Distribution of *hobo* Transposable Elements in Natural Populations of *Drosophila melanogaster*[1]

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Forty-six strains derived from American and French natural populations of *Drosophila melanogaster* were tested for the presence and activity of *hobo* elements by using Southern blotting and a gonadal dysgenesis assay. The oldest available strains exhibited weak detectable hybridization to the *hobo*-element probe and revealed neither *hobo*-activity potential nor *hobo*-repression potential. In contrast, all recently collected strains harbored *hobo* sequences and revealed a strong *hobo*-repression potential but no strong *hobo*-activity potential. On the basis of restriction-enzyme analysis, old strains appear to have numerous fragments hybridizable to *hobo* sequences, several probably conserved at the same locations in the genome of the tested strain and others dispersed. In recently isolated strains, and unlike the situation in the published sequence of the cloned $hobo_{108}$ element, a *Pvu*II site is present in the great majority of full-sized *hobo* elements and their deletion derivatives. When the genetic and molecular characteristics are considered together, the available evidence is consistent with the hypothesis of a worldwide *hobo*-element invasion of *D. melanogaster* during the past 50 years. Comparison of data from the I-R and P-M systems suggests that the putative invasion followed the introduction of the *I* element but preceded that of the *P* element. This hypothesis poses the problem of the plausibility of three virtually simultaneous element invasions in this species. Such a possibility might be due to a modification of the genetic structure of American populations of *D. melanogaster* during the first part of the 20th century.

Introduction

Among the mobile elements that have been studied in *Drosophila melanogaster*, three independent systems (*I*, *P*, and *hobo*) can produce a number of germ-line abnormalities referred to as "hybrid dysgenesis" (Kidwell et al. 1977). The *hobo* system has been implicated in such genetic instabilities as germ-line hypermutability, chromosomal aberrations, and gonadal dysgenesis (Lim 1979; Yannopoulos et al. 1983, 1987; Blackman et al. 1987).

The *hobo* element was cloned and characterized by McGinnis et al. (1983) and subsequently sequenced by Streck et al. (1986). This $hobo_{108}$ sequenced element contains 3,016 bp, including 12-bp inverted terminal repeats. The functional *hobo* element produces an 8-bp duplication of the target site on insertion into the genome and contains both a 2-kb main and other, smaller open reading frames. Molecular analyses have revealed two classes of strains, as defined by their *hobo* elements (reviewed in Blackman and Gelbart 1988). H strains contain 3.0-kb full-sized elements and nu-

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merous smaller derivatives, whereas E strains lack all such elements but show some weakly hybridizing sequences. The number of complete *hobo* elements in the genome of H strains is usually low, 2–10 copies (Streck et al. 1986; Blackman et al. 1987), whereas the number of defective *hobo* elements is 30–75 elements/genome, although, unlike the situation in *P* elements, there are only a few size classes typical of different H strains. The physical structure, genomic distribution, and genetic behavior of the *hobo* system are all similar to those of the *P*-element system. However, the extent of the similarity between *P* and *hobo* is not clear. There are not many data on hybrid dysgenesis and the pattern of *hobo* distribution in natural populations of *D. melanogaster.*

In the present paper we report the genetic and molecular analysis of a temporal survey of *D. melanogaster* strains by using a gonadal dysgenesis assay and Southern blots to determine the presence and activity of *hobo* sequences in these populations. Differences observed between older collected strains and more recently collected ones are examined in the light of genetic and molecular results, in order to understand the evolutionary history of the *hobo* system in *D. melanogaster.*

Material and Methods

Drosophila Strains

Fifty strains collected during 1935–89 in the Americas and in France were analyzed at the genetic and molecular levels. These strains were known for their P-M status. They were classified as P (harboring *P* elements with a strong activity), Q (with *P* elements but weak activity), M′ (with deleted *P* elements and no activity) and M (without *P* elements) (Anxolabéhère et al. 1988).

