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Original article

Hobo elements and their deletion-derivative sequences in *Drosophila melanogaster* and its sibling species *D simulans*, *D mauritiana* and *D sechellia*

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Summary – *Hobo* elements are a family of transposable elements found in *Drosophila melanogaster* which present a specific deletion-derivative element (*Th*) in the majority of the current natural populations. Present data has resolved the *Th* element into two elements *Th*1 (1 510 bp) and *Th*2 (1 490 bp) and specified the regions within which the deletion breakpoints lie. *Hobo* homologous sequences were analysed in the sibling species *D simulans*, *D mauritiana* and *D sechellia*. In the full-sized element a *Pvu*II site was shown in *D simulans* and *D mauritiana* as it exists in *D melanogaster*, but was not observed in *D sechellia*. Specific deleted-derivative elements were also characterized for the three sibling species: *h del sim* (1 080 bp), *h del maur* and *h del sech* (1 130 bp each) and their breakpoint regions plotted on a restriction map. The functioning of these elements is discussed.

hobo / transposable element / evolution / *Drosophila melanogaster* complex

Résumé – Les éléments transposables *hobo* et leurs dérivés délétés chez *D melanogaster* et chez les espèces jumelles *D simulans*, *D mauritiana* et *D sechellia*. *La famille des éléments transposables* hobo *existe chez* Drosophila melanogaster *et présente une classe spécifique d'éléments délétés* (Th) *observés dans la majorité des populations actuelles. Cette classe d'éléments a été résolue en 2 sous ensembles : éléments* Th*1 (1 510 pb) et* Th*2 (1 490 pb) dont les régions de délétion interne ont été précisées. Des séquences homologues à l'élément* hobo *ont été analysées chez les espèces jumelles* D simulans, D mauritiana *et* D sechellia. *Les éléments complets de* D simulans *et* D mauritiana *possèdent un site de restriction* Pvu *II comme cela a été décrit chez* D melanogaster, *mais ce site n'a pas été observé chez* D sechellia. *Des éléments délétés spécifiques ont été analysés :* h del sim *(1 080 pb),* h del maur *et* h del sech *(1 130 pb) et leurs régions de délétion interne ont été précisées par carte de restriction. Le problème du rôle et de la fonction de ces éléments est discuté.*

hobo / élément transposable / évolution / complexe *Drosophila melanogaster*

* Correspondence and reprints

INTRODUCTION

Hobo elements are a family of transposable elements which can be mobilized within the germline of *Drosophila melanogaster*. In this species, strains containing *hobos* may have 3.0 kb complete elements and numerous smaller derivatives of the element (Streck *et al*, 1986; Yannopoulos *et al*, 1987; Blackman and Gelbart, 1988; Louis and Yannopoulos, 1988). Molecular analyses have revealed the presence of a specific deletion-derivative element, the *Th* element, in all current strains of *D melanogaster* examined throughout the eurasian continent (Periquet *et al*, 1989a).

D melanogaster is not the only species in which *hobos* have been found. Homologous sequences have been detected in the sibling species *D simulans* and *D mauritiana* which contain what appear to be complete copies in addition to several internally deleted sequences (Streck *et al*, 1986). In this paper, the presence and the pre-eminence of specific deleted-derivative sequences in each of the four sibling species *D melanogaster, D simulans, D mauritania* and *D sechellia* are reported. Their structure is analysed and the maintenance of the activity of *hobo* elements during evolution is discussed.

MATERIALS AND METHODS

The species and the tested strains of *Drosophila* originated from our collection of flies sampled in the eurasian continent for *D melanogaster*, and from the collection of the BGE and CGM Laboratories of the CNRS at Gif/Yvette for *D simulans, D mauritiana* (163.1) and *D sechellia* (228).

Standard techniques were used for DNA extraction, gel electrophoresis, blotting, hybridization and ligation (Maniatis *et al*, 1982).

