn meal-agar-sugar medium, while the larvae used for salivary gland squashes were raised in a medium consisting of 10% glucose, 10% heat-killed yeast and 3% agar.

Salivary gland polytene preparations and *in situ* hybridizations were essentially carried out as described by Saunders *et al.* (1989). Probes were labelled with Bio-16-UTP (BCL-Boehringer Mannheim) by priming with random oligonucleotides. Probes used in hybridization analysis were *pπ25·1* (O'Hare & Rubin, 1983), a recombinant carrying the full-length *P* element plus genomic DNA from cytogenetic region 17C and the recombinant *pHcSac*, a 3016-bp full-length *hobo* element, subcloned in the *Sac* I site of *pUC9*, flanked by genomic DNA derived from the cytogenetic region 96E (Stamatis *et al.* 1989). Hybridization was detected using Avidin DH and biotinylated horseradish peroxidase H reagents (Vector Laboratories). The 17C and 96E labels were used as positive controls of *P* and *hobo* hybridizations, respectively. *P* and *hobo* insertions were mapped on the basis of the photographic

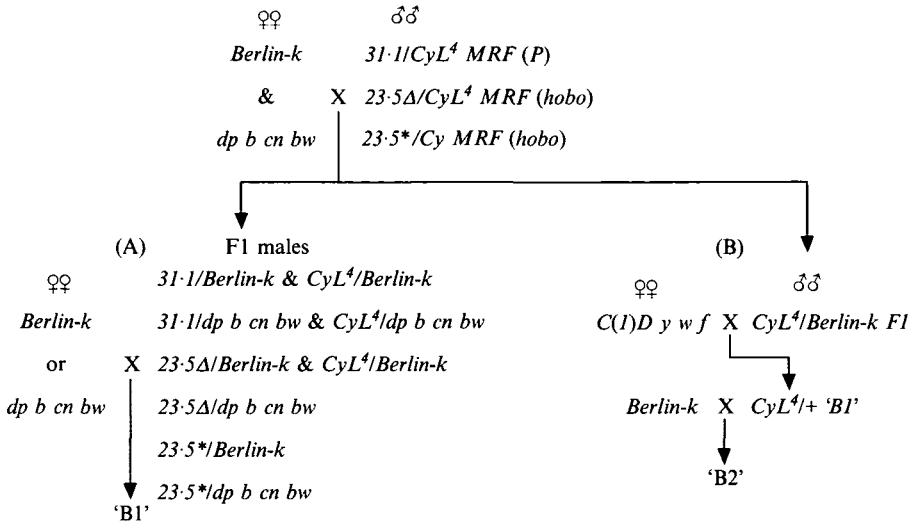

Fig. 1. Mating scheme used for the estimation of *P* and *hobo* transposition rates on the X chromosomes of *Berlin-k* and *dp b cn bw* ME strains per one (A) and per two (B) dysgenic generations.

maps of Lefevre (1976) and the cytogenetic maps of Bridge (1938).

For the estimation of the female GD sterility induced by the *31·1/CyL⁴ MRF (P)*, *23·5/CyL⁴ MRF (hobo)* and *23·5*/Cy MRF (hobo)* strains, F1 female progeny emerged from each cross of Fig. 1 at 25 °C were tested for GD sterility as described by Yannopoulos (1978). The frequency of the female sterility given in Table 3, was calculated as the percentage of the atrophic ovaries scored {number of dystrophic ovaries/(number of dissected females × 2) × 100}. Male sterility was calculated as the percentage of the tested F1 males that did not produce any progeny when crossed individually to three virgin *Berlin-k* or *dp b cn bw* females. The sterile males were also examined for rudimentary testis. The transposition rates and the values of sterility were not measured simultaneously. The test of significance applied is the evaluation of the ratio *d* (difference between two independent Poisson varieties) = $\bar{X}1-\bar{X}2/\sqrt{(\bar{X}1/N1+\bar{X}2/N2)}$, ($d > 1{\cdot}96$, $P < 0{\cdot}05$) (Parker, 1976).

3. Results

In order to analyse the transposition behaviour of the *P* and *hobo* elements of the *31·1/CyL⁴ MRF (P)*, *23·5Δ/CyL⁴ MRF (hobo)* and *23·5*/Cy MRF (hobo)* strains, when crossed to *Berlin-κ* and *dp b cn bw* laboratory strains, we counted the *P* and *hobo* insertions on the X chromosomes produced by the dysgenic males shown in Fig. 1. The results are summarized in Table 1.