Harwich^{Y} and 23.5*/Cy were the reference strains used in the gonadal dysgenesis assays. Harwich^{Y} is a derivative Harwich strain obtained from Dr. Yannopoulos's laboratory and is a P strain in the P-M system. The 23.5*/Cy strain bears the male recombination factor 23.5 MRF that was found to induce hybrid dysgenesis (Yannopoulos et al. 1987). It is a Q strain in the P-M system.

hobo System and Gonadal Dysgenesis Assay

The cross between 30 Harwich^{Y} females and 30 23.5*/Cy males at 25°C was carried out as a control. This cross results in ~70% gonadal dysgenesis in the wild daughters. The reciprocal cross led only to very low (~1%) levels of gonadal atrophy. The gonadal dysgenesis obtained in these conditions has been attributed to the activity of complete *hobo* elements present in the 23.5*/Cy strain (Stamatis et al. 1989).

Determination of *hobo* status of tested strains was based on standard tests for measuring induced gonadal dysgenesis. Thirty virgin individuals of the strain tested were mated en masse at 25°C as follows: A cross—♀♀ Harwich^{Y} × ♂♂ tested; A* cross—♀♀ tested × ♂♂ 23.5*/Cy.

After eclosion of the F1, flies were collected and allowed to mature for 3 d. At least 50 wild females were then taken at random for dissection. The percentage of dysgenic ovaries [100 × (number of dysgenic ovaries/total number of ovaries scored)] was used to classify the strain. The A cross provides a measure of the *hobo*-activity potential of a tested strain. The A* cross provides a measure of *hobo*-repression potential. A high percentage of dysgenic ovaries in the progeny from an A cross defines the strain as H^{+}, whereas such a high percentage in the progeny from the A* cross defines an H^{-} strain.

As Harwich^{Y} is a strong P strain, all the dysgenic ovaries found at 25°C in the

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progeny of the A cross can be imputed to the *hobo* activity of the tested strain, because the P cytotype of Harwich^{Y} females represses gonadal dysgenesis induced by the *P* elements of the tested strain. On the other hand, 23.5*/Cy is a Q strain which generates only 1% gonadal dysgenesis in the P-M system when crossed at 25°C. Consequently the level of gonadal dysgenesis found in the progeny of A* crosses can be imputed to the *hobo* system (Yannopoulos et al. 1987). Similar results—but with a lower level of induced gonadal dysgenesis—were obtained with the Oregon R^{s} strain of Sparrow (an M strain harboring active *hobo* elements).

On the basis of this gonadal dysgenesis assay, three classes of strains can be defined. H^{+} strains (such as 23.5*/Cy) reveal their *hobo*-activity potential (>5% gonadal dysgenesis) when crossed with Harwich^{Y} (cross A) and reveal their *hobo* repression potential (<5% gonadal dysgenesis) when crossed with 23.5*/Cy (A* cross). H$^{\circ}$ strains show weak *hobo*-activity potential (<5% gonadal dysgenesis) in an A cross but normal *hobo*-repression potential in an A* cross, whereas H^{-} strains lack both *hobo*-activity and -repression potentials (<5% gonadal dysgenesis in an A cross and >5% gonadal dysgenesis in an A* cross).

Hybridization Techniques

The presence of *hobo* sequences was tested by Southern blot analysis using the 2.6-*Xho*I internal restriction fragment (see fig. 1) of the pHcSac plasmid as a probe (Stamatis et al. 1989). Genomic DNA was extracted from 200 female flies/strain and was digested with *Xho*I. Southern blots were performed using standard techniques (Maniatis et al. 1982, p. 382). Hybridization were performed in 0.45 M NaCl, 0.045 M trisodium citrate (3 × SSC), 0.2% Ficoll 400, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.1% lauryl sulfate (SDS) overnight at 65°C. Filters were washed at high stringency (1 × SSC, 0.1% SDS at 65°C).

