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Transmission pattern of *hobo* transposable element in transgenic lines of *Drosophila melanogaster*

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Summary

This study is an attempt to trace the fate of *hobo* elements in the genomes of E strains of *Drosophila melanogaster* that have been transfected with pHFL1, a plasmid containing an autonomous *hobo*. Such long-term population studies (over 105 generations) could be very useful for better understanding the population and genomic dynamics of transposable elements and their pattern of insertions. Molecular analyses of *hobo* elements in the transfected lines were performed using Southern blots of *Xho*I-digested genomic DNAs. The complete element was observed in all six injected lines. In two lines we observed, at generation 100, two deleted elements, which did not correspond to *Th1* and *Th2*. The results obtained by the *in situ* method show that the number of hybridization sites increases in each line and prove that the *hobo* element may be amplified in an RM genome. The *hobo* activity does not seem to be systematically correlated with the number of *hobo* elements. After generation 85, the evolution of the *hobo* element's insertion site number depends on the injected line. In all lines, the total number of insertions remains quite small, between 0 and 11. *Hobo* elements are located on each of the chromosomal arms. We describe 'hotspots' – insertion sites present in all lines and in all generations. On the 3R arm, a short inversion appeared once at generation 85.

1. Introduction

Among the mobile elements in *Drosophila melanogaster*, three independent systems (*P*, *I and hobo*) may produce a number of aberrant germ-line events, such as high mutation rate, infertility and elevated levels of chromosome instability (Blackman & Gelbart, 1988). Streck *et al.* (1986) have described the transposable element *hobo*, which is about 3 kb in length and has inverted sequences at each end. In this system, three classes of strains have been characterized:

H strains contain 3 kb full-sized elements and, usually, smaller derivatives (Blackman & Gelbart, 1988); current natural strains and most laboratory strains are of the H genotype (Streck *et al.*, 1986; Yannopoulos *et al.*, 1987; Blackman & Gelbart, 1988; Louis & Yannopoulos, 1988; Periquet *et al.*, 1989*a*, *b*).

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DH strains contain only deleted elements, shorter than 3 kb (Ho *et al.*, 1993; Calvi & Gelbart, 1994). *E strains* lack any detectable elements.

Yannopoulos *et al.* (1987), Blackman *et al.* (1987) and Pascual & Periquet (1991) have suggested that *hobo* elements are responsible for the aberrant dysgenic properties of the F_1 females obtained after crosses between E and H lines.

In various studies (Periquet *et al.*, 1989*a*, *b*; Boussy & Daniel, 1991) *Drosophila melanogaster* strains collected since about 1970 from Europe, central Asia, China, and from North and South America, have all been found to be H whereas some strains caught earlier were E. This pattern, similar to that observed for the *P* element (Anxolabehere *et al.*, 1988), has been interpreted as resulting from a recent invasion of *D. melanogaster* populations by *hobo* elements.

In natural populations of *D. melanogaster*, some transposons are present at inversion breakpoints. For

instance, Eanes *et al.* (1992) have shown that *P* elements accumulate in unique inversions and Lyttle & Haymer (1992) have described the probable role of the *hobo* transposable element in the origin of endemic inversions. The cosmopolitan inversions observed by the same authors appear to show no association with the *hobo* element. However, in the study by Zabalou *et al.* (1994), three of the five cosmopolitan inversions were found to have *hobo* insertions at or very near one of the two breakpoints. In the same way, in laboratory strains, the presence of a *hobo* element in all the rearrangement (inversion and deletion) breakpoints observed by Ho *et al.* (1993) supports the view that interaction between *hobo* elements in the *Uc-1 X* chromosome may give rise to the rearrangements. More recently Eggleston *et al.* (1996) investigated the structure of chromosomal inversions mediated by transposable elements in the *Uc-1* X chromosome. All six inversions analysed by *in situ* hybridization had homology to *hobo* probes at both breakpoints. After molecular studies, they concluded that their results are consistent with a model in which *hobo*-mediated inversions result from homologous pairing and recombination between a pair of *hobo* elements in reverse orientation.

In view of the results above, it is interesting to know whether the *hobo* element can invade the populations as the *P* element does, even when it is not introduced on chromosomes. In a previous report (Ladeveze *et al.*, 1994), we presented data which showed that, in *hobo* transfected lines, the element can increase its frequency during the first 50 generations after transfection. Moreover, specific hotspots of insertion seemed to appear at the level of the polytene chromosome. The purpose of the present work was to follow the fate of introduced *hobo* elements from generation (G) 50 to G100, to observe the evolution of the element (complete or deleted, active or inactive), and to observe the number of insertion sites on the polytene chromosomes and the characteristics of the transfected lines (induction of transposition or sterility). The analysis of the genomic distribution of the *hobo* element by *in situ* hybridization to chromosomes allowed us to localize the chromosomal sites, to determine insertion polymorphism and to have a better appreciation of the *hobo* hotspots.