Genomic DNA were digested either by *Xho*I to show the presence of the 2.6-kb fragment characteristic of potentially complete *hobo* elements, or by the double digest *Bam* H1 plus *Bgl* II, which do not cut the *hobo* element, in order to obtain an approximation of the total number of elements. Other enzymes were subsequently used to search for the presence of the corresponding restriction sites in the specific deletion-derivative elements. DNA samples were run on either 0.7% to 1.2% agarose gels or on 4% agarose Nuesieve gels according to the size of the fragments analyzed, and blotted onto Hybond-N membranes.

Hybridizations were carried out overnight at 65°C in 5 × SSC, 10 X Denhardt's solution, 0.1% SDS with the ^{32}P labelled *Xho*I fragment of 2.6-kb obtained from the pHcSac plasmid (Stamatis *et al*, 1989). Filters were washed for 40 min at 65°C in 3 × SSC, then for 2 × 20 min at 65°C in 1 × SSC or 0.5 SSC. In this way the procedure will promote and maintain DNA hybrids between probe and target when the two have a sequence similarity of 95% or more.

RESULTS

Our analysis is based on knowledge of the complete *hobo* element from *D melanogaster*, for which a restriction-enzyme map is shown in figure 1. To test for *hobo* sequences, *Xho* I digests of genomic DNA from strains of the different species

were probed with the *Xho*I fragment from the complete *hobo* element contained in the plasmid pHcSac. With this combination, full-sized *hobo* elements produce a 2.6-kb fragment with homology to the probe. A defective element with an internal deletion spanning between the two *Xho*I sites will produce a fragment smaller than 2.6-kb. On the other hand, elements having an insertion sequence between these two sites or having lost one (or both) *Xho*I sites will generally give a fragment larger than 2.6-kb.

