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# Dynamics of the *hobo* transposable element in transgenic lines of *Drosophila melanogaster*

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**Summary**

The impact of the *hobo* transposable element in global reorganization of the *Drosophila melanogaster* genome has been investigated in transgenic lines generated by injection of *hobo* elements into the Hikone strain, which lacked them. In the present extensive survey, the chromosomal distribution of *hobo* insertion sites in the line 28 was found to be homogeneous and similar for all chromosomal arms, except 3L, when compared with other transgenic lines. However, some original features were observed in this line at the genetic and chromosomal levels. Several hotspots of insertion sites were observed on the X, second and third chromosomes. Five sites with a high frequency of *hobo* insertions were present on the 3L arm in most individuals tested, suggesting the action of selection for *hobo* element in some sites. The presence of doublets or triplet was also observed, implying that *hobo* inserts can show local jumps or insertions in preferred regions. This local transposition occurred independently in 11 specific genomic regions in many individuals and generations. The dynamics of this phenomenon were analysed across generations. These results support the use of the *hobo* system as an important tool in fundamental and applied *Drosophila* genetics.

## 1. Introduction

Transposable elements can contribute to genome evolution (McDonald, 1998) and the way they invade the genome and are regulated is one of the major questions in population genetics. It is very likely that elements, such as *P*, *I* and *hobo* have invaded the *Drosophila melanogaster* genome of most worldwide populations in 50 years, suggesting a rapid invasion. Experiments with the *I* and *P* factors have shown, however, that the copy number can rapidly reach a peak at which it stabilizes. The first events of such invasion from the first generation following the injection are not yet fully understood. In order to approach this question, several experiments have been done with experimental transfected lines in this *hobo* system. *Hobo* elements belong to the same family of transposons as *Ac*. The impact of the *hobo* element in the *Drosophila melanogaster* genome has been shown in independent transgenic lines generated by injection of *hobo* elements in the Hikone strain, which initially lacked them (Ladevèze *et al*., 1994, 1998*a*).

In the present study, we focused on the quantitative analysis of the *hobo* sites distribution between generations for one of these lines (line 28) which showed the highest number of *hobo* insertions. Dynamics of the *hobo* element were studied by following transposable element activity and mobility for 110 generations; this represents a survey over 5 years. The element distribution revealed some original features including hotspots for insertion, sites with high frequencies of insertion, and local jumping of elements, which may have an important impact in genomic evolution. Local transposition concerns neighbour sites and could be used in targeting *hobo* element insertions to specific regions very near other *hobo* inserts. We discuss the possible roles of genetic drift and selection, the two phenomena which may be

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implicated in the original pattern observed in line 28, and compare our results with the data published for natural populations.

## 2. Materials and methods

### (i) *Strain used and microinjection*

*Drosophila melanogaster* embryos were transformed by microinjection as previously described (Spradling & Rubin, 1982). The strain used as receptor was Hikone, a strain devoid of *P* homologous sequences and devoid of functional *I* and *hobo* elements. The plasmid pHFL1 (Blackman *et al*., 1989) (200 $\mu$g/ml) was injected just before pole-cell formation in the posterior ends of 1000 embryos from the Hikone strain (Ladevèze *et al*., 1994). The pHFL1 plasmid contains one autonomous *hobo* element and adjacent genomic DNA from the cytogenetic locus *94E*, cloned in the pBLUESCRIPT KS plasmid. After hatching, the embryos were transferred to standard *Drosophila* food medium and maintained at 25 °C. Thirty independent lines were obtained by crossing individually one injected fly with one non-injected Hikone fly. The lines were analysed at generation 2 using the Southern blot technique to check for the presence of the *hobo* element. The seven *hobo*-positive lines obtained were maintained at 25 °C during the following generations by 100–150 individuals. Line 28, which showed the highest number of *hobo* insertions at generation 50, was chosen for an extensive survey of the dynamics of its hobo elements during the following 110 generations.