2. Material and methods

(i) *Strain used and microinjection*

Drosophila melanogaster embryos were transformed using the microinjection technique described by Spradling & Rubin (1982). The strain used as a receptor was Hikone, an E strain in the *hobo* system, which is also devoid of *I* and *P* homologous sequences. The posterior ends of 1000 embryos from Hikone were injected just before pole-cell formation with plasmid pHFL1 (200 μg/ml) (Ladeveze *et al.*, 1994; Galindo *et al.*, 1995). The microinjected plasmid pHFL1 (Blackman *et al.*, 1989) contains one autonomous *hobo* element and some adjacent genomic DNA from cytogenetic locus 94E, cloned in the pBLUESCRIPT KS plasmid.

After hatching, the embryos were transfered to standard *Drosophila* food medium and maintained at 25 °C. Thirty independent lines were founded by crossing one of the injected flies with one non-manipulated Hikone. These lines were analysed at generation 2 using the Southern blot technique. Six *hobo*- positive lines (5HH, 9HH, 11HH, 15HH, 22HH and 26HH) were obtained and maintained at 25 °C 'en masse' independently during the following generations.

(ii) *Southern blotting*

Standard techniques were used for DNA extraction, gel electrophoresis, blotting and hybridization (Maniatis *et al.*, 1982). Genomic DNA of adult flies was digested by *Xho*I: this enzyme cuts near each end of the *hobo* sequence yielding at 2·6 kb fragment characteristic of complete *hobo* elements. After electrophoresis on a 1% agarose gel, transfer and hybridization were performed on Appligene membranes. Hybridizations were carried out overnight at 65 °C in 5× SSC, 10× Denhardt's solution, 0·1% SDS. The membranes were washed during 40 min at 65 °C in 3× SSC, then 2×20 min at 65 °C in 1× SSC. The filters were then exposed to X-ray film for 1 or 2 days.

(iii) In situ *hybridization*

In situ hybridization of biotinylated probes (Boehringer kit) to salivary gland polytene chromosomes was adapted from Engels *et al.* (1986). This technique permits identification of the sites of homologous DNAs. The pHFL1 plasmid used in the microinjection step was also used as a probe for the *in situ* hybridizations. Depending on the strain and the generation, two to seven larvae were screened.

(iv) *Hobo activity and repression tests*

According the standard procedure (Yannopoulos *et al.*, 1987; Galindo *et al.*, 1995), we measured the *hobo* element activity potential of each line by crossing, at 25 °C, 30 males from the injected lines with virgin females from the reference E line Oregon-K. About 50 F_1 females were dissected (Galindo *et al.*, 1995). The *hobo* activity potential was estimated as the number of dysgenic ovaries divided by the total number of ovaries scored multiplied by 100. The higher the

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G0 ♂ ♂ putative H-Tpase strain × *y*, *w*, H[*w*+, haw1]♀♀

↓

[**G1** 1♂ (*y*, *w*, H[w^+, haw1]/Y; putative H-Tpase chromosome/+)] × 2♀♀ (*w*, sn^3)

↓

G2

phenotype	interpretation
[*w*, *sn*]♂[white eye]	normal segregation
[w^+]♀[pale orange eye]	normal segregation
[w^+, *sn*]♂[pale orange eye]	transposition
[w^{++}]♀[deep maroon eye]	transposition
[*w*]♀[white eye]	excision

Fig. 1. Inserted *hobo* elements show a transposase activity. The integrated *hobo* elements were examined for their abilities to mobilize a genetically marked *hobo* element lacking transposase activity. Using the assay described in the figure, we observed that P[ry^+, HBL1], a full-length element used as control (Calvi *et al*., 1991), causes transpositions of H[w^+, haw1] in 15% of the germlines tested (Table 4).

percentage the higher is the activity potential of the tested line. *Hobo* repression potential was measured in a similar way by the percentage of dysgenic ovaries in 50 females from crosses at 25 °C between males from the reference H lines (23·5*/Cy) and virgin females from the lines tested. The lower this percentage, the higher is the repression potential of the tested line.