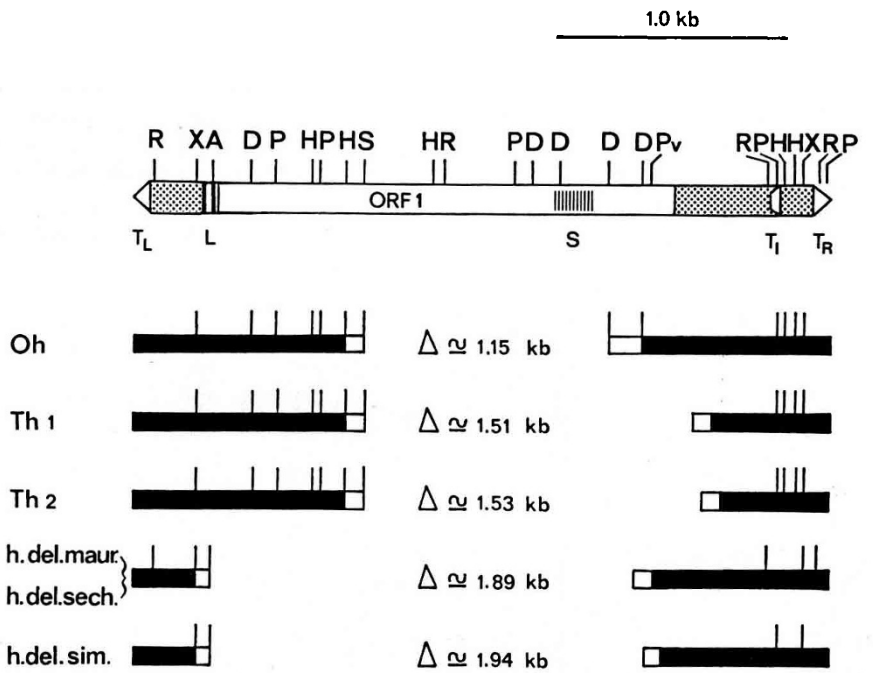

Fig 1. Depiction of the complete *hobo* element of *D melanogaster* (Streck *et al*, 1986) and the deletion-derivatives of the four sibling species, *D melanogaster (Oh, Th1, Th2), D mauritiana (h del maur), D sechellia (h del sech)* and *D simulans (h del sim).* At the top, a map of *hobo* of *D melanogaster* shows restriction sites and major structural features. Each T repeat is represented by a white arrowhead, each L repeat by a thick vertical bar, and each S repeat by a thin vertical bar. The ORF1 region is white and the rest of the element is shaded. Below this element are maps of the different specific deletion-derivatives. The sequence homologous to *hobo – D melanogaster* that is present in a derivative is shown as a black horizontal bar, the sequence that is missing is shown as a gap, and the region within which a deletion breakpoint lies is shown as a white horizontal bar. Each restriction site whose location was mapped is indicated by a vertical bar positioned below the analogous site in *hobo – D melanogaster.* The only differences shown for these elements are the large internal deletions, ignoring possible smaller sequence modifications (Restriction sites : *Rsa* I (R), *Xho* I (X), *Ava* II (A), *Dde* I (D), *Pst* I (P), *Hae* III (H), *Sal* I (S), *Pvu* II (Pv).)

With this approach we will able to assess the intactness of the *Xho*I fragment but of course not the left and right ends of the elements. Finally, operating under

the assumption that the restriction sites in *hobo* sequences from other species are not dramatically different from those found in the cloned $hobo_{108}$ element of *D melanogaster* (Streck *et al*, 1986), this approach allows the investigation of the *hobo* sequences present in sibling species.

Hobo *sequences in* D melanogaster *and sibling species*

Southern blot analyses of genomic DNA, digested by *Bam* HI plus *Bgl* II, from *D melanogaster, D simulans, D mauritiana* and *D sechellia*, confirm the presence of *hobo* sequences in these sibling species (Streck *et al*, 1986; Daniels *et al*, 1990). As these enzymes do not cut the *hobo melanogaster* sequence, they allow a rough estimation of the total number of *hobo* sequences in the tested strains. These values range from 25 to over 30 for *D melanogaster* and *D simulans* H strains, and from 15 to 20 for *D mauritiana* and are about 25 for *D sechellia* (data not shown).

Figure 2 shows the results of genomic DNA samples, digested by *Xho* I, of different species and subjected to Southern blot analysis with the 2.6-kb *hobo* probe.

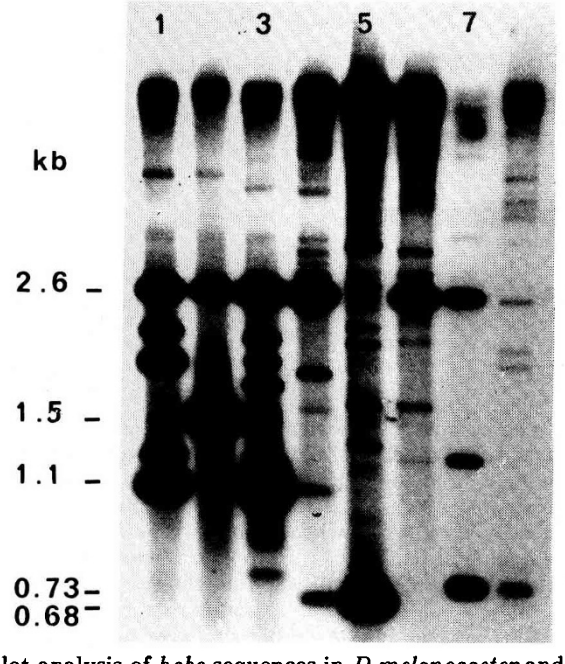

Fig 2. Southern blot analysis of *hobo* sequences in *D melanogaster* and its sibling species. DNA samples were digested with *Xho* I. Lanes 1-3 contain DNA from *D melanogaster* as control (1) 23.5*/Cy, (2) Oregon R^s, (3) Tours (1982). Other samples are as follows, *D simulans* : (4) Villeurbanne 77, (5) C.G.M. Gif, (6) Nasr'Allah 83; *D mauritiana*: (7) 163.1; *D sechellia*: (8) 228.

In *D melanogaster, hobo*-containing strains (lanes 1 to 3) show the presence of the 2.6-kb and band corresponding to the putative full-size element, as well as several

deletion-derivative elements. Two of the latter are frequently found in the different *D melanogaster* strains. The *Oh* element (from the Oregon R^s strain, see also Streck *et al*, 1986) gives a 1.5-kb fragment and corresponds to a 1.9-kb element with an internal deletion of about 1.1-kb. The *Th* element (Periquet *et al*, 1989a) gives a 1.1-kb fragment and corresponds to a 1.5-kb element having an internal deletion of about 1.5-kb.