(i) P *and* hobo *transposition rates associated with the activity of* 31·1 MRF

The highest *P* insertion frequency per dysgenic generation occurred on the *X* chromosome of the *31·1/dp b cn bw* dysgenic males. The insertion frequency (i.f.) was calculated to be 2·95, (line 1). However, in these males, not a single *hobo* insertion was observed on 28 X chromosomes examined. In contrast, the number of *P* insertions observed on the *X* chromosomes of the *31·1/Berlin-k* males were significantly lower: the i.f. being 1·40 ($d = 3{\cdot}55$, $P < 0{\cdot}05$) (line 2). Furthermore, in these dysgenic males *hobo* transposition did occur, giving an i.f. of 0·43 (line 2). A similar behaviour was exhibited by the *P* and *hobo* elements in the F1 dysgenic males *CyL⁴/dp b cn bw* and *CyL⁴/Berlin-k*, although here the corresponding insertion frequencies were much lower (i.f. for *P* = 1·64 and 0·97) ($d = 2{\cdot}09$, $P < 0{\cdot}05$), i.f. for *hobo* = 0·08 and 0·27 respectively, lines 3 and 4).

The transposition rates of the *P* and *hobo* elements were also counted in the heterozygous *CyL⁴* derived from the cross ♀♀ *Berlin-k* X *31·1/CyL⁴* ♂♂, after two dysgenic crosses. In these 'B2' *CyL⁴/+* males (line 5), the corresponding *P* and *hobo* insertion frequencies were 1·94 and 1·90 respectively; the *P* insertions were almost doubled, while the *hobo* insertions increased almost sevenfold in one additional dysgenic generation (these frequencies may be an underestimate because a number of putative lethal insertions would have been removed from the 'B2' males). Thus, although, there is a significant difference between the number of *P* and *hobo* insertions on the X chromosome of the F1 *CyL⁴/Berlin-k* males ($d = 3{\cdot}55$, $P < 0{\cdot}05$) (line 3), the *P* and *hobo* insertions were almost similar after two dysgenic generations.

(ii) P *and* hobo *transposition rates associated with the* 23·5 MRF *activity*

Analysis of the activity of the *23·5 MRF* crosses revealed that the *hobo* transposition frequency exceeds that of *P*. More precisely, *P* transposition was absent

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Table 1. P *and* hobo *elements inserted on the X chromosomes of* Berlin-k *and* dp b cn bw *ME strains in dysgenic crosses with the* 31·1 MRF (P) *and* 23·5 MRF (hobo) *strains per generation (lines 1–4 and 6–10) and per two generations (line 5), at 25 °C. The table also includes the mean number* ± S.D. *(standard deviation) of* P *and* hobo *insertions scored in the corresponding F1 dysgenic male larvae*

	P elements				*hobo* elements			
Genotype of the dysgenic males[a]	No. of insertions scored	No. of X chromosomes examined	Insertion frequency	No. of *P* insertions in F1 ♂♂ larvae[c]	No. of insertions scored	No. of X chromosomes examined	Insertion frequency	No. of *hobo* insertions in F1 ♂♂ larvae
1 31·1/dp.[b]	65	22	2·95	27·6 ± 1·0	0	28	0	81·2 ± 1·4
2 31·1/B-k	35	25	1·40	27·6 ± 1·0	12	28	0·43	81·2 ± 1·4
3 CyL^4/dp.	36	22	1·64	32·1 ± 1·4	2	25	0·08	93·8 ± 1·3
4 CyL^4/B-k	33	34	0·97	32·1 ± 1·3	8	30	0·27	93·8 ± 1·3
5 CyL^4/+[d] 'B2'	64	33	1·94	ND	59	31	1·90	ND
6 23·5Δ/B-k	1	28	0·04	23·1 ± 1·3	53	29	1·83	59·2 ± 1·7
7 23·5Δ/dp.	0	27	0	23·1 ± 1·3	5	27	0·19	59·2 ± 1·7
8 CyL^4Δ/B-k	0	29	0	11·0 ± 2·1	5	34	0·15	20·2 ± 1·6
9 23·5*/B-k	4	29	0·14	52·8 ± 1·7	11	27	0·41	43·6 ± 1·4
10 23·5*/dp.	7	27	0·26	53·8 ± 1·7	4	29	0·14	43·6 ± 1·4

[a] Transpositions occurring in the F1 (lines 1–4 and 6–10) and F2 dysgenic males (line 5) were detected in the next generation by backcrossing them to *Berlin-k* or *dp b cn bw* virgins. For more details see text and Fig. 1.

[b] dp. stands for the *dp b cn bw* chromosome and B-k for the *Berlin-k* strain.

[c] Five chromosome preparations for each inducer chromosome were used to calculate the mean number of insertions.

[d] + refers to the second chromosome from the *C(1)D y w f* strain.

ND, not determined.