(v) *Transposase assays*

It is known that not all full-sized *hobo* elements are functional (Blackman *et al*., 1989). The integrated *hobo* elements were thus examined for their ability to mobilize a target genetically marked with a *hobo* element lacking transposase activity. In order to obtain results comparable with those of other authors, *hobo* transposase assays were conducted following the method of Calvi *et al*. (1991) by crossing G0 males containing the putative transposase source to females of the *y*, *w*, H[w^+, haw1] strain containing only two target elements of the X chromosome (Fig. 1). The [w^+, haw1] individuals have pale orange eyes. The resulting G1 males containing the haw1 target and the putative transposase source were crossed individually with two [*white*, $singed^3$] tester females to detect transposition and excision in their germlines. G2 [w^+, *sn*] males with pale orange eyes and [w^{++}] females with deep maroon eyes were scored as transpositions, and [*w*] females as excisions. Because the parental G1 X chromosomes are differentially marked by *y* and sn^3, transposition and excision events can be distinguished from exceptional segregation of the mini-$white^+$ marker due to G1 nondisjunction or non-virgin G1 mothers. All crosses were performed at 25 °C. The transposition activity was estimated following Calvi *et al*. (1991) by the G1 germline rate based upon the number of fertile germlines tested that gave rise to one or more progeny. For one estimate, only progeny giving [w^+] (pale orange) males were taken into account, in order to calculate a rate similar to that of Calvi. A second statistic scored all progeny with either [w^+] males, [w^{++}] females or both in order to sum all transposition events. It is worth noting that as these tests for transposition are based on the number of fertile germlines tested that gave rise to one or more progeny showing transposition, the observed rate may vary with sample size of offspring. However, in the present experiments the variation in offspring number was reduced (from *c*. 50 to *c*. 100 individuals) and the estimate obtained may be taken as a correct approximation.

As a reference strain bearing wild-type *hobo* transposase we used one transformed with the element *P* [ry^+, HBL1] (P-HBL1) constructed by Calvi *et al*. (1991). This element contains a small deletion of the 3′ end of *hobo*, rendering it stable but leaving intact transposase coding regions. The rate of transposition catalysed by this reference strain *P* [ry^+, HBL1] (42D/E-Cy^O) was compared with the rate obtained in the males of the different transfected lines.

3. Results

Molecular analyses of *hobo* elements were performed in the second generation of the selected lines by Southern blots of *Xho*I-digested genomic DNAs extracted from each line (Ladeveze *et al*., 1994; Galindo *et al*., 1995). Six lines (5HH, 9HH, 11HH, 15HH, 22HH and 26HH) were selected because all six DNA samples gave hybridization signals for one band corresponding to the *Xho*I-*Xho*I internal restriction fragment (2·6 kb of *hobo* element: Ladeveze *et al*., 1994; Galindo *et al*., 1995). Line 5HH gave a signal weaker than the others. Other injected lines gave no observable hybridization signal.

During the study, the selected lines were followed by Southern blotting and *in situ* hybridization analyses for both the *hobo* and the *P* elements, the latter in order to check for contamination of the lines. As Hikone is an EM strain and our current *Drosophila* stocks HP, any contamination could be detected by the unexpected presence of *P* elements in the transfected lines. This makes the contamination of our transfected lines extremely unlikely.

In Southern blot analyses from generation 2 to 120, all the selected lines, except 5HH, showed the 2·6 kb *Xho*I internal fragment corresponding to the complete *hobo* HFL1 element (Ladeveze *et al*., 1994; Galindo *et al*., 1995). Before generation 85, no smaller restriction fragments that might have originated from an internal

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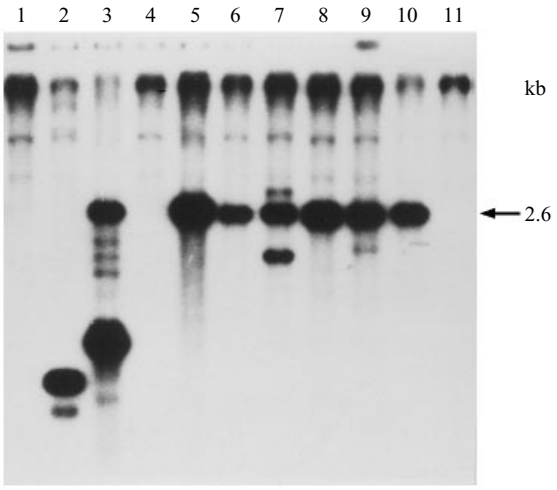

Fig. 2. Southern blot analysis of *hobo* sequences in injected strains of *D. melanogaster*. Lane 1 corresponds to DNA from the injected line 5HH at around generation 120; lanes 2 and 3 to control DH strain and control H strain respectively used as positive control; lane 4 to the original Hikone strain used as negative control; lane 5 to 26HH line at 20 °C; and lanes 6–11 contain DNA from the injected lines 26HH, 22HH, 15HH, 11HH, 9HH, 5HH at 25 °C at around generation 100.

deletion were observed. However, after that generation several bands appeared very intensely in some lines. At generation 100, most of them corresponded to fragments smaller than 2·6 kb (2·2 and 2·1 kb respectively in 11HH and 22HH), but one was larger (3 kb in 22HH) suggesting a more complex rearrangement (Fig. 2). It is noteworthy that no new fragment was shared between lines and that none of them had a size similar to the fragments generated by the *Th* elements (1·1 kb and 1·5 kb).