Results

Genetical Analysis

The genetic and molecular results for the strains analyzed are shown in tables 1 and 2. The results of gonadal dysgenesis assays indicate that the majority of strains derived from natural populations in the Americas (table 1) and France (table 2) before the mid-1950s are unable to give dysgenic ovaries in the progeny of A crosses and cannot repress *hobo* activity in A* crosses. They are basically H^{-} strains. In contrast, almost all populations collected after the mid-1950s show *hobo*-repressor

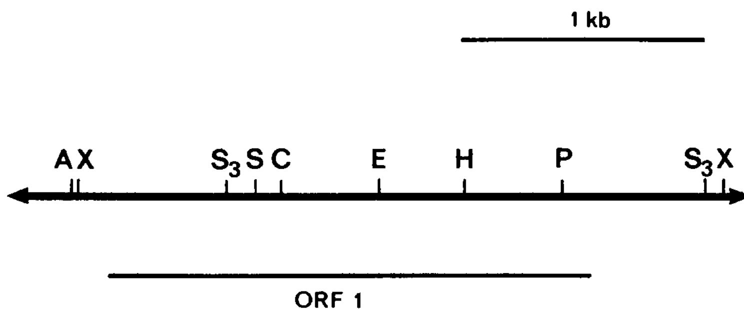

FIG. 1.—Structure of *hobo* element, with endonuclease cleavage sites of enzymes. A = *Acc*I; X = *Xho*I; S_3 = *Sau*3AI; S = *Sal*I; C = *Cla*I; E = *Eco*RV; H = *Hin*dIII; P = *Pvu*II.

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Table 1
Molecular and Phenotypic Characteristics in *hobo* System, for Strains Collected in North and South America between 1925 and 1989

Year(s), Strain	Strength of Hybridization to *hobo* Fragment[a]				*hobo* Activity Potential[b]	*hobo* Repression Potential[c]	P-M Status[d]
	2.6	1.5	1.1	Other			
Controls:							
NA, 23.5*/Cy	S	W	O	I	70	1	Q
NA, Oregon R[s]	I	S	O	E	20	1	M
NA, Harwich[Y] (United States of America)	W	E	I	I	0	70	P
1935, Canton-S (United States of America)	O	O	O	E	0	20	M
1936, Inbred (United States of America)	E	O	O	E	...	...	M
1938, Lausanne S (United States of America)	E	O	O	E	0	80	M
1938, NB1, Connecticut	O	O	O	E	0	30	M
1938, Cockaponsett Forest (United States of America)	O	O	O	E	0	32	M
1938, IF-38, Idaho Falls, Idaho	W	O	O	W	0	0	M′
1950–55, Boa Esperance, Brazil	E	E	O	E	...	...	M
1950–55, Gruta, Argentina	O	O	O	E	0	10	M
1952, 731C, St. Paul	I	O	O	E	1	35	M
1952, 91C, St. Paul	W	S	W	E	12	4	M′
1954, NO1, New Orleans	E	E	O	E	...	...	M
1954, RC1, Riverside (United States of America)	W	I	O	E	...	...	M
1954, BV1, Blasksburg (United States of America)	W	O	I	I	...	...	P
1957, SC1, Santiago, Chile	O	O	O	E	0	71	M
1957–58, Ica, Peru	O	O	I	I	0	23	M′
1962, BOG1, Bogota	O	O	O	E	0	20	M′
1963, RVC2, Riverside (United States of America)	O	O	O	W	...	...	M
1966, 4B, Mammoth Cave	I	O	W	I	0	2	M
1966, 10D, South Carolina	W	O	W	W	1	3	M
1967, Harwich, Massachusetts	E	O	W	I	1	0	P
1970, Marion (United States of America)	O	O	W	I	0	8	M′
1971, SH-71G, New Jersey	I	E	I	I	...	...	M′
1980, NY2, New York	I	W	W	I	0	5	P
1981, Furnace Creek, California	W	O	I	W	0	0	P
1982, Raleigh, North Carolina	I	O	I	I	0	0	P
1989, Chicago	I	W	I	I	0	0	P

[a] S = strong; I = intermediate; W = weak; E = extremely weak; O = hybridization undetectable.
[b] Measured as % gonadal dysgenesis in diagnostic cross ♀ Harwich[Y] × tested ♂.
[c] Measured as % gonadal dysgenesis in diagnostic cross ♀ tested × ♂ 23.5*/Cy.
[d] According to Anxolabéhère et al. (1988).