### (ii) *Genetics and molecular tests*

The *hobo* status of line 28 was measured by crossing the line with Hikone and 23·5*/*Cy* as in Ladevèze *et al*. (1994) and Galindo *et al*. (1995). 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, which cuts near each end of the *hobo* sequence, yielding a 2·6 kb fragment characteristic of complete *hobo* elements. After electrophoresis on a 1 % agarose gel, transfer and hybridization were performed on Appligene membranes. Hybridization was carried out overnight at 65 °C in 5 × SSC, 10 × Denhardt solution, 0·1 % SDS. The membranes were washed for 40 min at 65 °C in 3 × SSC, then 2 × 20 min at 65 °C in 1 × SSC. Filters were then exposed to X-ray film for 1 or 2 days.

### (iii) In situ *hybridization*

*In situ* hybridization (ISH) of biotinylated probes (Boehringer kit) to salivary gland polytene chromosomes was adapted from Engels *et al*. (1986). The pHFL1 plasmid used in the microinjection step was used as a probe for the *in situ* hybridizations. About five larvae from the line 28 were screened every 10 generations.

## 3. Results

### (i) *Genetic and molecular analyses*

The genetic test showed that from generation 50 (G50) onwards line 28 had a very high repression potential

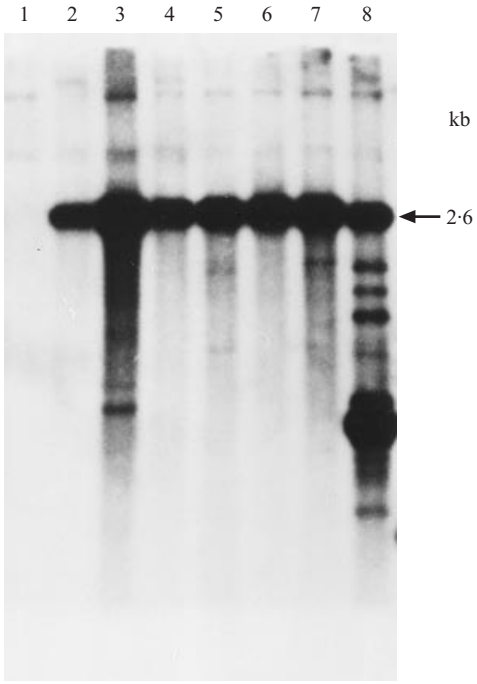


Fig. 1. Southern blot analysis of *hobo* sequences in line 28 at different generations. Lane 1 corresponds to the Oregon K strain as negative control, lanes 2–7 to line 28 at G35, G115, G145, G156, G159 and G161 respectively, lane 8 to a positive control strain (Tours 82).

Table 1. *Mean number of* hobo *inserts per individual in line 28 through generations*

| Generation | No. of inserts Mean (min.–max.) | *n* |
|---|---|---|
| G50 | 7·25 (4–12) | 4 |
| G59 | 6·25 (2–12) | 4 |
| G64 | 9·75 (5–15) | 4 |
| G79 | 4 (2–6) | 3 |
| G87 | 8·7 (5–16) | 3 |
| G98 | 11·7 (5–19) | 6 |
| G117 | 9·71 (5–14) | 7 |
| G129–134 | 13·4 (5–21) | 5 |
| G147 | 14·3 (11–17) | 3 |
| G157–G160 | 16·9 (7–21) | 8 |

*n*, number of larvae analysed.