The number of *hobo* elements was estimated during the evolution of the selected lines at different generations (from the 8th to the 105th) by the *in situ* method. The total number of sites per genome observed until generation 50 remained small, at from four to six. This trend persisted between generations 50 to 85 with a general diversification of the insertion sites (Fig. 3), which are scattered on all chromosomes (Fig. 4). For example, around generation 100, the average number of insertions has decreased dramatically to 2 in 22HH and 26HH, or less than 0·5 in 5HH; it has increased slowly to 4–5 in 11HH and remains around 5 in 9HH and 15HH. In fact, the total number of elements seems to decrease after reaching a maximal value of 6 to 9.

During the evolution of the lines, a total of 239 different insertion sites were observed. They appear to be more or less randomly distributed on each of the chromosomal arms (Figs. 4, 5). However, the *hobo* element was more frequently inserted on the 2L and 3R arms (24·7% and 22·3% respectively, over a total of 373 insertions observed for generations 50, 80 and 100; Table 1) and, in particular in 38C, 94E and 96C. Comparatively few sites were found in the 3L arm (15·5%) and, in particular, from the chromocentre to 70C. Only three insertions and two sites were observed on chromosome 4. In each line very few sites were observed to be conserved during evolution. However, comparison between the lines revealed that 12 sites presented a relatively high frequency of insertion and were occupied in all six independent lines (Fig. 4): these were 31A and 38C on 2L; 54A, 56E and 57B or 2R; 66B and 70C on 3L; and 94E, 96C, 96E, 98B and 99A on 3R. Moreover, *hobo* insertions were observed in 30B and 50C in five of the six lines. These 14 sites, common to every injected line up to generation 100, are called putative hotspots.

Within one of the lines (22HH), a study of the variability of the site distribution was carried out at generation 95. Table 2 shows a total of 50 different sites: 10 on the X chromosome, six on 2L, 17 on 2R, seven on 3L, eight on 3R and two on 4. Some of the sites were present in several larvae, while others were seen only once. These results can be compared with the similar study made at generation 50 (Ladeveze *et*

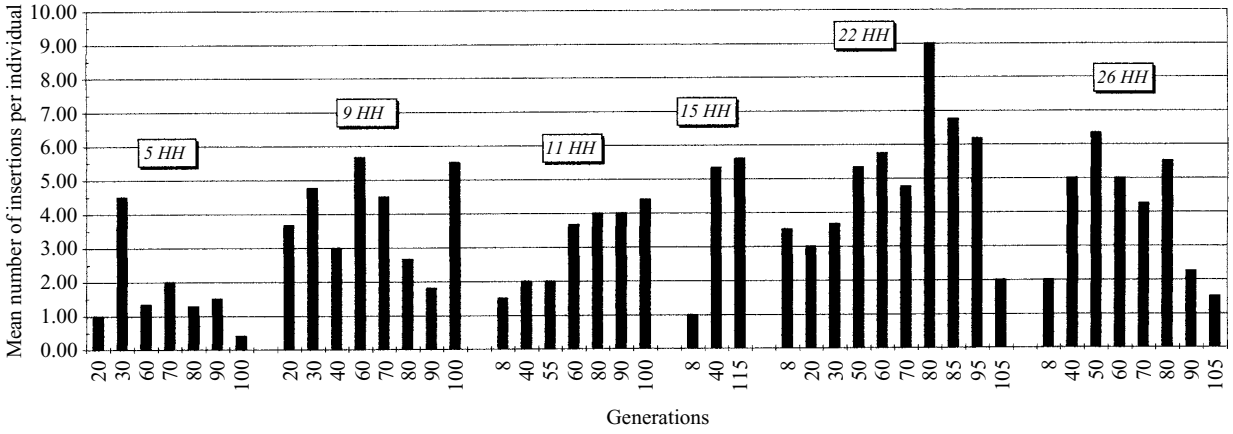

Fig. 3. Pattern of *hobo* inserts number in transgenic lines.