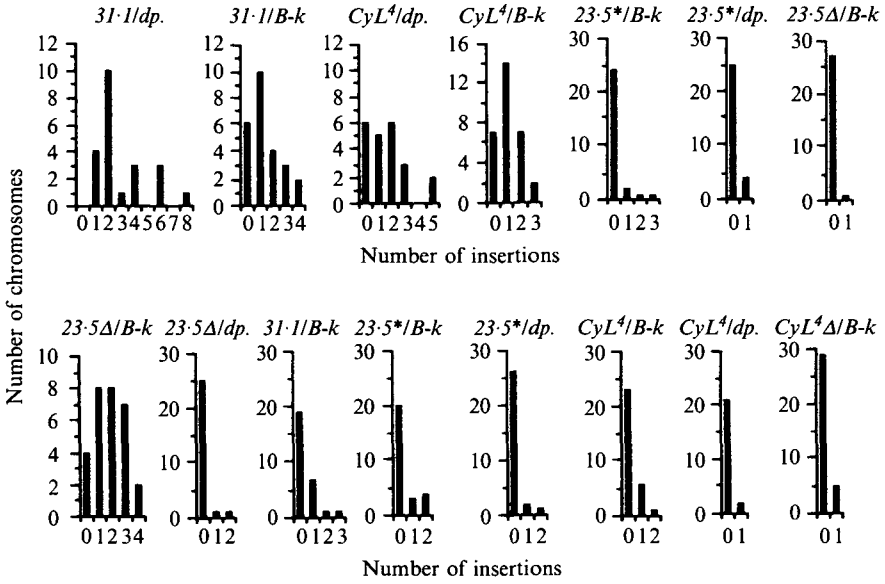

Fig. 2. Distributions of the number of *P* and *hobo* insertions per X chromosome per generation (the dysgenic males without insertions are not included).

or very low in *23·5Δ/Berlin-k* and *23·5Δ/dp b cn bw* dysgenic males (lines 6 and 7), but higher in the corresponding heterozygotes with *23·5* MRF* chromosome: *23·5*/Berlin-k* gave an i.f. of 0·14 (line 9) while for *23·5*/dp b cn bw* the i.f. was 0·26 (line 10). The highest transposition rate of *hobo* (i.f. = 1·83) was observed on the X chromosome of the *23·5Δ/Berlin-k* dysgenic males (line 6). The corresponding *23·5Δ/dp b cn bw* males gave a much lower transposition rate (i.f. = 0·19, line 7) ($d = 6{\cdot}31$, $P < 0{\cdot}05$). The *hobo* insertion frequency observed in the *CyL⁴/Berlin-k* was also low (i.f. = 0·15, line 8). Finally, the *hobo* transposition rates observed in heterozygous *23·5*/Berlin-k* males was almost threefold higher than that observed in the *23·5*/dp b cn bw* heterozygotes: i.f. = 0·41 and 0·14 respectively (lines 9 and 10) ($d = 1{\cdot}93$, $P = 0{\cdot}054$ very close to the statistically significant level).

The transposition rates observed in the heterozygous males *31·1/dp b cn bw*, *CyL⁴/dp b cn bw*, and *23·5Δ/Berlin-k* which exhibited high values of male sterility, may be an underestimate because of a possible selective elimination of some transposition events in the germ line with a high rate of transposition; GD sterility can affect more often germlines which suffer high transposition rates. Such an assumption implies, of course, the limitation that for the observed sterility responsible is mainly the X chromosome.

Taking into consideration the data given in Table 1, it becomes clear that on average the *hobo* transposition rate is higher in *Berlin-k* heterozygotes than in the *dp b cn bw* ones, while the opposite is true for the transposition of *P* elements. Since none of the chromosome preparations having more than one hybridization site had identical sites, the possibility that premeiotic events affected the estimated insertion frequencies is negligible.

The distribution of the number of *P* and *hobo* element insertions on the X chromosomes of the dysgenic males examined in one dysgenic generation (see Table 1) given in Figure 2, did not deviate significantly from Poisson expectations (data not shown).

The mean number of *P* and *hobo* elements borne by the F1 heterozygous *31·1 MRF* and *23·5 MRF* males, and contributing to the X chromosome transposition, is also given in Table 1 (column 4 for *P* and column 8 for *hobo*). These numbers include only the *P* and *hobo* elements borne by one second, one third and one fourth homologous chromosome, since all the heterozygous males of Table 1 inherit their X chromosome from their mothers.