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Table 2
Molecular and Phenotypic Characteristics in *hobo* System of Strains Collected in France between 1938 and 1989

Year(s), Strain	Strength of Hybridization to *hobo* Fragment[a]				*hobo* Activity Potential	*hobo* Repression Potential	P-M Status
	2.6	1.5	1.1	Other			
1938, Banyuls	O	O	O	E	0	73	M
1942, Champetières	E	O	O	E	0	41	M
1945, Paris	E	O	O	E	0	88	M
1946, Charolles	O	O	O	E	0	17	M
1952, Banyuls	O	O	O	E	0	8	M
1957, Ajaccio (Finosello)	E	O	O	E	0	7	M
1965, Marseillan	W	O	O	I	0	3	M
1967, Tautavel	W	E	W	I	0	0	P
1968, Lapeyrouze	O	O	W	W	0	3	M
1969, Kerbinou	W	W	I	I	0	25	P
1970, Le Chatelet	I	O	S	I	0	0	P
1973, Le Mesnil	I	O	S	W	0	0	Q
1976, Brouilly	I	O	S	I	...	...	Q
1976, Tostes	I	O	S	E	...	...	Q
1978, Mèze	I	O	S	I	0	2	Q
1982, Tours	I	O	S	I	0	0	Q
1983, Tautavel	I	O	S	E	0	0	Q
1986, Ste. Geneviève	I	O	S	W	0	0	Q
1988, Tours	W	O	S	E	0	0	Q
1989, Sarlat	W	O	S	W	0	0	Q

Note.—See footnotes to table 1.

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activity in the A* cross but do not reveal *hobo*-dysgenic activity in the A cross. We have called these strains "H°." However, at least some of them were probably polymorphic, with some H^+ individuals (as has been shown for other recently collected European strains from Greece, France, Spain and the USSR) in which some sublines were able to induce ≤20% gonadal dysgenesis (Yannopoulos et al. 1987; Bazin et al. accepted; G. Periquet, unpublished data). Finally, no strong H^+ strains were found in our survey.

Molecular Analysis

The presence and structure of *hobo* elements was analyzed by Southern blotting (tables 1 and 2 and fig. 2 and 3). At normal exposure most of the strains collected before the mid-1950s are devoid of both the 2.6-kb fragment corresponding to the full-sized *hobo* element and the smaller derivatives. These strains appear to be typical E strains—although they are not totally devoid of *hobo*-hybridizable sequences, as some clear bands were detected above the 3-kb level. However, after long exposure (4 d; fig. 2), some clear fragments, the size of which is <2.6-kb, were revealed in almost all strains. Moreover, in several strains (e.g., Lausanne S, NB1, RC1, and Champetières) a fragment of 2.6 kb was revealed. As our DNA samples were prepared from ~200 flies, these results suggest that the old strains are probably polymorphic, with some *hobo*-hybridizable sequences being occasionally present. Although the exact nature of these sequences remains to be determined, these strains cannot strictly be described as empty of *hobo*-like sequences.

Most of the strains collected after the mid-1950s are H strains with putative full-sized and defective *hobo* elements. Among the defective elements, one class was ob-

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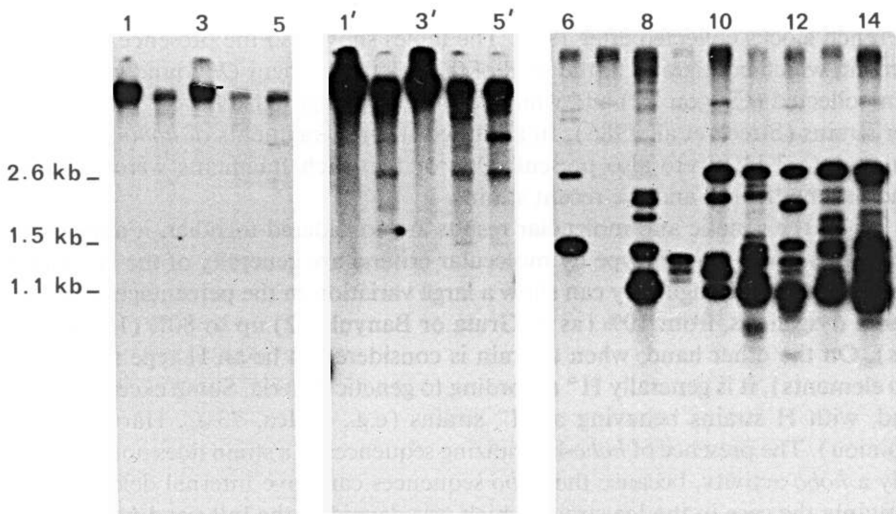