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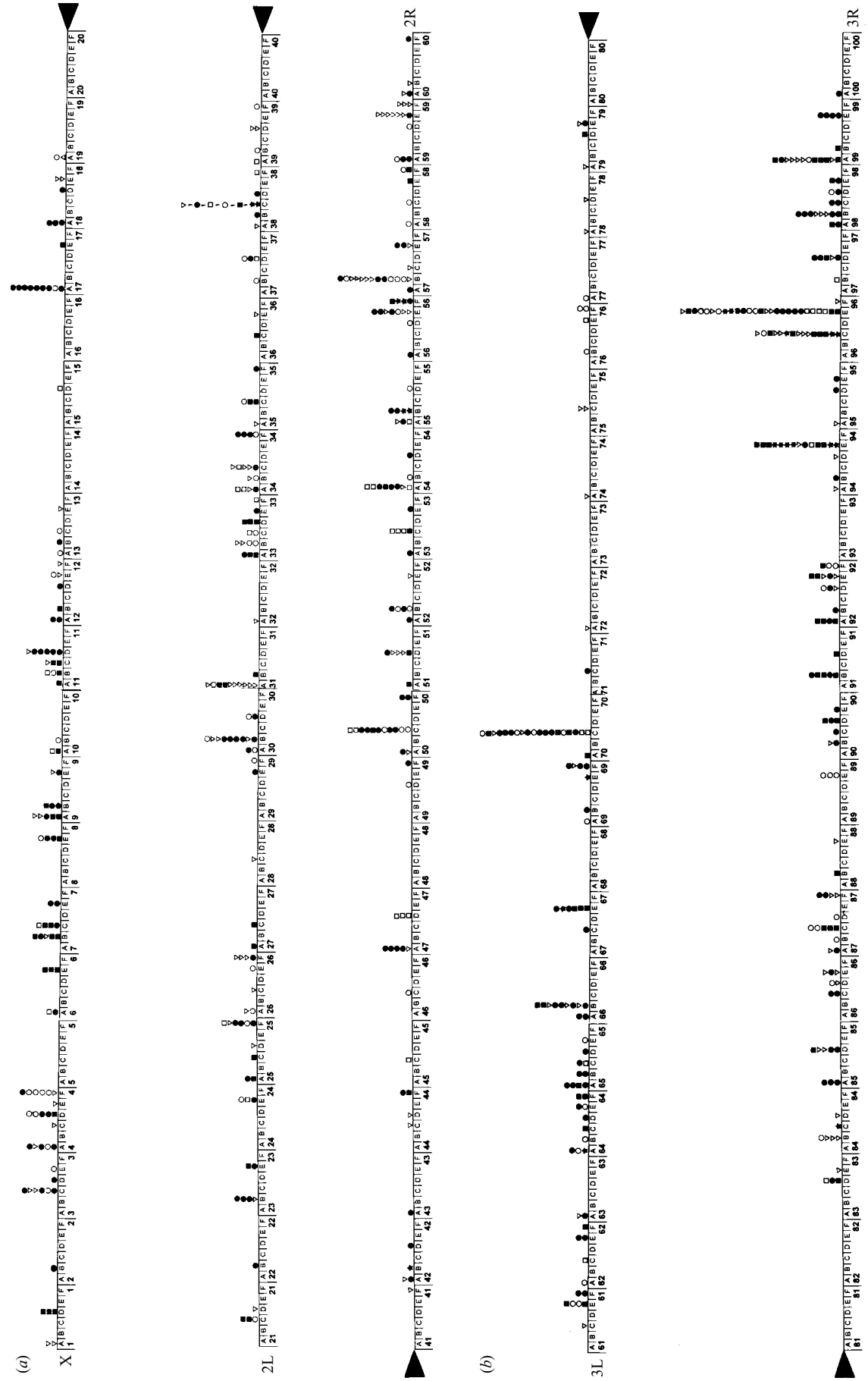

Fig. 4. (*a*) Distribution of *hobo* hybridization sites on the X, 2L and 2R chromosomes. (*b*) Distribution of *hobo* hybridization sites on the 3L and 3R chromosomes. Arrowhead, position of centromere. ★, 5HH strain; ■, 9HH strain; ○, 11HH strain; □, 15HH strain; ●, 22HH strain; ▽, 26HH strain.

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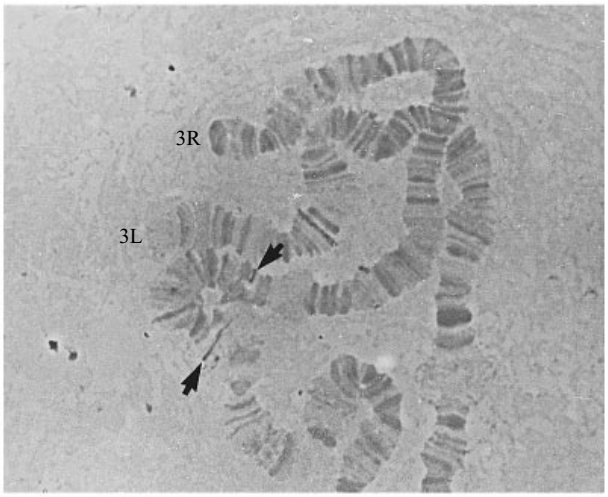

Fig. 5. *In situ* hybridization with *hobo* to 22HH chromosomes, carrying 3R inversion. *Hobo* elements are present at one breakpoint (90C) and close to the second breakpoint of the inversion (arrows).

al., 1994). We observe that very few insertion sites are common to generations 50 and 95, due to an increase in both the number of insertions per larvae and the total number of sites occupied. At generation 50, insertions per larvae range from four to seven, and at generation 100 from one to 11. The total number of insertions increased from generation 50 (from 97 in generation 50 to 186 in generation 85). However, at generation 105 these numbers have decreased (Fig. 3) compounded with sample size.

At generation 85, an inversion was observed on the 3R arm of the 22HH line. Its breakpoints were 90C and 92E with a *hobo* element to 90C, and a *hobo* element close to the second breakpoint in 92F1-2 (Fig. 5). This short inversion was observed only once, as was the first inversion detected on the 2R chromosome arm at G52 in the 26HH line (Ladeveze *et al.*, 1994).

