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hobo transposable elements in *Drosophila melanogaster* and *D. simulans*

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Summary

Genomic patterns of occurrence of the transposable element *hobo* are polymorphic in the sibling species *Drosophila melanogaster* and *D. simulans*. Most tested strains of both species have apparently complete (3·0 kb) and smaller *hobo* elements (H lines), but in both species some strains completely lack such canonical *hobo* elements (E lines). The occurrence of H and E lines in *D. simulans* as well as in *D. melanogaster* implies that an hypothesis of recent introduction in the latter species is inadequate to explain the phylogenetic occurrence of *hobo*. Particular internally deleted elements, the approximately 1·5 kb *Th1* and *Th2* elements, are abundant in many lines of *D. melanogaster*, and an analogous 1·1 kb internally deleted element, *h del sim*, is abundant in most lines of *D. simulans*. Besides the canonical *hobo* sequences, both species (and their sibling species *D. sechellia* and *D. mauritiana*) have many *hobo*-hybridizing sequences per genome that do not appear to be closely related to the canonical *hobo* sequence.

1. Introduction

The transposable element *hobo* was originally described by McGinnis, Shermoen & Beckendorf (1983) from an insertional mutant of the *Sgs-4* glue protein gene of *Drosophila melanogaster*. In the genus *Drosophila*, DNA sequences hybridizing to *hobo* probes have an extremely limited occurrence among species, being found only in the *melanogaster* species group, and there only in some *montium* subgroup species and in the *melanogaster* subgroup (Daniels, Chovnick & Boussy, 1990*a*). The strongest hybridization is found in the members of the *melanogaster* species complex (*D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia*), and only these species have sequences corresponding to the described *hobo* sequence in size and restriction enzyme sites. The *hobo* element is transpositionally active in *D. melanogaster*, and has been implicated in a system of hybrid dysgenesis (Yannopoulos, Stamatis & Eeken, 1986; Yannopoulos *et al.* 1987; Blackman *et al.* 1987; Louis & Yannopoulos, 1988; Blackman & Gelbart, 1989; Stamatis *et al.* 1989).

Variability of *hobo* elements among lines of *D. melanogaster* was first demonstrated by Streck, MacGaffey & Beckendorf (1986). They showed that six lines differed in their genomic complements of *hobo* elements. Upon digestion by *Xho* I and probing with a *hobo* probe, genomic DNAs from three of their lines showed a strong band of 2·6 kb corresponding to multiple copies of full-sized *hobo* elements (see Fig. 1), and several bands corresponding to smaller *hobo* elements, differing among the lines in sizes and densities. They called lines with such patterns 'H' (for '*hobo*') lines. The other three lines they tested showed no such bands; they called these 'E' (for 'empty') lines. They also tested four lines of *D. simulans* and three of *D. mauritiana*, finding bands in each corresponding to full-sized and smaller *hobo* elements.

Periquet *et al.* (1989*a*, *b*, 1990) have described genomic patterns of occurrence of *hobo* among a total of 54 lines of *D. melanogaster* from different eras and from around the world, especially from North America, France, the USSR and the People's Republic of China. They found an historical pattern of occurrence, with E lines most prevalent in North America before 1950 and in France before 1960, and with only H lines found thereafter. They interpret the historical pattern as suggesting a recent introduction of *hobo* into the species, as has been postulated for the *P* element (Kidwell, 1983, 1986; Anxolabéhère, Kidwell & Periquet, 1988).

In order to determine more about the distributions of *hobo* elements in *D. melanogaster* and *D. simulans*,

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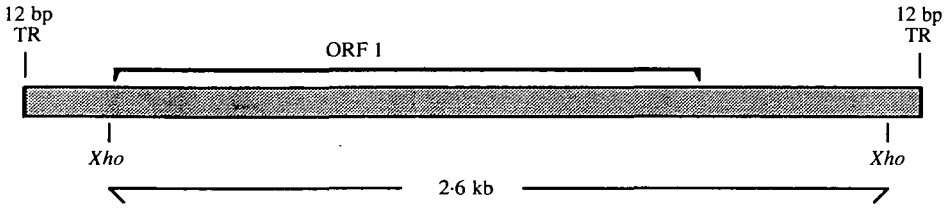