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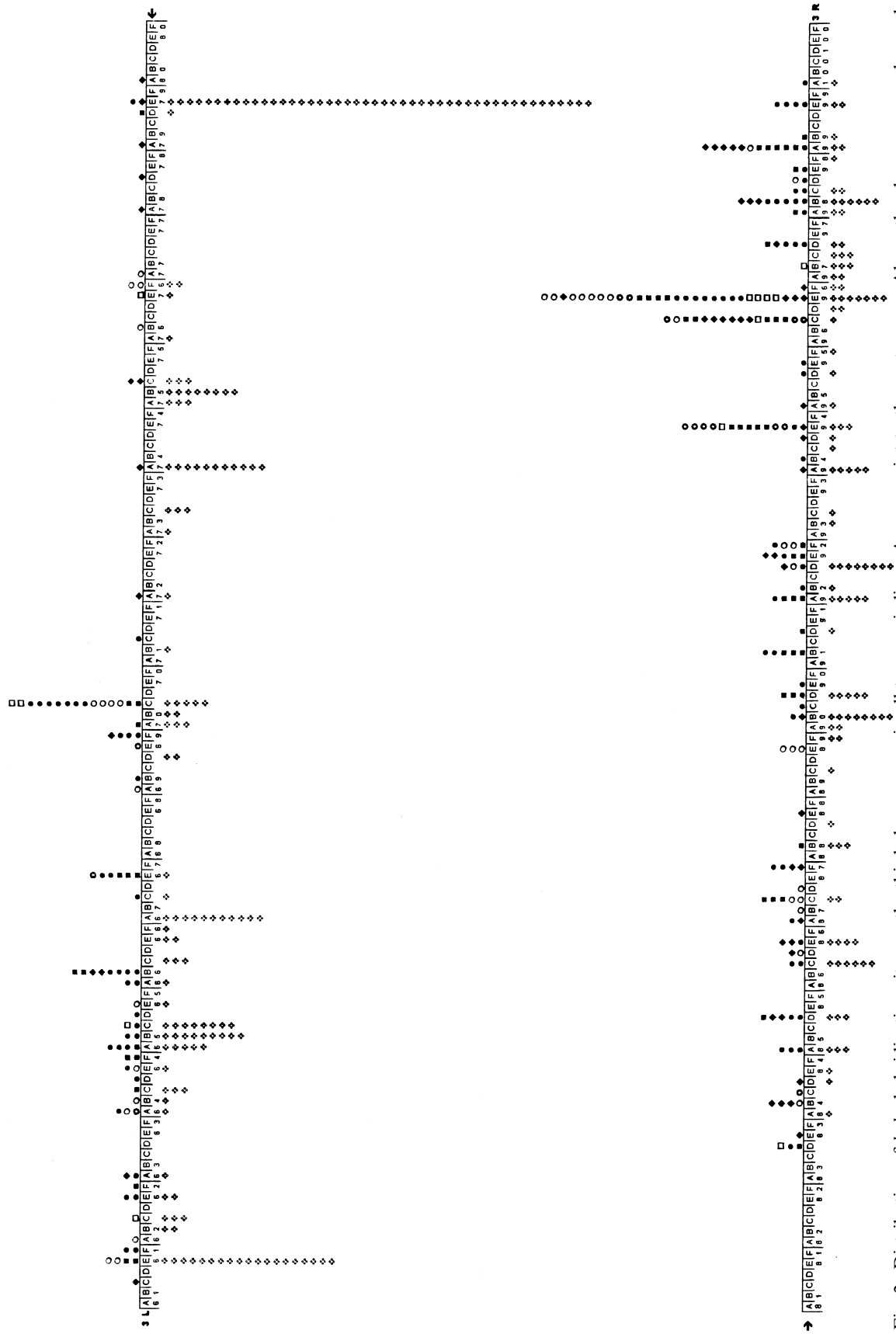

Fig. 2. Distribution of *hobo* hybridization sites on the third chromosome in all transgenic lines. Arrows point to the centromere. Above the chromosome: observed distributions in lines 5 (✪), 9 (■), 11 (○), 15 (□), 22 (●) and 26 (◆); below the chromosome: observed distribution in line 28 (✤).

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for *hobo* elements (5–10% of induced GD sterility versus 70–80% for the control strain devoid of repressive potential), but no *hobo* activity potential was detected at all, including at generation 160 (data not shown).

Southern blot analyses of line 28 from generation 35 to 160 showed the 2·6 kb *Xho*I internal fragments expected for complete *hobo* HFL1 element (Fig. 1). From generation 35 to 85, no smaller restriction fragments that might have originated from internal deletions were observed. After G85, some bands appeared corresponding to deleted elements, but they were always very rare and none became fixed.