FIG. 2.—Southern blot analysis of *hobo* elements in *Drosophila melanogaster* American strains. The 2.6-kb *Xho*I fragment derived from full-sized *hobo* elements and the 1.5-kb and 1.1-kb fragments corresponding to the deleted-derivatives *Oh* and *Th* elements are indicated. Lane 1, Canton-S 1935. Lane 2, Lausanne-S 1938. Lane 3, NB1 1938. Lane 4, Boa Esperanca 1950–55. Lane 5, NO1 1954. Lanes 1′–5′, Same strains after a long exposure (4 d). Lane 6, RC1 1954. Lane 7, SC1 1957. Lane 8, Ica 1957–58. Lane 9, RVC2 1963. Lane 10, 4B 1966. Lane 11, SH-71G 1971. Lane 12, NY2 1980. Lane 13, Raleigh 1982. Lane 14, Chicago 1989.

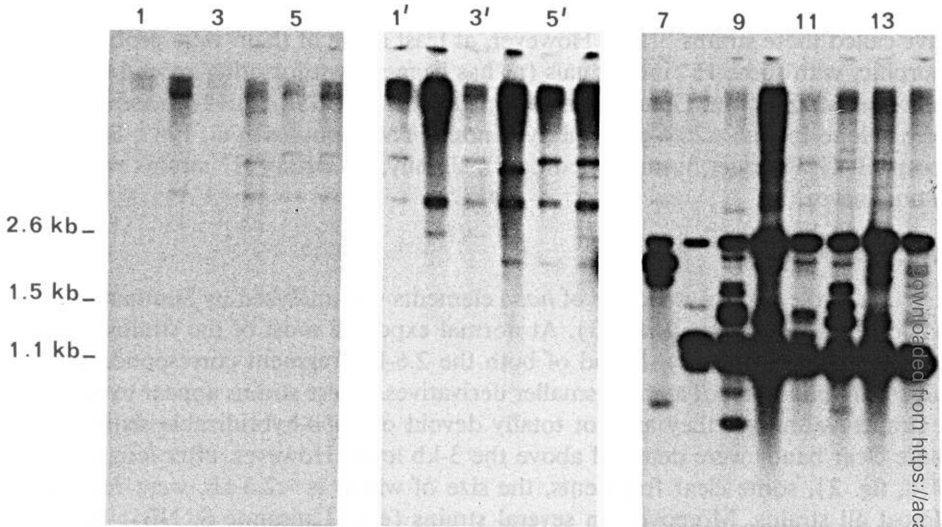

FIG. 3.—Southern blot analysis of *hobo* elements in *Drosophila melanogaster* French strains. Lane 1, Banyuls 1938. Lane 2, Champetières 1942. Lane 3, Paris 1945. Lane 4, Charolles 1946. Lane 5, Banyuls 1952. Lane 6, Ajaccio 1957. Lanes 1′–6′, Same strains after a long exposure (4 d). Lane 7, Marseillan 1965. Lane 8, Tautavel 1967. Lane 9, Kerbiniou 1969. Lane 10, Le Chatelet 1970. Lane 11, Tostes 1976. Lane 12, Mèze 1978. Lane 13, Tautavel 1983. Lane 14, Tours 1988.

served in the majority of strains tested. This class gives a 1.1-kb fragment corresponding to the 1.5-kb deleted *Th* element currently found in all natural Eurasian strains (Periquet et al. 1989*a*). This band appears for American stocks collected after 1953 and for French stocks collected after 1966. The tables show also the presence of a 1.5-kb fragment, which comigrates at the level of the deleted element *Oh* found in the oldest strain collected (Oregon R^s) and which at one time might also have been present in other strains (Streck et al. 1986). In all tested strains, fragments of *hobo*-hybridizing sequences (>3 kb) were also present. One or two such fragments were frequently found among the old and the recent strains.