The results of the genetic tests of the independent *hobo*-transfected lines maintained at 25 °C are presented in Table 3. The activity and repression potentials changed progressively. At generation 50 it seemed that these lines were on a plateau. In the next 50 generations, large fluctuations were observed, and high GD potential was not acquired. In fact, the general levels of activity and repression show a decreasing trend. The activity potential was always low (between 0 and 10 % of induced GD sterility). The

Table 1. *Number of insertion sites of the* hobo *element*

Generation	Arm	X	2L	2R	3L	3R	4	Total
G50–60	Total no. of insertions	13	26	25	18	17	0	99
G80–85		27	34	19	16	33	0	129
G100–105		22	32	31	24	33	3	144
Total		62	92	75	58	83	3	373

Table 2. *Polymorphism of* hobo *insertion sites in 22HH strain at generation 100*

Chromosome	X	2L	2R	3L	3R	4
Slide 1	0	38C	50A 50C	69B 79E	96E	0
Slide 2	12A	30B 38C	49F 57B 59A	66B	95E 99E	0
Slide 3	16F	30A 33A 38C	55A 55B 60F	0	90E	0
Slide 4	7C 16F	34F 38C	0	65B	98C	0
Slide 5	3C 17A	23B	50F	70C	97D 98B	0
Slide 6	17A	23B 30B	57A	70C	98B	0
Slide 7	17A	0	47A 50F	69F 70C	0	102D
Slide 8	2B 4A 4D	30B 34F 38C	54A 56D	65A 65B	98A	0
Slide 9	0	30B	47A 52B 53A 53E 56E	0	0	102B
Slide 10	0	30A	0	0	0	0
Slide 11	0	38C	50C	0	97D	0
Slide 12	4F 12D	0	0	0	99E	0
Slide 13	0	38C	0	0	0	0

This table shows several sites presenting a high frequency of insertion in all five independent lines.
Hobo is also inserted in some other sites not described above, but the majority of these sites have already been described as putative hotspots by Bolshakov *et al.*, Genet. Sel. Evol., 1994; Yannopoulos *et al.*, Genet. Res. Camb., 1994.

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Table 3. *GD test in the* hobo*-transfected lines at different generations*

		Generation							
		4–11	12–15	22	26–30	40	50	80–85	110–120
5HH	A	0–5%	39	34	57	57	58	12	0
	A*	90–98%	30	–	75	29	29	44	40
9HH	A	0–5%	31	51	47	59	60	12	9
	A*	90–98%	57	70	31	30	24	75	73
11HH	A	0–5%	28	58	28	40	40	28	11
	A*	90–98%	82	67	47	46	28	33	35
15HH	A	0–5%	2	35	42	59	60	11	10
	A*	90–98%	59	73	57	30	28	42	73
22HH	A	0–5%	2	30	47	53	55	24	10
	A*	90–98%	59	70	65	42	24	42	53
26HH	A	0–5%	13	–	45	30	38	25	6
	A*	90–98%	71	70	70	24	24	77	60
Control		61	69	85	60	99	85	74	89

The percentage of dysgenic ovaries are given in A for the measure of *hobo* potential activity and in A* for *hobo* susceptibility of the tested lines. The control results are given by the percentage of dysgenic ovaries in the F_1 [+] flies from the crosses Oregon-K females and 23·5*/Cy males. Lines were maintained at 25 °C.

Table 4. *Transposase activity of the* hobo*-transfected lines*

	Germlines with transpositions[a]					
					Germline rates[b]	
Line tested	1 [w^+] males	2 [w^+] males and [w^{++}] females	3 [w^{++}] females	Total of germline tested	% (a)	% (b)
CyHBL1 (control)	34	7	8	322	12·7	15·2
5HH	0	0	0	69	0	0
9HH	6	3	1	47	19·1	21·3
11HH	7	0	2	34	20·5	26·5
15HH	11	5	4	116	13·8	17·2
22H	4	1	0	31	16·1	16·1
26HH	5	1	0	35	17·1	17·1

The integrated elements were examined for their ability to mobilize the donor inactive transposon H[w^+, haw1] (4F + 6D) and with the results obtained compared with the P[ry^+, HBL1] (42D/E Cy°) source of transposase used as control.
[a] Germlines with transposition were those that gave rise to one or more exceptional generation 2[w^+] males (1), both [w^+] males and [w^{++}] females (2), or only [w^{++}] females (3).
[b] Germinal rates are the percentage of germlines producing [w^+] males (a) or both [w^+] males and [w^{++}] females (b).
[w^+]: pale orange eye similar to the [w^+, haw1] phenotype.
[w^{++}]: deep maroon eye phenotype closer to a wild-type eye colour.

repression potential varied greatly from moderate (70% of induced GD sterility) to high (35%) but no total repression was acquired by any line.