In *D simulans, hobo*-containing strains (lanes 4 to 6) generally show the presence of a fragment comigrating with the 2.6-kb fragment of *D melanogaster* (lanes 4 and 6), although some strains may be devoid of this fragment (lane 5). Moreover, a characteristic deleted-derivative element is generally present in the recent strains studied (lanes 4 and 5). This fragment has been found in 10 strains collected from 1970 to 1990 in the Americas, Europe and Japan, but not in the African strain tested (lane 6). This *h del sim* element gives a fragment of 0.68-kb and corresponds to 1.1-kb element having an internal deletion of about 1.9-kb.

Finally, in *D mauritiana* and *D sechellia* (lanes 7 and 8), whose stocks are limited by the endemism of these species, fragments comigrating at the 2.6-kb level are also present, as well as characteristic fragments of deleted-derivative element comigrating at the 0.73-kb level. These fragments correspond to 1.1-kb elements with an internal deletion of about 1.9-kb. For the moment it is not possible to determine whether these specific deleted elements (called *h del maur* and *h del sech*) are identical or not.

Analysis of the specific deleted-derivative elements

Figure 3 shows the results for genomic DNA samples of various *D melanogaster* strains collected on several continents. These samples were digested by different restriction enzymes, in order to study for pattern similarity. If the *Th* element were present in the different strains, the patterns would be identical. As expected, the patterns were the same but, since the DNAs were run onto a 4% agarose gel in order to detect small fragments, in all cases a double band was present at the *Th* level. The *Th* element was therefore resolved into two elements. Figure 3 illustrates the results obtained with the enzymes *Xho*I plus *Sau* 3AI (lane A) and *Xho*I plus *Pst*I (lane B). With *Xho*I plus *Sau* 3AI three fragments of 0.6 kbp, 1.92 kbp and 0.1 kbp respectively are expected from the full-sized *hobo* element. With *Xho*I plus *Pst*I six fragments of 0.32 kbp, 0.18 kbp, 0.87 kbp, 1.12 kbp, 0.13 kbp and 0.99 kb are expected. The 4% agarose gel allows a clear discrimination of fragments between 300 bp and 700 bp, and shows the presence of a double band at the *Th* level. These analyses defined the two characteristic deleted-derivative elements called *Th1* and *Th2*.

By using differents sets of enzymes and Southern blot analyses, a restriction map of these two elements was obtained and their size was estimated by a logarithmic regression analysis of the band distance on the autoradiograms. The results are summarized in figure 1, which also gives the data obtained for the *Oh* element. These elements are deleted in the central part of the sequence and have the following approximate sizes : *Oh* (1870 bp), *Th1* (1510 bp), *Th2* (1490 bp).

In *D simulans, D mauritiana* and *D sechellia* similar analyses were performed to characterise the deleted-derivative elements. Results are summarized in figure 1. All