Fig. 1. The *hobo* transposable element, based on the sequence derived by Streck *et al.* (1986). The putatively full-sized element whose sequence they reported ($hobo_{108}$) was 3016 bases in length, with a single large open reading frame of 1·9 kb (indicated as ORF 1), and inverted terminal repeats (TR) of 12 bp. The cutting sites of the restriction enzyme *Xho* I, after base 286 and after base 2906, are indicated, as is the 2·6 kb fragment generated.

we probed Southern blots of DNA from a large number of lines of both species with a *hobo* probe. Some of the *D. melanogaster* lines were chosen to represent collections from different years and from different areas of the world, in order to detect possible spatial or temporal patterns of occurrence of *hobo*-hybridizing sequences. Our results corroborate the findings of Periquet and co-workers of considerable polymorphism in *D. melanogaster*, including the occurrence of the *Th1* and *Th2* elements (Periquet *et al.* 1989*b*, 1990) in many lines. We likewise found an historical pattern of occurrence, with E lines only among the oldest lines.

Our sampling of *D. simulans*, however, showed that it varied as much as did *D. melanogaster*, both in having considerable variability among H lines and in having E lines. This confounds a simple interpretation of the pattern in *D. melanogaster* as due to a recent introduction of *hobo* to the species, since one would also have to hypothesize a recent introduction into *D. simulans*.

In both *D. melanogaster* and *D. simulans*, we also found sequences that hybridized to the *hobo* probe at the moderate stringencies we used but that appear not to be closely related to the canonical *hobo* sequence. As originally noted by Streck *et al.* (1986), similar sequences are also present in both *D. mauritiana* and *D. sechelia*. The sequences imply that *hobo* is a member of a *hobo* superfamily of related elements.

2. Materials and methods

(i) *Lines tested*

The tested lines of flies were from the collections of A. Chovnick (U. Connecticut), of M. G. Kidwell (U. Arizona), or of the authors, or from the National Drosophila Species Resource Center (Bowling Green State University, Bowling Green, Ohio).

(ii) *DNA extraction, restriction enzyme digestion, Southern blotting, hybridizations, and autoradiography*

DNA was extracted and purified by standard techniques (essentially as in Daniels & Strausbaugh, 1986). One microgram of genomic DNA from each strain was digested with 5–10 units of the restriction enzyme *Xho* I, following the instructions of the manufacturer (Bethesda Research Laboratories). Fragments were electrophoretically separated by size in an agarose gel, then transferred to a nitrocellulose or nylon membrane filter (Nytran, Schleicher & Schuell) by Southern blotting (Maniatis, Fritsch, & Sambrook, 1982) or vacuum blotting (Olszewska & Jones, 1988). Hybridizations and washes were carried out using standard procedures (Rushlow *et al.* 1984; Boussy *et al.* 1988). After a 2 h pre-hybridization at 42 °C in 50% formamide, 25% 20× SSPE (3·0 M-NaCl, 0·2 M-$NaHPO_4$, 0·04 M-EDTA, pH 7·0), 1% SDS, 2·5% 100× Denhardt's solution [2% (w/v) Ficoll (type 400), 2% polyvinypyrrolidone, 2% bovine serum albumin (Pentax fraction V; Sigma)], and 250 μg/ml salmon testis DNA (type III, sodium salt; Sigma; denatured by boiling), hybridizations were carried out in a similar mix but with 500 μg/ml salmon testis DNA and with ^{32}P-labelled probe DNA (see below). Two post-hybridization washes were of 10 min each in 2× SSPE, 1% SDS at room temperature, followed by two washes of 30 min each in 0·1× SSPE, 0·5% SDS at 42 °C.

Autoradiography was performed at −70 °C with Kodak X-Omat AR-5 X-ray film and intensifying screens.

(iii) *Probe DNA*

To detect *hobo*-hybridizing sequences we used the plasmid pRG2.6X (a gift of R. Blackman). pRG2.6X carries the 2·6 kb *Xho* I fragment from a full-sized 3·0 kb *hobo* element (see Fig. 1) inserted in the *Sal* I site of the vector pUC8 (Blackman *et al.* 1987). The plasmid was radioactively labelled with ^{32}P-nucleotide by nick translation.