### (ii) *Localization of* hobo *inserts*

At generation 50, an average of seven sites per diploid genome was detected by ISH in line 28 (Table 1). This insertion site number had reached 12 at generation 98, and 62 generations later was 17. The total number of *hobo* inserts is 244 for chromosome 3, 151 for chromosome 2 and 88 for chromosome X. No insertion is observed on chromosome 4.

Fig. 2 shows the *hobo* distributions on chromosome 3 of line 28 and of the other six injected lines previously described (Ladevèze *et al.*, 1994, 1998*a*). The data across all of the generations were summed in this figure. The *hobo* localization appeared to be more or less randomly distributed on each of the chromosomal arms in the different lines. The total number of these different insertion sites observed in line 28 is 158. Their distribution is similar for all chromosomes except for the 3L arm. This arm, which contains only 17·5% of the total number of chromosomal bands (Sorsa 1988), shows a significantly higher number of sites (25·3% of the total number of inserts, with $P < 0{\cdot}01$, 1 ddl).

### (iii) *Distribution*: *hotspots*, *high-frequency inserts*

In several cases, the presence of a *hobo* insertion was observed in the same chromosomal site for different independent injected lines. This could arise either by chance or by preferential insertions (hotspot). A simulation program was constructed which give the occurrence of 0, 1, 2, *n* insertions in a site, when the insertions are at random for the 5000 possible sites corresponding to the number of chromosomal bands observed in *Drosophila melanogaster*. It was run for seven independents lines, each having a mean of 12 elements, and sampled for 11 generations. These parameters were similar to those in our experiments, except for the mean element number, which was chosen to be equal to the mean value observed (line 28 between G50 and G160). This choice maximizes the chance of having common insertion sites when they are selected at random and will give a higher confidence to reject this hypothesis if necessary. In such conditions, the running of simulations shows that random insertion in a site in three independent lines would occur with a frequency of $7 \times 10^{-3}$, in four independent lines with a frequency of $7 \times 10^{-4}$, and in five or more independent lines with a frequency of $6 \times 10^{-5}$. Clearly, the presence of a *hobo* insertion in the same site in four, five or more independent lines is not likely to be the result of a random process. We defined as a hotspot an insertion site that was observed at least in four independent lines.

Table 2. *Location and frequency of the hotspots found in at least five transgenic lines*

| IS: | 7C | 38C | 70C | 96C | 96E |
|---|---|---|---|---|---|
| F: | 0·020 | 0·290 | 0·080 | 0·057 | 0·120 |

IS, insertion site; F, frequency.

Taking into account all the seven transfected lines observed, we detected five hotspots in five or more independent lines (7C on the X, 38C on 2L, 70C on 3L and 96C and 96E on 3R; Table 2) and 17 hotspots in four independent lines (4D, 7B, 8E, 9A, 25F, 30B, 31A, 34A, 50C, 54A, 56E, 56F, 57B, 85D, 92D, 97B, 99A).

Nevertheless, insertions at such hotspots are not always present in all individuals and in all generations. The frequency of insertions at these loci is low ($< 0{\cdot}29$). This maximal frequency is obtained by dividing the number of insertions in each hotspot by the total number of individuals observed (Table 2).

On the other hand, in line 28, we observed five sites on the 3L arm (61E, 65B, 67A, 74A and 79E) with a high frequency of *hobo* insertion (Table 3). These sites are defined as sites where a high frequency of *hobo* insertion is found in a given generation and we will refer to them as high-frequency sites (HFS). Frequencies of larvae which had insertions at the different sites along generations are given in Table 3. It is worth noting that elements detected at a given site are either homozygous or heterozygous and these states cannot be distinguished. If Hardy–Weinberg equilibrium is assumed, the frequency of *hobo* inserts per site can be estimated, and the corresponding values are also given in Table 3. The 74A site was first observed at G50, 65B first at G98 and the three others at G87. Insertions at 61E and 79E were the most frequent and remained extremely frequent over the 73 generations considered. At the opposite extreme, insertions at 67A, which were initially at about 50%, were no longer observed at G160. Frequency of insertions at 74A did not vary over the generations, contrary to that of 65B HFS. A very recent observation, made at G186, reveals that