In order to check whether the transfected elements were functional, the assay of mobilization of the (haw1)-labelled *hobo* element was used, as previously described. In the control crosses, the P-HBL1 element catalysed one or more transpositions in 12·7–15·2% of the male germlines tested according to the used rate (Table 4). In the injected lines, tested between generations 95 and 105, all but the 5HH line gave similar or even slightly greater rates (13·8–26·5%), showing that the integrated elements are functional. These results are similar to those of Calvi *et al.* (1991). No somatic activity and no excision events were observed, as with the control P-HBL1 element.

4. Discussion

Since the microinjection technique avoids any contamination either by other transposable elements or by genomic information, any new properties acquired

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by the host genome must result from the introduced element. In view of the experiments presented here, the genomic invasion by *hobo* elements therefore appears autonomous.

It was previously shown that, in some crosses between E and H strains, the *hobo* element has the potential to transpose at high frequencies, and to non-homologous chromosomes (Yannopoulos *et al.*, 1994). Our results demonstrate that the *hobo* elements *per se* may promote high rates of infertility (hybrid dysgenesis). There was great variation in our GD test. In the same way, Bazin & Higuet (1996) showed that the occurrence of GD sterility depends on the H and E lines used. The strains tested here were the Hikone, 23·5*/Cy and HH lines. Hikone and 23·5*/Cy lines were always stable, but HH lines were dependent on the generation studied and presented a large variability of the number and localization of *hobo* insertion sites. This may explain why the GD results are so different at each generation and for each HH line.

It is striking that the acquisition of a high *hobo* activity potential and a high repression potential have not been completed after more than 100 generations. In fact, after a period of increase in the activity potential (up to the 50th generation), the GD status showed a global evolution towards a low activity potential with a very variable level of repression potential. This is a strong difference with the *P* element situation described by Anxolabehere *et al.* (1987), where the *P* activity potential and the low *P* repression were acquired between the 50th and the 100th generations in transfected *Drosophila melanogaster* lines. However, a considerable variation in the GD status was also observed (Montchamp-Moreau, 1990; Higuet *et al.*, 1996) with *P* elements transfected in *D. simulans*. This might be attributed to transposon–host genome interactions, which lead to a lower invading power of the *hobo* element than of the *P* element in *D. melanogaster*. These interactions might depend on the inserted elements, the type of insertion sites and the host genome.

Moreover we have shown that the inserted elements remain active 100 generations after their introduction into the host genome. Considering that the levels of transposition observed are similar or slightly superior to the levels obtained by Smith *et al.* (1993) with one complete element as the donor source of transposase, we can deduce that only a few active elements are actually present in the HH lines, which is in good agreement with the total number obtained by *in situ* observations. Its relationship with the gonadal dysgenesis remains to be clarified either in terms of the number and the quality of active elements or in terms of specific locations on the genome.

By Southern blot analysis, we always observed the 2·6 kb fragment that corresponds to the complete element, except in the 5HH line. Indeed, it is noteworthy that in the 5HH line neither complete nor deleted *hobo* elements were observed very soon after the first generation, although in this line we observed *in situ* hybridization signals with *hobo* probe. This discrepancy may be explained either by the fact that, in 5HH, the number of *hobo* insertion sites is very low (0 or 1 in general) or by the higher reliability of the *in situ* method. In the other lines, the presence of restriction fragments shorter than 2·6 kb, after the 85th generation, is interpreted as due essentially to internally deleted elements. They appear independently in the different lines and increase in frequency along the generations. Similar observations were made by Montchamp-Moreau (1990) with *P* element injected in lines of *D. simulans*. Indeed, the general trend in her experiments was an overall increase of the number of deleted elements with time, but more rapidly (from generation 5 to generation 60) than in our case.

In nature, most of the strains collected after the mid-1950s were H strains with some putative full-sized, and numerous deleted, *hobo* elements and with a high frequency of the 1·5 kb *Th* deletion derivative elements (Periquet *et al.*, 1989*a*). However, in the 11HH and 22HH lines, while deleted elements were also observed after the 85th generation, they were not of the *Th* type. Taking into account the total number of inserted elements in our lines after G85, these deleted elements were present in few copies per genome (< 5), instead of the higher number found in wild-type strains. This might be due to the short time of evolution of our lines and could explain why the regulation of *hobo* element was not acquired in our lines. Moreover, the absence of *Th* elements is in agreement with the hypothesis of the contribution of these elements to the regulatory mechanisms of the *hobo* system (Periquet *et al.*, 1989*a*). This is reminiscent of the results obtained for *P* element regulation; indeed, Corish *et al.* (1996) demonstrated that the almost complete repression of transposition in a Q population of *D. melanogaster* is the result both of the presence of *KP* elements with intermediate levels of repression and of the strong contribution of a single 2·6 kb *P* element deletion derivative called *SR* (strong repressor). As *KP* is responsible for the decrease in transposition, we suggest that in the absence of *Th* in our lines, *hobo* continues to transpose (by excision and insertion, in the majority of events) at a rate specific to the HFL1 element, which determines the low number of insertion sites. If so, only after the appearance of *Th* elements would strong regulation be established. On the other hand, the fact that the total number of *hobo* elements did not increase above five to six elements on the average is striking. One hypothesis is that the increase in the complete *hobo* elements number favours the process of excision against the process of transposition and integration. The presence of numerous deleted