(iv) *Stringencies of hybridization and washing*

We refer to the stringency of hybridization or washing of blots as a number approximating the percent identity of DNA strands that will be 50% annealed under the conditions used. We have used numerical relationships discussed by Wahl, Berger & Kimmel (1987) to define the '% Str' by the following equation:

$$\%\,\mathrm{Str} = 18{\cdot}5 - 41[\mathrm{G}+\mathrm{C}] - 16{\cdot}6\,(\log[\mathrm{M}]) + 500/L + 0{\cdot}62\mathrm{F} + T,$$

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where [G+C] is the mol fraction of guanine and cytosine residues in the hybridizing DNAs, [M] is the total concentration of monovalent cations (primarily Na^+ in most hybridization procedures), L is the length of duplexes formed, F is the percent formamide, and T is temperature of hybridization or washing (in °C). For our calculations, the mol fraction of guanine and cytosine residues in the canonical *hobo* sequence was 0·38 (Streck *et al.* 1986), and we assumed that the $500/L$ term was negligible. Our standard hybridization conditions were thus at a stringency of 76%, and our standard final wash was at a stringency of 75%. These conditions should yield hybridizations between probe and target only when the two have a sequence similarity of greater than about 76%.

3. Results

(i) hobo *in* D. melanogaster

Figure 2 shows DNA from ten laboratory lines of *D. melanogaster*, digested with *Xho* I and probed with the pRG2.6X probe. The 2·6 kb size class is marked. Five of the lines (Limpopo, Harwich 77, Bethalie, Oregon RC, and Swedish C) show hybridization to a 2·6 kb fragment, indicating potentially full-size *hobo* elements in their genomes, and to smaller fragments as well; they thus correspond to H lines of Streck *et al.* (1986), and the bands described (of 2·6 kb or less) correspond to full-sized or internally deleted derivatives of the canonical *hobo* sequence. Other lines (sd^{ry+} ry^{506}, y sn^w; bw st, Kalahari, MGR-1, and Samarkand) show no bands of 2·6 kb or smaller, even upon long exposure (data not shown), and thus correspond to E lines of Streck *et al.* (1986).

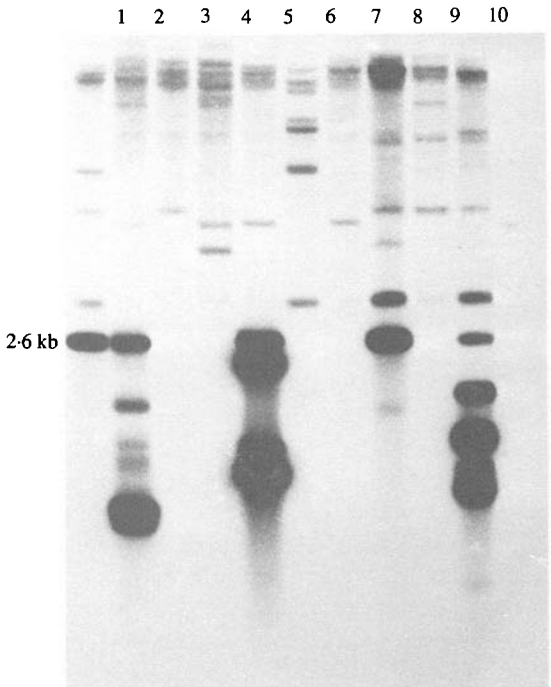

Fig. 2. *Xho* I digests of genomic DNA from 10 laboratory lines of *D. melanogaster*, probed with the pRG2.6X probe that carries the 2·6 kb *Xho* I fragment from $hobo_{108}$. The expected 2·6 kb internal *Xho* I fragment class from full-sized *hobo* elements is indicated. The strains are: lane (1) Limpopo; (2) Harwich 77; (3) sd^{ry+}; ry^{506}; (4) y sn^w; bw st; (5) Bethalie; (6) Kalahari; (7) MGR-1; (8) Oregon RC; (9) Samarkand; (10) Swedish C.