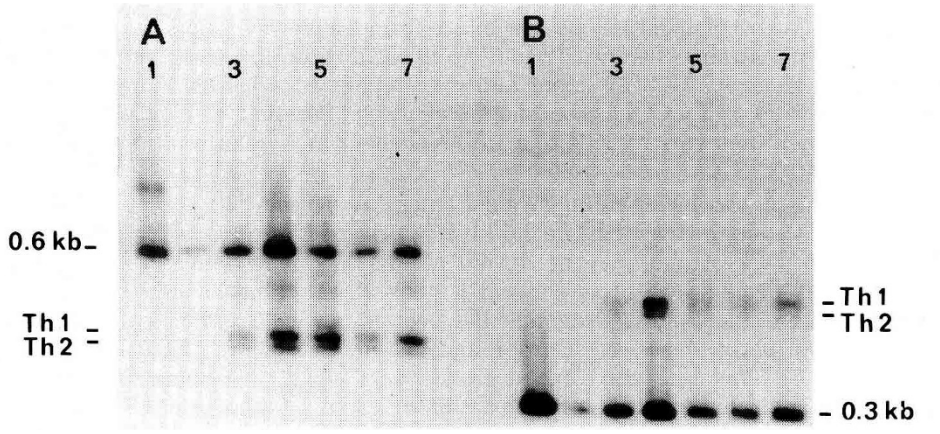

Fig 3. Southern blot analysis of the *Th* element in *D melanogaster*. DNA samples were digested with either *Xho*I plus *Sau* 3AI (lanes A) or *Xho*I plus *Pst*I (lanes B). For both digestions, strains collected from the Americas, France, USSR and China revealed the presence of two fragments corresponding to the two specific deleted-derivatives *Th1* and *Th2*. 1: Oregon R^s, 2: 731C (USA), 3: Furnace Creeck 1981 (USA), 4: Tours 1982 (France), 5: Taschkent 1982 (URSS), 6: Tongza 1987 (China), 7: Ica 1955-57 (Peru).

these elements are also internally deleted and have an approximate size of 1 130 bp (*h del maur* and *h del sech*), and 1 080 bp (*h del sim*). The breakpoints of the internal deletion are different for *D melanogaster, D simulans* and *D mauritiana*. However, at the level of this restriction map, no difference has been detected in the deleted elements of *D mauritiana* and *D sechellia*.

Analysis of the putative full-sized element

All the preceding experiments revealed good conservation of the restriction sites of *hobo* elements from the sibling species of *D melanogaster* as compared to the sequence of the cloned $hobo_{108}$ element. However, Bazin and Williams (personal communication) have recently found a non-described *Pvu*II site, at position 2227, of a *hobo* inserted element at the *vestigial* locus of *D melanogaster*. This site is extremely frequent in all current populations of *D melanogaster* as well as in the functional *hobo* element of the pHFL1 plasmid (Periquet, unpublished data). DNA samples of different species, alternatively digested by *Xho*I and *Xho*I plus *Pvu*II, were analysed by Southern blot. When the *Pvu*II site is present, the 2.62-kb fragment is therefore cut into two fragments of 1.94-kb and 0.68-kb respectively. The results (figure 4) show that the *Pvu*II site is also present in the putative full-sized elements of *D simulans* (but not in the US strain El Rio) and *D mauritiana*. For *D sechellia* the results are less clear. The pattern difference between the last two lanes shows that a *Pvu*II site is present in some *hobo* sequences but the absence of bands at 1.94- and 0.68-kb levels suggests the absence of this site in the putative full-sized element. These data pose the question of the fine structure of the *hobo* element in *D sechellia*.