(iii) *Location of the* P *and* hobo *insertions on the X chromosome*

The location of the *P* and *hobo* insertions across the X chromosome is presented in Figure 3. Visual inspection of the distribution of 245 *P* element insertions and 159 *hobo* element insertions reveals that the distribution of each family is not uniform over Bridges' subdivisions and that there are hot spots for both elements. In order to evaluate the putative hot spots for both the *P* and *hobo* insertions, the 95% confidence limits were used as a reference point {when the sample size is large (greater than 50), the actual distribution may be approximated by the normal distribution (Snedecor & Cochran, 1972)}. Subdivisions with number of insertions falling outside the range $\bar{X} + 1{\cdot}96$ S.D. ($\bar{X}$ = mean number of insertions/subdivision, S.D. = standard deviation) were considered as putative hot spots. The data are given in Table 2 along with the corresponding values for the *de novo P* insertions reported by Ajioka & Eanes (1989). The locations of

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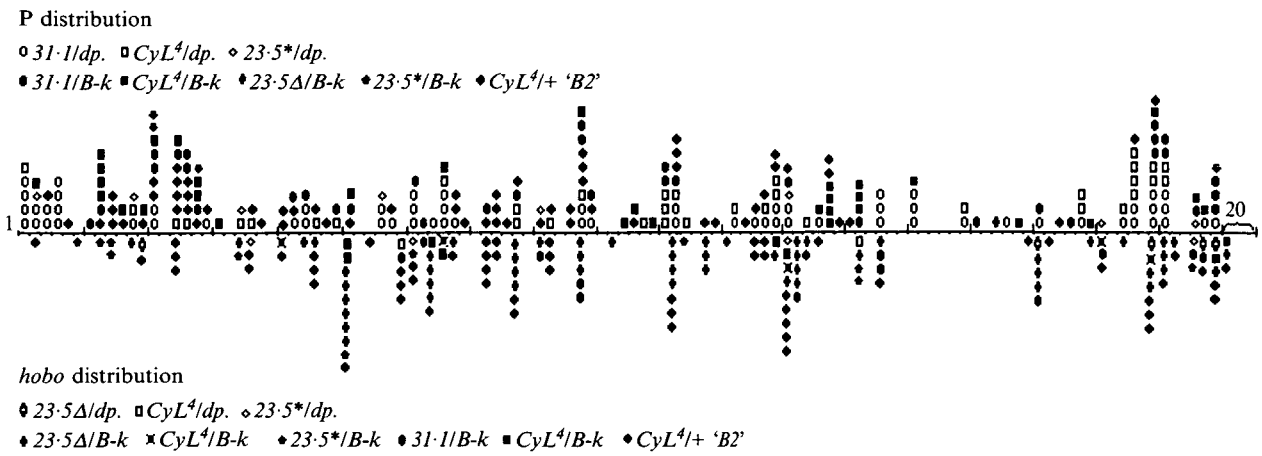

Open symbols denote insertions on the X of *Berlin-k* (*B-k*) strain and black symbols on the X chromosome of *dp b cn bw* (*dp.*) strain. The CyL^4 chromosome II comes from the *31·1/*CyL^4 *MRF* strain while the $CyL^4\Delta$ chromosome II from the *23·5Δ/*CyL^4 *MRF* strain.

Fig. 3. Chromosomal locations of de novo *P* and *hobo* element insertions along the X chromosomes of *Berlin-k* and *dp b cn bw* ME strains.

Table 2. *Mean values* ($\bar{X}$), *standard deviations* (S.D.), *sizes of the putative hot spots and the location of putative hot spots for the* P *and* hobo *insertion on the* X *chromosomes of* Berlin-k *and* dp b cn bw *given in Fig. 2*

X chromosomes	$\bar{X} \pm$ S.D.	Size of hot spots	Bridges' map location of putative hot spots
P elements			
Berlin-k	1·19 ± 1·57	⩾ 5	2B, 3A, 3C, 3D, 3E, 9E, 13E, 18F
dp b cn bw	0·94 ± 1·30	⩾ 4	1A, 1D, 7D, 12F, 18D, 18F, 19A
Total *P*	2·13 ± 2·22	⩾ 7	3A, 3C, 9E, 11B, 18D, 18F, 19A
Total *hobo*[a]	1·41 ± 2·02	⩾ 6	6A, 7C, 8E, 11B, 13A, 18F
Data of Ajioka & Eanes	2·14 ± 2·12[b]	⩾ 7	2C, 6D, 7D

[a] The *hobo* insertions are not given separately for the *Berlin-k* and *dp b cn bw* X chromosomes because of the limited number detected on the *dp b cn bw* X chromosome (11 insertions).
[b] These values were estimated from the data given in Fig. 1 of Ajioka & Eanes (1989).