Figure 3 is representative of our results for surveys of lines collected in particular geographic areas over several decades. It shows DNA from 19 strains of *D. melanogaster* from the eastern Mediterranean, Middle East and western Central Asia, arranged from left to right by the year of collection of the line from 1950 to 1982. The 2·6 kb size class is again indicated, as is a 1·1 kb size class corresponding to the *Th1* and *Th2* elements of Periquet (Periquet *et al.* 1989*a*, 1990). Some of the lines (VAL 50 (lane 1), QAA 52 (3), VAR 60 (6), DIL 64 (7) and PON 70 (11)) are E lines. The rest all show hybridization typical of H lines except LM (lane 5), which appears to lack a band at exactly 2·6 kb, although it has many bands of smaller size. Similar results were obtained from 84 other lines from northeastern North America (collections from 1929 to 1980), northwestern Europe (1925–1986), and from the Iberian peninsula (1962–1981) (not shown; IAB, unpublished data).

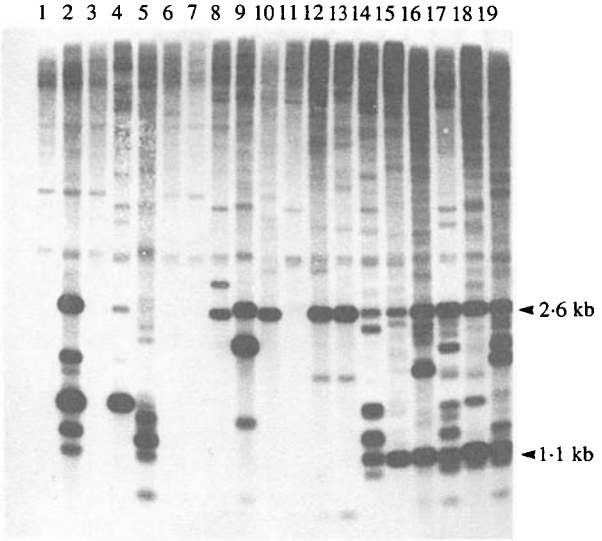

Fig. 3. *Xho* I digests of genomic DNA, probed with the pRG2.6X probe, from 19 strains of *D. melanogaster* collected in the eastern Mediterranean, Middle East and western central Asia, arranged according to the year of collection (from 1950 to 1982). The 2·6 kb fragment is indicated, as is the 1·1 kb fragment class corresponding to the *Th1* and *Th2* elements of Periquet *et al.* (1989*a*). The lines, their countries of origin, and years of collection are: lane (1) Valdagno, Italy, 1950; (2) Aspra, Italy, 1952; (3) Qiryat Anavim-A, Israel, 1952; (4) QI-2, Israel, 1954; (5) LM, USSR, 1960; (6) Varese, Italy, 1960; (7) Dilizhan, USSR, 1964; (8) Athens-2, Greece, 1965; (9) Athens-3, Greece, 1965; (10) Dilizhan, USSR, 1966; (11) Ponza, Italy, 1970; (12) Uman, USSR, 1970; (13) Krasnodar, USSR, 1974; (14) Schio, Italy, 1975; (15) Bizerte, Tunisia, 1978; (16) Krasnodar, USSR, 1980; (17) Qiryat Anavim-B, Israel, 1981; (18) Hacettepe, Turkey, 1982; (19) Kibris, Cyprus, 1982.