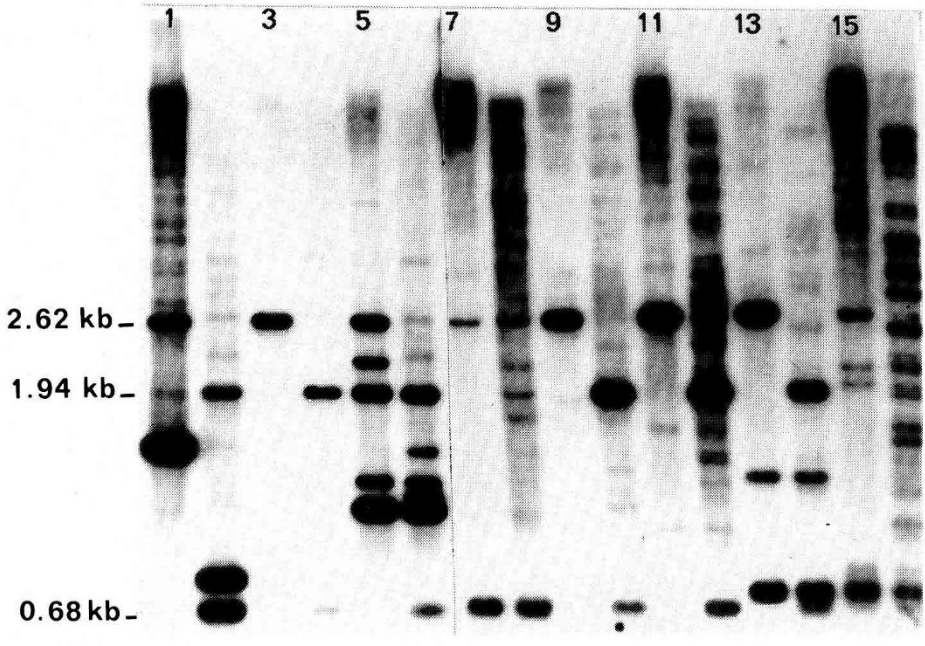

Fig 4. Screen for the presence of the *Pvu* II site in *hobo* sequences of *D melanogaster* and its sibling species. DNA samples were digested either with *Xho*I (odd-numbered lanes) or *Xho*I + *Pvu* II (even-numbered lanes). When present, the *Pvu* II site permits the breaking of the 2.62-kb *Xho* I fragment into two fragments of 1.94-kb and 0.68-kb respectively. Strains are as follows, *D melanogaster* (1-6): Oregon R^s (1,2); 731 C, USA (3,4); *23.5*/Cy* (5,6); *D simulans* (7-12): El Rio 83, USA (7-8); Villeurbanne 1977, France (9,10): Nasr'Allah 83, Tunisia (11,12); *D mauritiana*: 163.1 (13,14); *D sechellia*: 228 (15,16).

The presence of full-sized elements in the *D melanogaster* sibling species raises the problem of the functioning of these elements. In *D simulans*, the existence of different patterns of restriction fragments *Bam* HI plus *Bgl* II among strains suggests differences in the number and location of *hobo* elements which might be due to their mobility.

In *D mauritiana*, a former experiment made in order to obtain inter-specific hybrids between *D simulans* and *D mauritiana* proved meaningful for the present purpose. The cross involved a *D simulans* strain devoid of the 2.6-kb *Xho* I fragment and the present *D mauritiana* strain (fig 5). After 13 generations of free mass-mating, the hybrid flies were of the *D simulans* type, as is classically obtained in this type of inter-specific cross. DNA samples digested by *Xho*I of these G13 flies were analysed by Southern blot and showed the pattern of band characteristics of the *D simulans* elements, plus the presence of a new 2.6-kb band (fig 5). This result strongly supports the hypothesis of the presence of functional transposable *hobo* elements in *D mauritiana* which were able to be mobilized in the hybrid genome of the first generations of flies.

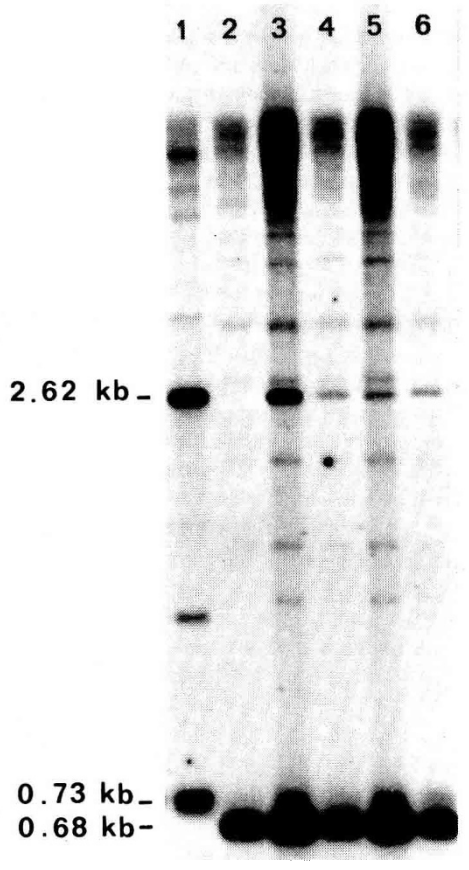

Fig 5. Southern blot analysis of the transfer of *hobo* elements from *D mauritiana* to *D simulans*. DNA samples were digested with *Xho*I. Lanes 1 and 2: parental strains *D mauritiana* (with the 2.62-kb fragment) and *D simulans* C.G.M.-Gif (without the 2.62-kb fragment). Lanes 3 to 6: hybrid strains from an initial cross of ♀ *D simulans* × ♂ *D mauritiana* (3 and 5) or from ♀ *D mauritiana* × ♂ *D simulans* (4 and 6), tested at the 4th generation (5,6) and the 13th generation (3,4). In all cases the parental *D simulans* pattern is observed but a 2.62-kb band is also present.