When the genetic and molecular results are considered together, it appears that strains considered to be E type by molecular criteria are generally of the H$^-$ type by genetic criteria, although they can show a large variation in the percentage of induced gonadal dysgenesis, from 10% (as in Gruta or Banyuls 52) up to 80% (LausanneS, Paris). On the other hand, when a strain is considered to be an H type strain (with *hobo* elements), it is generally H$^\circ$ according to genetic criteria. Some exceptions were found, with H strains behaving as H$^-$ strains (e.g., in Ica, 731C, Harwich^Y, and Kerbiniou). The presence of *hobo*-hybridizing sequences in a strain does not necessarily imply a *hobo* activity, because the *hobo* sequences can have internal deletions. This is certainly the case in the Ica strain, which was devoid of the full-sized *hobo* element (fig. 2). However, apparently full-sized *hobo* elements are present in the 731C, Kerbiniou, and Harwich^Y strains (figs. 2 and 3). The *hobo* elements present in these strains are probably inactivated by either a mutation or a microdeletion, even though they appear to be complete elements.

Given the classification of these strains in the P-M system (Anxolabéhère et al. 1988), the oldest strains are mainly of the E;M type, with the striking exception of

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the Oregon R^s strain (H; M). This H; M combination is rather rare; most of the recently collected stocks are H; P strains. The E; P combination was not found in any population.

hobo-homologous Sequences in the E Strains

To examine the distribution of the *hobo*-hybridizing fragments present in different populations, we carried out two complementary experiments. First, DNA samples from 12 E strains from the Americas, Europe, and Japan were blotted after double digestion by *Bam*HI and *Bgl*II, two enzymes that do not cut *hobo*.

The results are illustrated in figure 4, which presents the Southern blot analysis of 10 E strains and five H strains, separated by the control Oregon R^s strain. Although similar, the different strains do not have the same pattern, either for the number of bands (from ~20 to >30) or for the size of fragments (from <2 kb to 20 kb). These data suggest the presence of a few well-conserved *hobo* sequences at the same locations in the genomes of the tested strains (giving comigrating bands), as well as other sequences differently dispersed (giving the other bands).

In a second experiment, DNA from Lausanne S, Paris, and Hikon (an old Japanese E strain) was digested independently with three pairs of enzymes: *Ava* I+*Cla*I, *Sal*I + *Hin*dIII, and *Sau*3AI+*Eco*RV. Gels were run to reveal the presence of fragments similar to those expected from the complete *hobo* element (fig. 1). In the different strains there was an irregular presence of fragments of the expected size (fig. 5). The left-hand fragments *Ava*I-*Cla*I (0.83 kb) and *Sau*3AI-*Eco*RV (0.61 kb) appeared to be present in the three tested strains, while the right-hand fragment *Eco*RV-*Sau*3AI (1.32 kb) was only detected in the Paris strain, as was the internal fragment *Sal*I-

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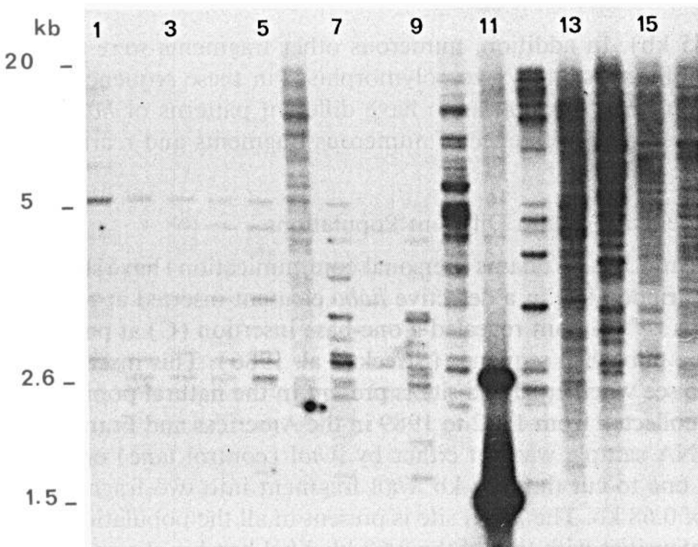