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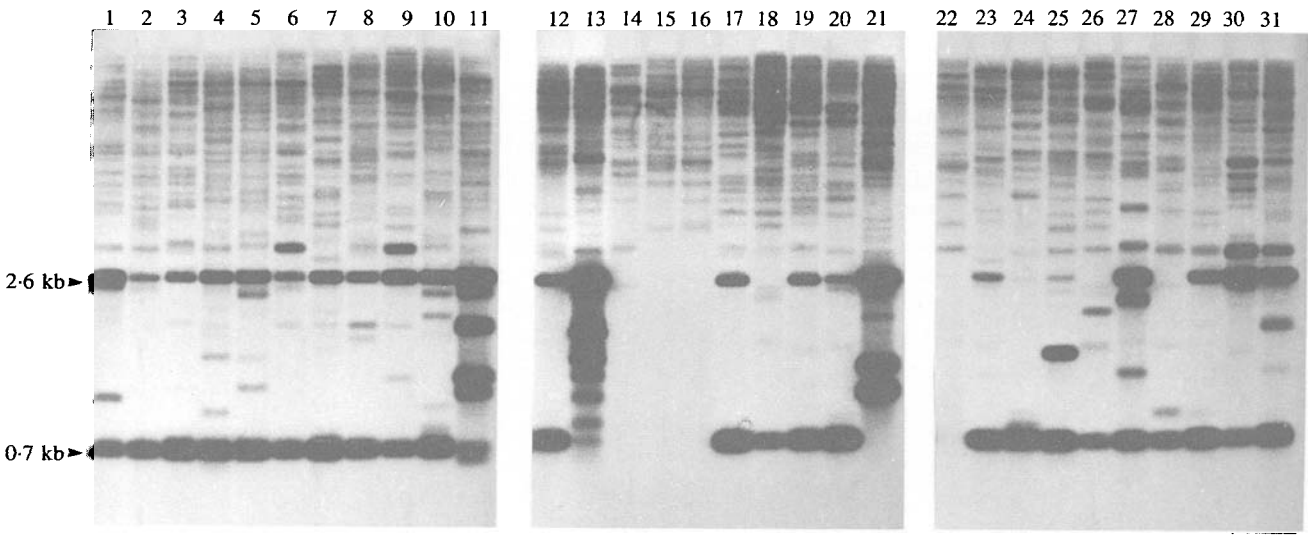

Fig. 4. *Xho* I digests of genomic DNA from 31 strains of *D. simulans*, probed with the pRG2.6X probe. The 2·6 kb fragment expected from full-sized *hobo* elements is indicated, as is a 0·7 kb fragment class. Lanes 1–10 represent strains from North America; lanes 11–21 from divers places; lanes 22–31 are of unknown provenance. Strain names, and origins as far as are known (in parentheses), are as follows (BG indicates a strain from the National Drosophila Species Resource Center): lane (1) A8-9 (North America); (2) DsChovnick (North America); (3) 8DS (North America); (4) Amherst (Massachusetts, 1967); (5) Weymouth (Providence, Rhode Island); (6) Archwhite (Lake Placid, Florida, 1981); (7) Chicago (Illinois); (8) DsTuc2 (Tucson, Arizona, 1985); (9) BG 14021-0251.6 (California); (10) BG 14021-0251.8 (Mexico); (11) BG 14021-0251.3 (Honduras); (12) BG 14021-0251.0 (Haiti); (13) BG 14021-0251.1 (Guyana); (14) BG 14021-0251.2 (Colombia); (15) BG 14021-0251.5 (Peru); (16) Lima (Peru); (17) Hawaii (Hawaii); (18) BG 14021-0251.4 (Australia); (19) Australia (Australia); (20) BG 14021-0251.7 (Cook Islands); (21) Szpb5A (South Africa); (22) b pm (unknown); (23) e ru (unknown); (24) f; pm net; st e (unknown); (25) jv st pe (unknown); (26) K18(Lhr) (unknown); (27) mf (unknown); (28) se (unknown); (29) w^a (unknown); (30) y w (unknown); (31) att-X y w (unknown).

(ii) hobo *in* D. simulans

Figure 4 shows *Xho* I digests of DNA from 31 lines of *D. simulans*. Since the year of collection is not available for most of these lines, we have grouped them by area of the world where such information was available. The 2·6 kb band expected from full-size *hobo* elements is indicated, as is a strong band in many of the lines of 0·7 kb in size. This 0·7 kb band corresponds to multiple copies of an internally deleted *hobo* element of about 1·1 kb that Periquet *et al.* (1990) have called *h. del. sim.* Most lines are H. Three lines [BG 14021-0251.4 (lane 18), K18 (Lhr) (lane 26), and se (lane 28)] do not have a 2·6 kb band at all, but have a strong band at 0·7 kb and a few other faint bands of less than 3 kb. A few lines [BG 14021-0251.5 (lane 15), Lima (lane 16), and perhaps BG 14021-0251.2 (lane 14) and b pm (lane 22)] are E.

(iii) *High-molecular-weight* Xho *I bands in all lines of both species*

Many bands of greater than about 3 kb (and of up to greater than 20 kb) are present in all lines tested of both species (Figs. 2–4), whether H or E. The precise pattern differs between lines in both *D. melanogaster* and *D. simulans*, but the overall pattern of many such bands is similar in both these species. The bands were prominent at the ≈ 76% stringency used, but were somewhat less prominent at higher stringencies (e.g. at ≈ 90% stringency, most such bands were still visible, but their intensities were less, whereas those corresponding to canonical *hobo* sequences were not less intense; data not shown).