DISCUSSION

The severe conditions of stringency and the normal exposure used in our experiments confirmed the presence of extremely well conserved *hobo* sequences among *D melanogaster* and its sibling species (Streck *et al*, 1986; Daniels *et al*, 1990). The existence of specific deleted-derivative elements appears to be a feature of the *hobo* family, with the presence of a majority class in almost all current populations of the cosmopolitan species *D melanogaster* and *D simulans*. These internally deleted elements are different for each species, but in both cases they have lost the majority of

the ORF1 and are probably non functional, which makes one wonder why they are present in such large quantities in these species. This may be due to the recurrent formation of specific deletions from the complete *hobo* element. The presence of the two *Th1* and *Th2* elements, which differ by about 20 bp, in numerous populations of *D melanogaster*, shows that the mechanisms of such recurrent deletions might be very precise and would implicate preferential breaking sites. On the other hand, the specific deleted-derivative elements might play a role in the regulation of the activity of the complete *hobo* element, as has been shown for the *KP* element in the P-M system (Black *et al*, 1987; Jackson *et al*, 1988). Consequently their presence in many populations would implicate a rapid spread of these non-recurrent *Th* elements, aided by a selective advantage.

Although *Oh, Th1* and *Th2* are not present in the other three species, other derivative-deleted elements are found in these species. In any case, the massive presence of such derivative-deleted elements is also an argument in favor of the maintenance of active *hobo* elements in *D simulans* and of the putative role of such derivative elements well adapted to the genome of this species. These activities are also suggested for *hobo* elements of *D mauritiana* and are corroborated by the high degree of similarity implicated by our conditions of stringency.

Contrary to results presented by Daniels *et al* (1990), our *D sechellia* strain shows the presence of one fragment migrating at the 2.6-kb level, but the pattern of the other bands resemble Daniels'. The difference might be due either to a stochastic loss of this element in a derived sub-line of the 228 strain, or to an excision due to its mobility. Moreover, the fact that *hobo* elements in *D sechellia* appear to present differences in sites which are common to the other three species could be related either to an ancient divergence of this species from the other ones, or to an evolution by genetic drift in this island species. In any case, sequencing of the elements of the sibling species will be necessary to determine their fine structure and the relatedness between species.

At the phylogenetic level, *hobo* sequences appear to be limited to the *melanogaster* and *montium* subgroups (Daniels *et al*, 1990). The present data corroborate the strength of the relatedness between the members of the *melanogaster* complex, as opposed to the weakness and the lack of information of the relationships among the *hobo* hybridizing sequences found in the *montium* subgroup. These authors suggest two hypothetical scenarios to account for the current distribution of *hobo* sequences in these subgroups. The first proposes a single introduction of *hobo* elements into the common ancestral lineage. The second proposes two introductions, one into the common ancestral lineage and another one specific to the *melanogaster* complex.

When considering only the *melanogaster* complex and the existence of several *D melanogaster* strains devoid of almost all *hobo* elements (essentially in the oldest strains collected from natural populations), the presence of active *hobo* elements in all the current populations of this species poses the problem of the origin of *hobo* elements (Periquet *et al*, 1989b; Pascual and Periquet, 1991). As proposed, the active *hobo* element of *D melanogaster* may have originated either from internal recombination-reactivation of deleted *hobo* elements in *D melanogaster* itself, or by horizontal transfer from a foreign species.

Present data show that the best candidate for such a transfer is *D simulans* which is also a cosmopolitan species non-vicariant of *D melanogaster*. Clearly sequence

analyses of *hobo* elements between these two species will be of help in understanding the evolutionary history of *hobo* elements.

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