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Table 3. *High-frequency sites (HFS) on the 3L arm in line 28: means of the observed frequencies per individual along generations*

| | Sites | | | | |
|---|---|---|---|---|---|
| Generations | 61E | 65B | 67A | 74A | 79E |
| G87–G117 | (a) 0·670 | (a) 0·270 | (a) 0·470 | (a) 0·130 | (a) 0·600 |
| $n = 15$ | (b) 0·426 | (b) 0·146 | (b) 0·272 | (b) 0·067 | (b) 0·368 |
| G129–G147 | (a) 0·880 | (a) 0·500 | (a) 0·130 | (a) 0·130 | (a) 1·00 |
| $n = 8$ | (b) 0·654 | (b) 0·293 | (b) 0·067 | (b) 0·067 | (b) 1·00 |
| G157–G160 | (a) 0·680 | (a) 0·250 | (a) 0·00 | (a) 0·130 | (a) 1·00 |
| $n = 8$ | (b) 0·434 | (b) 0·134 | (b) 0·00 | (b) 0·067 | (b) 1·00 |

*n*, number of individuals analysed.
(a) Frequency of larvae with insertion at the HFS site.
(b) Frequency of *hobo* inserts per site assuming Hardy–Weinberg equilibrium.

Table 4. *Local transposition (doublets and a triplet) observed through 100 generations*

| X | 2L | 2R | 3L | 3R |
|---|---|---|---|---|
| 20A1–2 | 26A1–2 | 54A1–2 | 64C1–2 | 85D1 |
| 20A4–5 | *26B1–2* | *54A3* | *64B12–13* | *85D2* |
| | 34A1–2 | 56E1–2 | 75B1–2 | |
| | *34A3* | *56E3* | *75B3* | |
| | 38C[a] | 56F6–7 | 79E1–2[b] | |
| | *37C* | *57A1–2* | *79E3–4* | |
| | | | *79D* | |

The second and third inserts are indicated in *italic*.
[a] Hotspot sites.
[b] HFS site (see text for details).

*hobo* was not detected in 67A and 74A, but was always present in the other sites, with similar frequencies.

Hotspots and HFS are different, but 61E and 79E are observed also in two other transgenic lines, 65B and 74A only in one another. 67A is the one HFS only present in line 28. Moreover we noted that 74A corresponds to an inversion breakpoint in both natural (Stalker, 1976) and experimental populations (Ladevèze et al., 1998*b*).

(iv) *Local jumping*

During the evolution of the lines, several *hobo* inserts appeared to be the result of a neighbouring copy, generally at the nearest band or at few bands from a first insertion. Table 4 shows the presence of doublet or triplet *hobo* inserts on all chromosomal arms, except chromosome 4. The first detection of doublets was observed in one individual, at G50, on the 3L arm. First observed at 64C1-2, a second hybridization was then obtained at 64B12-13. The same doublet was present at the following generation.

For the other doublets or triplet, the initial insertions were first observed at 26A1-2, 38C and 54A1-2 (G50), 85D1 (G79), 79E1-2 (G87) (Fig. 3*a*), 56E1-2, 56F6-7 and 75B1-2 (G98), and 34A1-2 (G134) (Table 4). For 20A, it was not possible to determine which was the first site because neither of the two inserts was observed without the other.