4. Discussion

(i) *Historical pattern of* hobo *occurrence in* D. melanogaster

Our results corroborate the historical pattern described by Periquet *et al.* (1989*b*), in which only a few older lines (pre-1955, in their study) were E lines, whereas all more recent lines were H. Following Streck *et al.* (1986), we are interpreting *hobo*-hybridizing bands of 2·6 kb or less from an *Xho* I digest as representing full-sized or deletion-derived copies of the canonical *hobo* element. The latest E line we found was collected in 1970, in Ponza, Italy. We note that the absence of hybridization in a line can be interpreted as evidence of no *hobo* elements in the original collection (since the loss by genetic drift of all elements from a genome containing as many as 50 elements in 20 years is extremely unlikely (Engels, 1986). The presence of such hybridization, however, is not unequivocal evidence of the original presence of *hobo*. Contamination of an old line during its time in the laboratory could have occurred. Because of the possibility of contamination, it seems plausible that most or all early lines were originally E, as the

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simplest version of a recent introduction hypothesis would predict.

Besides the lines reported on here and the lines reported on by Periquet and co-workers, Boussy *et al.* (1989) reported on 30 recent (1983) Australian isofemale lines from six localities. All were H, but there was considerable variability between lines even from the same locality. This result has since been repeated with isofemale lines from a population in North America (unpublished data; S. Shapiro and I. A. B.). We interpret this variability among H lines as due to current activity of *hobo* elements in the species.

This historical pattern and current variability, then, suggest an introduction and spread of *hobo* sequences within recent times in *D. melanogaster*, as is probably the case for the *P* element (Anxolabéhère *et al.* 1988). Periquet *et al.* (1989*b*) have developed this idea for *hobo*, suggesting that such an introduction of *hobo* must have occurred before about 1950 in the Americas, since *hobo* elements seem to have preceded *P* elements in the species. Periquet *et al.* (1990) have further suggested that the likely donor species is *D. simulans*. If this were true, *D. simulans* should appear to have had *hobo* elements longer than has *D. melanogaster*, and should thus have a more stabilized pattern of occurrence among lines.

(ii) D. simulans *is as variable as* D. melanogaster

Our results for *D. simulans*, however, show *D. simulans* to be fully as variable as *D. melanogaster*, especially in having both H and E lines. Thus there is no obvious direction of transfer between *D. melanogaster* and *D. simulans*. It is unfortunate that more collections of *D. simulans* with known collection dates are not available from the last five decades in order to determine if there is an historical pattern among *D. simulans* lines, similar to that in *D. melanogaster*. The presence of both H and E strains in both *D. melanogaster* and *D. simulans* suggests either independent recent introductions into each or fairly efficient mechanisms for ridding some genomes of *hobo* sequences in both species.

(iii) hobo *patterns of* D. mauritiana *and* D. sechellia *are similar to those of* D. simulans

Streck *et al.* (1986) tested four lines of *D. simulans* and three of *D. mauritiana*, finding in each bands corresponding to full-sized and smaller *hobo* elements. Daniels *et al.* (1990*a*) tested a single line each of *D. mauritiana* and *D. sechellia* for *hobo*-hybridizing sequences. The *D. mauritiana* line corresponded to an H line in having a band at 2·6 kb and many smaller ones; the *D. sechellia* line lacked a band at 2·6 kb but had many smaller bands, thus appearing similar to several of the *D. simulans* lines cited above. These results were corroborated by Periquet *et al.* (1990), but the *D. sechellia* isolate of the same line that they examined had a 2·6 kb band, so apparently full-sized *hobo* elements are present in that species as well.

It is worth noting that all the published tests of the species *D. mauritiana* and *D. sechellia* have been done on only three lines of the former and one of the latter (Streck *et al.* 1986; Daniels *et al.* 1990*b*; Periquet *et al.* 1990), all of which were H. Thus it is plausible that E lines exist in these species as well and have simply not been found. Thus a recent introduction into *D. melanogaster* has difficulties explaining the presence of *hobo* elements in the sibling species *D. simulans*, *D. mauritiana* and *D. sechellia* (Streck *et al.* 1986; Daniels *et al.* 1990*a*) without hypothesizing at least one independent introduction into that lineage after its divergence from *D. melanogaster*.