the *P* and *hobo* hot spots, generally do not coincide. Only two (11B and 18F) out of the 7 *P* and 5 *hobo* hot spots are common. Comparison of the *P* hot spots on the X chromosomes of *Berlin-k* and *dp b cn bw* (8 and 7 hot spots, respectively) shows that only one hot spot, at 18F, was common. Finally, comparison of our data on *P* distribution to those of Ajioka & Eanes (1989) which concern the distribution of *de novo P* insertions recovered from the dysgenic cross z^a w^{ch} X *π2* (*P*), showed that only one hot spot (6D) out of the 3 was common to the corresponding 7 hot spots on the X chromosome of the *dp b cn bw* strain (see Table 2). However, the *in situ* hybridization of Ajioka & Eanes (1989) was performed one or two generations after the primary dysgenic cross. Therefore, residual dysgenesis may have affected the copy number and distribution of the *P* insertions along the z^a w^{ch} X chromosome. Furthermore, differences were detected on the number of *P* insertions/division (the empty division 29 was omitted) on the X chromosomes of *Berlin-k* and *dp b cn bw*, by the contingency χ^2 test ($\chi^2 = 42{\cdot}85$, D.F. = 18. $P = 0{\cdot}0008$), for which the divisions 1, 2 and 3 with 14-3, 3-14 and 5-24 *P* insertions on the *Berlin-k* and *dp b cn bw* X chromosomes, respectively, are responsible. Leaving out these divisions, the rest do not show significant differences ($\chi^2 = 18{\cdot}5$, D.F. = 15, $P = 24$). The above data support the view that the integration sites for the *P* element could be influenced by the host strains. Because of the limited number of *hobo* insertions seen on the X chromosome of the *dp b cn bw* strain no adequate analysis could be carried out.

(iv) *GD sterility*

The female and male GD sterility caused by the *31·1/*CyL^4 *MRF* (*P*), *23·5Δ/*CyL^4 *MRF* (*hobo*) and *23·5*/Cy MRF* (*hobo*) males when mass-crossed to

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Table 3. *Frequencies of GD sterility in F1 females and males caused by* 31·1/CyL[4] MRF (P), 23·5Δ/CyL[4] MRF (hobo) *and* 23·5*/Cy MRF (hobo) *males when mass-crossed to virgin* Berlin-k *or* db b cn bw *females at 25 °C*

Genotype of the F1 progeny examined	Female sterility		Male sterility	
	No. of ♀♀ examined	Sterility (%)	No. of ♂♂ examined	Sterility (%)
31·1/dp.[a]	161	58·0	40	60·0
31·1/B-k	72	9·1	40	5·0
CyL[4]/dp.	75	48·0	30	37·0
CyL[4]/B-k	84	8·3	30	3·3
23·5Δ/B-k	90	85·0	40	32·5
23·5Δ/dp.	149	72·0	40	7·5
CyL[4]/B-k	114	10·0	30	3·3
23·5*/B-k	166	64·0	40	20·0
23·5*/dp.	122	25·0	30	12·5

[a] dp. stands for the *dp b cn bw* chromosome and B-k for the *Berlin-k* strain. From each cross the males and females tested for sterility are brothers and sisters. The sterile males were dissected and showed to possess dystrophic testis.

virgin *Berlin-k* and *dp b cn bw* females at 25 °C is given in Table 3. Comparing the data of *P* and *hobo* transposition with those of the GD sterility associated with the *31·1 MRF* and *23·5 MRF* activity, it becomes clear that there is a tendency for a positive relationship between these two parameters.

4. Discussion

The data of the present study suggest that the transposition rates of *P* and *hobo* are not only determined by their respective transposases and repressors, but also by host factors. As neither the *Berlin-k* nor the *dp b cn bw* strains carry any *P* or *hobo* sequences, the different transposition rates observed cannot be attributed to any difference in *P* or *hobo* repressors. In particular, specific *P* or *hobo* derivatives, such as *KP* which are involved in *P* regulation (Black *et al.* 1987) or *Th*, that may be implicated in *hobo* regulation (Periquet *et al.* 1989) cannot account for the differences in the rates of mobilization of the two elements. Both *P* cytotype (Sved, 1987) and *hobo* repressor (our unpublished data) need the presence of *P* and *hobo* elements to be developed or maintained. Thus, the differences must be due to other strain-specific factors such as host-encoded proteins involved in the *P* and *hobo* transposition processes either by enhancing or reducing the production of the *P* or *hobo* transposases, or through inactivation of *P* or *hobo* transposases, respectively. Based on this assumption the *dp b cn bw* strain must carry factors that either enhance *P*-element transposition or reduce *hobo* transposition. The opposite must be true with the *Berlin-k* strain factors; they must enhance the insertion rate of *hobo* while reducing that of *P*. That host-encoded proteins may be involved in the *P* element transposition process was shown by Rio and Rubin (1988) who isolated a host-encoded protein binding to a region of the terminal inverted repeats. The *P* transposase recognizes internal sites on *P* element DNA (Kaufman *et al.* 1989). The large differences in *P* insertion rates observed between the *31·1/dp p cn bw* and *31·1/Berlin-k* dysengic males (2·95 and 1·40 respectively) and the disparity in the transposition rates of *hobo* when comparing the *23·5Δ/Berlin-k* and *23·5Δ/dp b cn bw* hybrids (1·83 and 0·19, respectively) could perhaps lead to a genetic and molecular characterization of these factors. Two alternative, although not mutually exclusive explanations for these differences may be reflected. First, the chromatin structure of the ME strains: *P* elements are known to have a tendency to insert in the 5′ upstream regions of genes (Tsubota *et al.* 1985; Chia *et al.* 1986; Searles *et al.* 1986; Kelley *et al.* 1987), and it has also been shown that the site of integration of *P* elements affects their activity (Spradling & Rubin 1983; Levis *et al.* 1985; Eeken *et al.* 1991). Secondly, yet unknown hybrid dysgenesis systems could be implicated in the transposition behaviour of *P* and *hobo* elements.