As an example, a signal was found at 79E1-2 in G87 (Fig. 3*a*). In the five individuals studied at G117, two

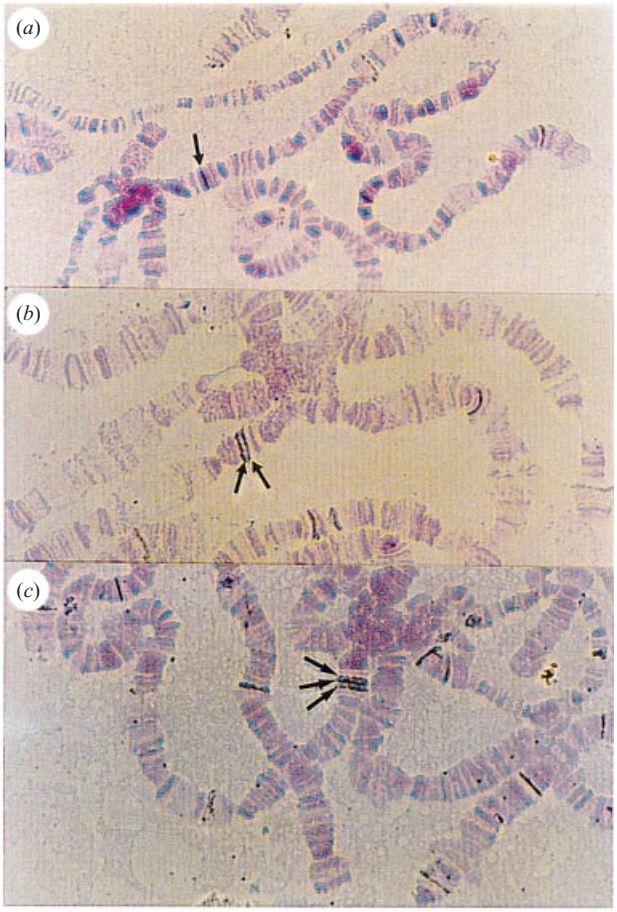


Fig. 3. Example of local multiple sites (arrows): *in situ* hybridization on the 3L arm. (*a*) 79E1-2; (*b*) doublet: 79E1-2 and 79E3-4; (*c*) triplet 79E1-2, 79E3-4 and 79D.

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insertion sites were present, at 79E1-2 and 79E3-4 (Fig. 3*b*). Ten generations later, identical signals were still present in all individuals. In G134, a third insert was detected at 79D, close to 79E (Fig. 3*c*). This triplet was not fixed as we still observed individuals with only the first two inserts in the same generation. In G160, none of the individuals studied showed all three inserts.

In summary, 11 doublets and one triplet were observed along the 110 generations analysed. Moreover, as shown in Table 2, one of them is a hotspot (38C) found in five or more different lines, and one is a high-frequency site (79E).

## 4. Discussion

Line 28 shows very interesting features compared with the other transgenic lines obtained from the same Hikone strain and previously analysed (Ladevèze *et al*., 1994, 1998*a*). First, the mean number of *hobo* elements per individual, from G50 to G160, is 4 times higher than that in the other transgenic lines. Although the mean number of *hobo* elements in the line 28 is still lower than the values reported for natural populations (between 50 and 60: Vieira *et al*., 1999), the distribution pattern appears very consistent with that encountered in the wild. Line 28 acquired a *hobo* status similar to the majority of current natural strains, with a high level of repression and no detectable activity potential. However, this observation does not imply the stopping of element mobility in the line, as the Gonadal Dysgenesic test for *hobo* activity is one which may produce no significant result when a low level of *hobo* transposition is still occurring (Bazin *et al*., 1999). At the molecular level, the vast majority of elements present in the genome correspond to full-sized elements, and very few deleted elements were observed. None of them correspond to the most frequently deleted element (TH) found in natural populations (Pascual & Périquet, 1991). These results suggest that to reach the state found in nature would require a higher number of generations.

### (i) *Hotspots*

Comparison with the other injected lines (Ladevèze *et al*., 1994, 1998*a*) reveals around 20 sites, occupied in at least four independent lines throughout the generations, that can be considered as hotspots. It is worth noting that *hobo* inserts are present in those sites in natural populations (Hoogland & Biémont, 1997), particularly 70C (universal hotspot, present in natural and experimental lines). In nature, the impact of hotspots might be of interest for chromosomal polymorphism, as they could appear in a recurrent way and become sites for chromosomal rearrangements. For example, 70C appears to be a breakpoint for inversions in natural populations in Spain (Roca *et al*., 1982) and the USA (Stalker, 1976), and also in some transgenic lines (Ladevèze *et al*., 1998*b*).