Both *D. mauritiana* and *D. sechellia* both show a strong band at about 0·75 kb, corresponding to the 1·15 kb *h del maur* element described by Periquet *et al.* (1990) in *D. mauritiana*. Figure 1 of Streck *et al.* (1986) also shows these bands in the three lines of *D. mauritiana* represented. These elements and the 1·1 kb *h del sim* elements in *D. simulans* may have special properties in the genomes of their hosts that lead to their presences at high frequency, as might the *Th1* and *Th2* elements in *D. melanogaster* (Periquet *et al.* 1989, 1990; G. Periquet, personal communication). Both seem analogous to the *KP* element in *D. melanogaster*, an internally deleted *P* element for which special regulatory or transpositional properties have been postulated (Black *et al.* 1987; Boussy *et al.* 1988; Jackson, Black & Dover, 1988).

(iv) *The phylogenetic* hobo *pattern is more like that of* I *elements than that of* P *elements*

The 'recent invasion' hypothesis (Kidwell, 1983, 1986) suggests that *P* elements only entered (or, at least, spread widely in) *D. melanogaster* within the last 40 years. This hypothesis is supported by data showing a lack of *P* elements in strains collected prior to 1950, and by the subsequent increase in frequency of *P*-element-bearing strains from all regions of the world; the last strain completely lacking *P* elements was collected in 1974 (Anxolabéhère *et al.* 1988). Even in current populations, with many *P* elements per genome, there is much variation between individuals in numbers of elements and their *P–M* system properties (Boussy *et al.* 1988; Izaabel *et al.* 1987; Ronsseray & Anxolabéhère, 1986; Simmons, 1986). Since inbred lines into which *P* elements are introduced by crosses (Good *et al.* 1989; Kidwell, Kimura & Black, 1988) or by transformation (Daniels *et al.* 1987; Montchamp-Moreau, 1990; Preston & Engels, 1989) tend to stabilize with respect to numbers of elements per genome after many generations, the species-wide lack of uniformity could be due to a lack of time to reach stability on a global scale. The recent invasion hypothesis is also consistent with the complete absence of *P*-element-hybridizing DNA in all

other members of the *melanogaster* species subgroup (Brookfield, Montgomery & Langley, 1984; Daniels *et al.* 1990*b*).

Arguments about the history of the *I* element in *D. melanogaster* cannot be as simple, since *I*-element-hybridizng DNA is found in all members of the *melanogaster* species complex (Bucheton *et al.* 1986) and, indeed, in virtually all members of the *melanogaster* species group (Stacey *et al.* 1986). It has been proposed that 'reactivation' of *I* elements may have occurred by recombination between different defective *I* elements (Bucheton *et al.* 1984). It is also possible that the apparent historical pattern seen among strains collected in different eras (less *I* activity among older strains, especially those pre-dating 1940; Bregliano & Kidwell, 1983; Kidwell, Frydryk & Novy, 1983) is the result of loss of elements in inbred laboratory culture [the 'stochastic loss' hypothesis of Engels (1981, 1986, 1989)].

The *hobo* element's phylogenetic pattern of occurrence seems more like that of the *I* element than that of the *P* element. Streck *et al.* (1986) showed that *hobo* sequences occur in the sibling species *D. simulans* and *D. mauritiana*, as well as in *D. melanogaster*. Daniels *et al.* (1990*a*) showed that the *hobo* elements of the *melanogaster* species complex (*D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia*) were canonical in size and *Xho* I sites, but that *hobo*-hybridizing sequences are also found in the other species of the *melanogaster* subgroup and in the related *montium* subgroup. Based on these patterns, either a recent reactivation or reintroduction hypothesis or a stochastic loss hypothesis seems most likely to explain the *hobo* element's occurrence.