FIG. 4.—Southern blot analysis of E and H strains of *Drosophila melanogaster*. DNA samples were digested with *Bam*HI+*Bgl*II (which do not cut the standard *hobo* element), except for Oregon R^s (lane 11), which was digested with *Xho* I in order to give the control fragments of 2.6 kb and 1.5 kb. Lane 1, Canton-S 1935. Lane 2, Lausanne S 1938. Lane 3, NB1 1938. Lane 4, NO1 1954. Lane 5, Banyuls 1938. Lane 6, Paris 1945. Lane 7, Charolles 1946. Lane 8, Banyuls 1952. Lane 9, Crimea (USSR) 1936. Lane 10, Kurumé (Japan) 1976. Lane 11, Oregon R^s 1925. Lane 12, 4B 1966. Lane 13, SH-71 1971. Lane 14, Chicago 1989. Lane 15, Marseillan 1965. Lane 16, Tostes 1976.

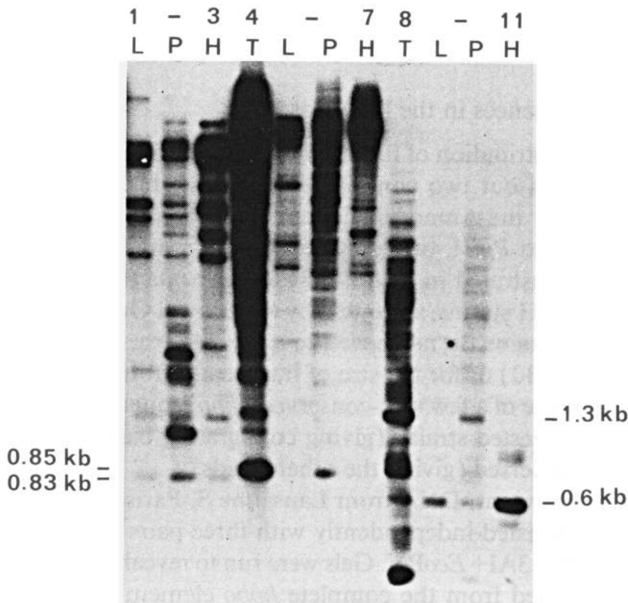

FIG. 5.—Southern blot analysis of E strains of *Drosophila melanogaster.* DNA samples were digested with *Ava*I+*Cla*I (lanes 1–3), *Sal*I+*Hin*dIII (lanes 4–7), and *Sau*3AI+*Eco*RV (lanes 8–11). Strains are as follows: Lausanne-S 1938 (L), Paris 1945 (P), Hikon (Japan) 1950–59 (H), and, as control, the H strain Tours 1982 (T). The conserved fragments expected from the sequence of the complete *hobo* sequence are indicated: *Eco*RV-*Sau*3AI (1.3 kb); *Sal*I-*Hin*dIII (0.85 kb); *Ava*I-*Cla*I (0.83 kb); *Sau*3AI-*Eco*RV (0.6 kb).

*Hin*dIII (0.85 kb). In addition, numerous other fragments were detected, showing the presence of a substantial size polymorphism in these sequences. In conclusion, most of the old E strains appear to have different patterns of *hobo*-hybridizing sequences, suggesting the presence of numerous fragments and rearrangements of the standard sequence.

Presence of the *Pvu*II Site in Different Populations

C. Bazin and J. A. Williams (personal communication) have shown the presence of a *Pvu*II restriction site in a defective *hobo* element inserted at the *vg* locus. Their analysis of this vg^{a1} mutant revealed a one-base insertion (C) at position 2227, compared with the published sequence (Streck et al. 1986). This insertion produced the *Pvu*II site. To see whether such a site is present in the natural populations, we tested 12 H strains collected from 1952 to 1989 in the Americas and France (figs. 6 and 7).