The rates of transposition of *P* elements per X chromosome per generation observed in this study are among the highest reported for *P* strains. Under conditions of hybrid dysgenesis, Bingham *et al.* (1982) estimated rates of *P* transposition of about 0·80 per X chromosome per generation. In reciprocal, non-dysgenic crosses, the transposition rate was much lower and estimated to be 0·29. On the other hand, Ajioka & Eanes (1989) observed an average rate of 2·27 per X chromosome; this frequency may be an overestimate since the *in situ* hybridization was performed one or two generations after the primary dysgenic cross and the residual dysgenesis may have increased the number of the copy insertions. The transposition rate estimated for the *31·1/dp b cn bw*

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males was 2·95, a value approaching the transposition rate of 3·45 observed by Robertson *et al.* (1988) when defective *P* elements on the second chromosome of the M′ Birmingham strain were mobilized by a transposase-producing *P* strain carrying the construct *P*[*ry*$^{+}$*Δ2-3*](*99B*).

The rates of transposition of the *hobo* elements reported in this study are also higher than those indirectly estimated by Harada *et al.* (1990). Like *P*, *hobo* has the potential to transpose at high frequencies and to non-homologous chromosomes.

The seven-fold increase in the rate of transposition of the *hobo* elements in the 'B2' *CyL*^{4}/+ males can be attributed either to the fact that the number of the active *hobo* element(s) were increased because of the transposition during the first dysgenic cross, or they moved to new sites where they were more active. Moreover, the dysgenic conditions could affect the chromatin structure making the *hobo* elements to be more prone to transposition or the chromosomes to be more susceptible to *hobo* insertions.

The finding of *hobo* transposition in the *31·1/B-k*, *CyL*^{4}*/dp b cn bw* and *CyL*^{4}*/B-k* heterozygous males (lines 2, 3 and 4, Table 1) suggests the existence of active *hobo* elements in these heterozygotes, that are able to express the transposase gene. The *31·1/CyL*^{4} *MRF* (*P*) strain also contains complete *hobo* elements in its genome, but it was previously shown that although these seemed to be inactive, the strain depicted strong *hobo* repressor abilities (Yannopoulos *et al.* 1986, 1987). The *hobo* transposition rate itself can not be taken as a criterion for the induction of dysgenic phenomena. While *31·1/Berlin-k* exhibited a *hobo* transposition rate of 0·43 and *23·4Δ/dp b cn bw* an i.f. of only 0·19, the first has no *hobo* dysgenic activity while the latter induce high frequencies (Yannopoulos *et al.* 1986, 1987). However, *hobo* transposition rates associated with *23·5 MRF* activities show a positive relationship with the rates of the GD sterility observed in males.

The observed transposition of the *P* elements in *23·5*/Berlin-k* and *23·5*/dp b cn bw* males, is in agreement with previous results which had revealed that some dysgenic mutations induced by *23·5 MRF* strains are due to the mobility of *KP* elements (Monastirioti *et al.* 1989). However, these strains do not induce *P*-mediated GD sterility and destabilize the *singed-weak* (*sn*^{w}) allele (Engels, 1981) in extremely low frequencies (Yannopoulos *et al.* 1988; Stamatis *et al.* 1989). Taking into consideration these findings and the observation of *P* transposition at a rate of 0·29 under non dysgenic conditions (Bingham *et al.* 1982) it can be suggested that a threshold quantity of *P* transposase is needed for the appearance of dysgenic anomalies (such as sterility).

The different distribution of the *P* elements on the X chromosome of the *dp b cn bw* and *Berlin-k* strains suggest that for the same element, the distribution is influenced by host factors. These factors may operate by determining the chromatin structure. The observations that the two ME strains (*dp b cn bw* and *Berlin-k*) affect differently the *P* and *hobo* transposition rates suggest that the putative host factors involved in their mobilization processes are different, a fact supporting the view that these two families of transposable elements are independent of each other (Stamatis *et al.* 1989).