Our current results cast doubt on any simple explanation. They show that apparently non-equilibrium conditions (large variation among lines in numbers and types per genome) exist in both *D. melanogaster* and *D. simulans*. The existence of E lines in both species is especially telling, since active *hobo* elements would be expected to transpositionally 'infect' E chromosomes, converting them to H. The recent world-wide spread of *P* elements argues for nearly global panmixia of *D. melanogaster*. It therefore seems unlikely that E chromosomes could have persisted for long in the species without encountering H chromosomes and being converted. Thus, if we are to accept an invasion or reactivation hypothesis, we must hypothesize at least two invasions or reactivations, one into or in *D. melanogaster* and one into or in the *simulans–mauritiana–sechellia* lineage.

If we accept two invasions, there remains the difficulty of explaining how *D. simulans* could have both H and E lines if the *hobo* introduction occurred prior to the divergence of *mauritiana* and *sechellia* from *simulans* one to 3 million years ago (Cariou, 1987). Alternatively, we can assume only a single non-recent introduction into the ancestor of the *melanogaster* species complex. We then must formulate an explanation for the apparent non-equilibrium state of *hobo* elements in both *D. melanogaster* and *D. simulans*. It seems unlikely that *hobo* elements would have remained in non-uniform states in both species since their divergence 2–5 million years ago (Cariou, 1987). Some model of active genomic purging is needed; genetic drift, as suggested in the stochastic loss hypothesis of Engels (1981) is not adequate (Engels, 1986, 1989). More complex hypotheses, with introductions into isolated subpopulations of *D. simulans* and *D. melanogaster*, are possible, of course.

Periquet *et al.* (1990) have suggested yet another introduction hypothesis: that the current active *hobo* elements in *D. melanogaster* entered the species recently from *D. simulans*. Since both *D. melanogaster* and *D. simulans* have both H and E lines, it is not obvious which should be considered the donor species and which the recipient, or whether another species, perhaps outside the genus, was the source for a horizontal transmission into both species (and also into *D. mauritiana* and *D. sechellia*).

(v) *Interpretation of the high-molecular-weight* Xho *I bands*

The bands of greater than about 3 kb (and of up to greater than 20 kb) seen in all lanes in *Xho* I digests of both *D. simulans* and *D. melanogaster* (Figs. 2–4) are also present in *D. mauritiana* and *D. sechellia* (Streck *et al.* 1986; Daniels *et al.* 1990*a*). The bands were described by Streck *et al.* (1986) in their E lines as well as their H lines of *D. melanogaster* as 'a heterogeneous collection of light bands of hybridization, most of which correspond to *Xho* I fragments larger than 8 kb'. The stringencies of hybridization and washing used by Streck and co-workers were ≈ 76 and ≈ 81%, respectively, and thus were comparable to ours.

The bands clearly represent multiple copy sequences, and they differ in location between lines, even between isofemale lines collected from the same locality (I.A.B., unpublished data; D. Y. Shapiro and I.A.B., unpublished data). This suggests that they are transposable elements in their own right. They do not seem to be canonical *hobo* elements; either they are much larger than canonical 3·0 kb *hobo* elements and all differ in size, or they lack one or both *Xho* I sites and therefore do not give a characteristic internal band with this enzyme. They may differ from the canonical *hobo* sequence either in overall similarity or in having only small regions of similarity, since at very high stringency they are less visible on a blot (data not shown). (This, plus perhaps poor transfer of high molecular weight DNA to a blot, may explain why other workers have not considered them critically.)

The pattern of occurrence of these bands in both H and E lines is reminiscent of the pattern of putatively degenerate *I* elements seen among R and I strains of *D. melanogaster* (Bucheton *et al.* 1984; Bucheton, 1990). Are the *hobo*-hybridizing bands relicts of *hobo*s

introduced long ago into the melanogaster species complex, and since highly degenerated? Were the *hobo* elements we now recognize as canonical reconstituted from such degenerate sequences by recombination or gene conversion, as has been hypothesized for active *I* elements? Or are they another family of transposable elements altogether, that diverged from *hobo* prior to either's entry into the *melanogaster* species complex, and that thus currently bear only partial similarity to *hobo*? Are these bands related to the (less numerous) bands seen in the *yakuba* and *erecta* species complexes and in the montium subgroup species (Daniels *et al.*, 1990*a*)? Do all these bands represent a superfamily of related elements? Answers to these and similar questions will only be forthcoming when the DNAs of these bands are cloned and sequenced, and the sequences compared between strains and species and to that of the canonical *hobo*.

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