hobo elements might be necessary for regulation. In any case, if the regulation capacity in the *hobo* system is connected with the total number of active and deleted elements, we might expect an oscillation of the number of complete *hobo* elements until an increase of the deleted elements number leads to the system stabilization.

By *in situ* hybridization, we observed that *hobo* elements were scattered on each of the chromosomal arms (X, 2L, 2R, 3L, 3R and 4). In a previous paper (Ladeveze *et al*., 1994), we showed that no sites were detected either in chromosome 4 or in the chromocentre. We explained the absence of *hobo* element insertion sites on chromosome 4 by the restricted number of elements in these experiments. Confirming this hypothesis, 50 generations later, *hobo* elements have indeed invaded the chromosome 4. However, unlike the majority of transposable elements, no *hobo* site was yet detected in the chromocentre, which contains heterochromatin. The study of Pimpinelli *et al*. (1996) confirms this observation: they suggest that *P* and *hobo* elements have been recently invading the genome and thus are not yet present in the heterochromatin, compared to the other transposable elements.

Concerning the nature of the insertion sites, the results reported here indicate that *hobo* elements are located all along the chromosomes, with the same preference for some regions we described in our previous article, such as 54A to 57B on the 2R arm or such as 94A to 100A on the 3R arm for example. This observation may have important implications when *hobo* elements are used as insertional mutagens or tagging genes. Whereas the 1A site on chromosome X is a specific integration site of *P* and *I* elements, it does not seem to be a specific site of *hobo* although we noted this insertion site sometimes in some injected lines. Most of the *hobo* insertion sites detected in our experiments have already been mentioned in *hobo* literature (Harada *et al*., 1990; Lyttle and Haymer, 1992; Ho *et al*., 1993; Smith *et al*., 1993; Bolshakov *et al*., 1994; Yannopoulos *et al*., 1994; Zabalou *et al*., 1994; Kozhemyakina & Furman, 1995). Only two sites on the X chromosome (1D and 18D), seven sites on the 2L arm, three on the 2R, two on the 3L and three on the 3R have not yet been described in the literature, representing 11% of total *hobo* insertion sites observed in our experiments. Zabalou *et al*. (1994) observed chromosomal insertions of *hobo* elements along the X, second and third chromosomes in natural populations. There is a tendency for accumulation in certain chromosome regions, as we have demonstrated here. We observed 14 other 'hotspots'. The 70C locus was observed by Smith *et al*. (1993), Bolshakov *et al*. (1994), Zabalou *et al*. (1994) and Kozhemyakina & Furman (1995). The 50C hotspot has been already described by Kozhemyakina & Furman (1995). In the study by Zabalou *et al*. (1994), *hobo* elements were also inserted in 31A, 50C, 54A, 56E, 57B, 66B, 94E, 96C, 98B and 99A loci. Smith *et al*. (1993) have shown that 96E, 98B and 99A have *hobo* insertions. Bolshakov *et al*. (1994) described also 57B. It is thus likely that some regions of the genome have particular chromosomal environments (DNA sequence or local chromatin structure) that are more suitable for the insertion of different elements (Biémont, 1992).

Lim and collaborators demonstrated in extensive studies that *hobo* transposable elements are responsible for producing genetic unstability (UcX) in the X chromosome (Lim, 1988; Ho *et al*., 1993; Sheen *et al*., 1993). However, in our study we did not find any rearrangement on the X chromosome. We observed an inversion in two larvae from two different lines: one presented the insertion of *hobo* elements at its breakpoints (Ladeveze *et al*., 1994), the other showed only one *hobo* integration site to one breakpoint (90C) and the other (92F1-2) close to the second breakpoint (92E). The 92E locus is a *hobo* insertion site frequently described in the literature. These two intrachromosomal rearrangements affect two different chromosomes. They have not been described in the literature, either in natural or in laboratory populations of *Drosophila melanogaster* (Ashburner & Lemeunier, 1976; Lemeunier & Aulard, 1992; Lyttle & Haymer, 1992; Zabalou *et al*., 1994). In the study of Harada *et al*. (1990), all inversions observed have insertions of the *hobo* element at these breakpoints. We may of course have missed some rearrangement in our study. However, the number of rearrangements should be low, considering that during 100 generations and with six lines, only two inversions were observed. Although the evidence is incomplete, there is little doubt that transposable elements, and *hobo* in particular, play a significant role in chromosome evolution.

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