### (ii) *HFS*

In line 28, sites presenting a high frequency of *hobo*s (HFS) are 61E, 65B, 67A, 74A and 79E. 67A had such frequencies only over a short period of time (around 30 generations) and this temporary increase in frequency was probably due to genetic drift. However, the other HFS are always present after 80 or 100 generations. Of course, as the population size ($N$) is maintained at around 100–150 individuals per generation, genetic drift cannot be ruled out. As about 15 inserted sites were followed in this line during the experiment, one of them could well have been fixed or nearly fixed by chance. However, this would be less plausible for the three sites, but the fact that they are found on the same chromosome could explain a common increase in frequency due to genetic drift. Another explanation is that a selective process might have taken place, favouring the presence of *hobo* elements in these sites. This could be due to an advantage either for each of the HFS sites, or for one of them, which should have driven up the frequencies of the others by a hitchhiking effect. In this case the 79E site would appear to be the best candidate. It was the first to be a triplet in our experiments and had always the highest frequency. It is worth noting that none of these sites is a hotspot, but are present in natural populations (Hoogland & Biémont, 1997). Moreover, one of them (74A) corresponds to a breakpoint of an inversion that appeared in that line (Ladevèze *et al*., 1998*b*).

### (iii) *Local transposition*

In this study we found twin sites (doublets and a triplet) scattered in the chromosomal arms. These twin sites can be explained either by transposition of an element elsewhere in the genome to a preferred chromosomal region or by local jumping of an element already present in the vicinity of the site in question. In any case single 'starting sites' are first detected and then followed by doublets or a triplet during further generations. The local jumping sites affect 'old sites': doublets are observed 30 or more generations after the appearance of the insertion in the starting site.

We note that all chromosomes could be affected by local jumping which took place either for HFS, hotspots or 'normal' sites. More data are needed to establish whether this phenomenon is restricted, or not, to some sites of the chromosomes.

Such local jumping has already been suggested for *P* (Tower *et al*., 1993; Zhang & Spradling, 1993) and a variety of *Ac* and *Ds* elements located at several different starting sites (Dooner & Belachew, 1989; Kermicle *et al*., 1989; Osborne *et al*., 1991; Athma *et*

*al*., 1992; Moreno *et al*., 1992) and *hobo* elements (Sheen *et al*., 1993; Smith *et al*., 1993). Smith *et al*. (1993) described putative local jumps, associated with the 55A *hobo* enhancer trap insertion, from section 51 to 58, that is about 3000 kb apart. However, our data show another type of local jump: at short distances, around 100 kb, from one band of the polytene chromosome to the closest one in most cases. These results agree with the recent observation by Newfeld & Takaesu (1999) of local transposition of a *hobo* element within the *decapentaplegic* locus of *Drosophila melanogaster* around 25 kb further. Our study extends this observation to several chromosomal sites and numerous individuals *in vivo*. It is the first observation in one population of *Drosophila melanogaster* of many sites of local transposition. The twin sites occupied by *hobo* element, the determination of the starting site and the further sites, across generations, are established in this study for the first time.

In conclusion, local transposition reported in this paper is used in targeting *hobo* element insertions to specific regions very near other *hobo* inserts. Our data provide additional information about the distribution of these targeting and starting sites. They also demonstrate that the hotspot 38C and the HFS 79E are starting sites for local transposition. This phenomenon therefore appears useful for enhancing the rate of insertional mutation within predetermined regions of the genome. In such ways the dynamics of *hobo* elements might have an impact on the evolution of the *Drosophila* genome, with rearrangements as previously described (Ladevèze *et al*., 1998*b*) and with some regions modified by both doublet and triplet insertions. These results support the use of the *hobo* system as an important tool in fundamental and applied *Drosophila* genetics.

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