Our results revealed that factors, other than the ones encoded by *P* and *hobo* elements themselves, can influence the transposition rates of these mobile elements. Identification and characterization of these factors would contribute greatly to the understanding of *P* and *hobo* transposition regulation in *Drosophila melanogaster*.

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References

Ajioka, W. J. & Eanes, W. F. (1989). The accumulation of P-elements on the tip of the X chromosome in populations of *Drosophila melanogaster*. *Genetical Research* **53**, 1–6.

Bingham, P., Kidwell, M. G. & Rubin, G. M. (1982). The molecular basis of hybrid dysgenesis: the role of the P-element, a P-strain specific transposon family. *Cell* **29**, 995–1004.

Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP elements repress P induced hybrid dysgenesis in *Drosophila melanogaster*. *The EMBO Journal* **6**, 4125–4135.

Blackman, R. K. & Gelbart, W. M. (1989). The transposable element *hobo* of *Drosophila melanogaster*. In *Mobile DNA* (ed. D. Berg and M. Howe), American Society for Microbiology Publications, pp. 523–529.

Blackman, R. K., Grimaila, R., Koehler, M. M. D. and Gelbart, W. M. (1987). Mobilization of hobo elements residing within the decapentaplegic gene complex: suggestion of a new hybrid dysgenesis system in *Drosophila melanogaster*. *Cell* **49**, 497–505.

Bridges, C. B. (1938). A revised map of the salivary gland X cromosome of *D. melanogaster*. *The Journal of Heredity* **29**, 11–13.

Calvi, B. R., Hong, T. J., Findley, S. D. & Gelbart, W. M. (1991). Evidence for a common evolutionary origin of inverted repeat transposons in Drosophila and plants. *Cell* **86**, 465–471.

Chia, W., Howes, G., Martin, M., Meng, Y. B., Moses, K. & Tsubota, S. (1986). Molecular analysis of the *yellow* locus of Drosophila. *The EMBO Journal* **5**, 3597–3605.

Daniels, S. B., Boussy, I., Tuckey, A. A., Carillo, M. & Kidwell, M. G. (1988). Variability among "true M" lines in P–M gonadal dysgenesis potential. *Drosophila Information Service* (*DIS*) **66**, 37–39.

Eggleston, W. B., Johnson-Schlitz, D. M. & Engels, W. R. (1988). P–M dysgenesis does not mobilize other transposable elements in *D. melanogaster*. *Nature* **331**, 368–370.

Eeken, J. C. J. (1982). The stability of mutator (MR)-induced X-chromosomal recessive visible mutations in *Drosophila melanogaster*. *Mutation Research* **96**, 213–224.

Eeken, J. C. J., Roneyn, R. J., de Jong, A. W. M.

Yannopoulos, G. & Pastink, A. (1991). Characterization of MR (P) strains of *Drosophila melanogaster*: the number of intact P elements and their genetic effect. *Genetical Research* **58**, 211–223.

Engels, W. R. (1989). P elements in *Drosophila*. In *Mobile DNA*. (ed. D. E. Berg and M. M. Howe) American Society for Microbiology Publications; pp. 437–484.

Fawcett, D. H., Lister, C. K., Kellett, E. & Finnegan, D. J. (1986). Transposable elements controlling I–R hybrid dysgenesis in *D. melanogaster* are similar to mammalian LINEs. *Cell* **47**, 1007–1015.

Finnegan, D. J. (1989). *I* factors in *Drosophila melanogaster* and similar elements in other eukaryotes. In *Mobile DNA*. (ed. D. Berg and M. Howe). American Society for Microbiology Publications, pp. 503–517.

Harada, K., Yuruhiro, K. & Mukai, T. (1990) Transposition rates of movable elements in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **87**, 3248–3252.

Karess, R. E. & Rubin, G. M. (1984). Analysis of P element function in *Drosophila*. *Cell* **38**, 135–146.

Kelley, M. R., Kidd, S., Berg, R. L. & Young, M. W. (1987). Restriction of P element insertions at the *Notch* locus of *Drosophila melanogaster*. *Molecular and Cell Biology* **7**, 1545–1548.

Kidwell, M. G., Kidwell, J. F. & Sved, J. A. (1977). Hybrid dysgenesis in *Drosophila melanogaster*: A syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* **36**, 813–883.

Kaufman, P. K., Doll, R. F. & Rio, D. C. (1989). *Drosophila* P element transposase recognizes internal P element DNA sequences. *Cell* **59**, 359–371

Lefevre Jr, G. (1976). A photographic representation and interpretation of the poletene chromosomes of *Drosophila melanogaster* salivary glands. In: *Genetic and Biology of Drosophila. Vol. 1a* (ed. M. Ashburner and E. Novitski) pp. 31–66. New York.

Levis, R., Hazelrigg, T. & Rubin, G. M. (1985). Effects of genomic position on the expression of transduced copies of the *white* gene of *Drosophila*. *Science* **229**, 558–561.

Lindsley, D. L. & Grell, E. H. (1968). Genetic variation of *Drosophila melanogaster*. Carnegie Institute of Washington Publication, No. 627.

Louis, C. & Yannopoulos, G. (1988). Transposable elements involved in hybrid dysgenesis in *Drosophila melanogaster*. *Oxford Surveys in Eukaryotic Genes*, Vol. 5, pp. 205–250.

Monastirioti, M., Hatzopoulos, P., Stamatis, N., Yannopoulos, G. & Louis, C. (1988). Cohabitation of KP and full-length P elements in the genome of strains of *D. melanogaster* inducing P-M-like hybrid dysgenesis. *Molecular and General Genetics* **215**, 94–99.

O'Hare, K. & Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

Parker, E. R. (1976). Introductory Statistics for Biology. Edward Arnold Ltd.

Periquet, G., Hamelin, M. H., Bigot, Y. & Kai, H. (1989). Presence of the deleted *hobo* element *Th* in Eurasian populations of *Drosophila melanogaster*. *Genetics Selection Evolution* **21**, 107–111.

Rio, D. C. & Rubin, G. M. (1988). Identification and purification of a *Drosophila* protein that binds to the terminal 31-base-pair inverted repeats of the P transposable element. *Proceedings of the National Academy of Sciences, USA* **85**, 8929–8933.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D., Benz, W. K. & Engels, W. R. (1988). A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**, 461–470.

Searles, L. L., Greanleafg, A. L., Kemp, W. E. & Voelker, R. A. (1986). Sites of P element insertions and structures of P element deletions in the 5′ region of *Drosophila melanogaster RPII215*. *Molecular and Cell Biology* **6**, 3312–3319.

Saunders, R. D. C., Glover, D. M., Ashburner, M., Siden-Kiamos, I., Louis, C., Monastirioti, M., Savakis, C. & Kafatos, F. (1989). PCR amplification of DNA microdissected polytene from a single chromosome band: a comparison with conventional microcloning. *Nucleic Acids Research* **17**, 9027–9037.

Snedecor, W. G. & Cochran, G. W. (1972). Statistical methods (Sixth Edition). The Iowa State University Press.

Spradling, A. C. & Rubin, G. M. (1983). The effect of chromosomal position on the expression of the *Drosophila* xanthin dehydrogenase gene. *Cell* **34**, 47–57.

Stamatis, N., Yannopoulos, G. & Pelecanos, M. (1981). Comparative studies of two male recombination factors (MRF) isolated from a southern Greek *Drosophila melanogaster* population. *Genetical Research* **38**, 125–135.

Stamatis, N., Monastirioti, M., Yannopoulos, G. & Louis, C. (1989). The P-M and 23·5 MRF (hobo) systems of hybrid dysgenesis in *Drosophila melanogaster* are independent of each other. *Genetics* **123**, 379–387.

Sved, J. (1987). Hybrid dysgenesis in *Drosophila melanogaster*: evidence from sterility and Southern hybridization tests that P cytotypes are not maintained in the absence of P factors. *Genetics* **115**, 121–127.

Tsubota, S., Ashburner, M. & Schedl, P. (1985). P element induced control mutations at the *r* gene of *Drosophila melanogaster*. *Molecular and Cell Biology* **5**, 2567–2574.

Woodruff, R. C., Blount, J. L. & Thompson, Jr. J. N. (1987). Hybrid dysgenesis in *D. melanogaster* is not a general release mechanism for DNA transpositions. Science **237**, 1206–1208.

Yannopoulos, G. (1978). Studies on the sterility induced by the male recombination factor 31·1 MRF in *Drosophila melanogaster*. *Genetical Research* **32**, 239–247.

Yannopoulos, G. & Pelecanos, M. (1977). Studies on male recombination in a southern Greek *Drosophila melanogaster* population. *Genetical Research* **29**, 231–238.

Yannopoulos, G., Stamatis, N. & Eeken, J. C. J. (1986). Differences in the cytotype and hybrid dysgenesis inducing abilities of different P strains of *Drosophila melanogaster*. *Experientia* **42**, 1283–1285.

Yannopoulos, G., Stamatis, N., Monastirioti, M., Hatzopoulos, P. & Louis, C. (1987). Hobo is responsible for the induction of hybrid dysgenesis by strains of *Drosophila melanogaster* bearing the male recombination factor *23·5 MRF*. *Cell* **49**, 487–495.