Each DNA sample was cut either by *Xho*I (control lane) or by *Xho*I+*Pvu*II. *Pvu*II allows one to cut the 2.62-kb *Xho*I fragment into two fragments, one of 1.94 kb and one of 0.68 kb. The *Pvu*II site is present in all the populations tested, and, by comparing intensities with that of the 2.62-kb *Xho*I band in the control line, we conclude that the large majority of the potentially full-sized *hobo* elements in these strains possess the *Pvu*II site. Comparisons between the pattern of the control lane and that of the sample double-digested with *Xho*I-*Pvu*II showed that the *Pvu*II site is frequent in both larger and smaller elements. This site is also present in the complete and functional *hobo* element inserted into the pHFL1 plasmid, as well as in the elements inserted into the pHcSac and pRG 2.6X plasmids (data not shown). It appears that

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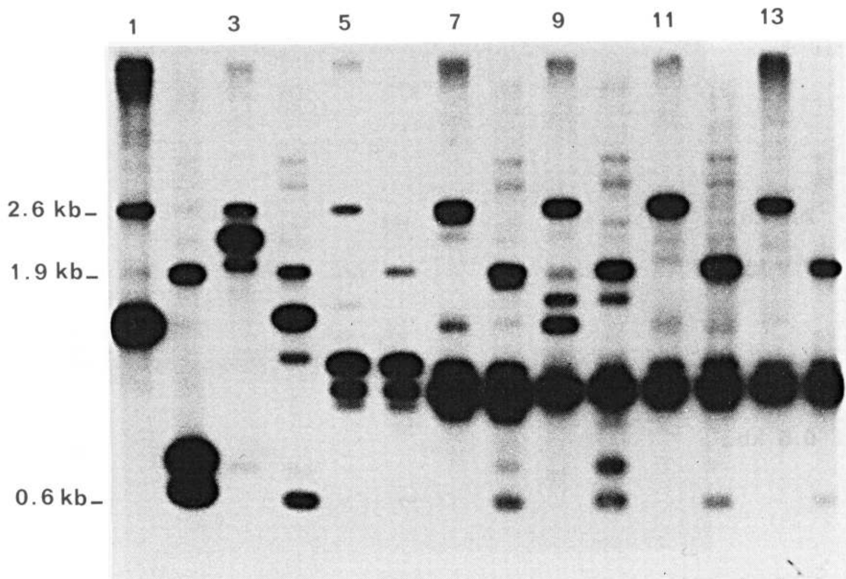

FIG. 6.—Southern blot analysis of presence of *Pvu*II site in *hobo* element of *Drosophila melanogaster* American strains. DNA samples were digested with either *Xho*I (odd-numbered lanes) or *Xho*I+ *Pvu*II (even numbered lanes). Strains are as follows: Oregon R^s 1925 (lanes 1 and 2); BV1 1954 (lanes 3 and 4); RC1 1954 (lanes 5 and 6); 4B 1966 (lanes 7 and 8); SH-71 1971 (lanes 9 and 10); NY2 1980 (lanes 11 and 12); Chicago 1989 (lanes 13 and 14).

the *hobo* element cloned into *pH 108* that was sequenced by Streck et al. (1986) does indeed have a C at position 2227 (M. Calvi and W. M. Gelbart, personal communication).

Discussion

Our survey of the *hobo* status of samples collected from natural populations of *Drosophila melanogaster* revealed both a clear difference between the oldest and the more recently collected strains and a correlation between the genetical and molecular analyses. H^- strains that show a high percentage of gonadal dysgenesis in the progeny from the A* cross are generally devoid of *hobo* hybridizable sequences (i.e., are E strains). Although some E strains do have deleted or rare *hobo* hybridizable sequences, we shall continue to use the nomenclature until improved molecular methods distinguish between the different types of E strains. Strains with a low *hobo* repressor potential can be found among these E strains; it is possible that their long period under laboratory conditions has resulted in the accumulation of gonadal dysgenesis modifying agents.

Strong H^+ strains [e.g., 23.5*/Cy (Yannopoulos et al. 1987) and Oregon R^s (Blackman et al. 1987)] apparently have several active *hobo* elements; we found no such strains among the lines we surveyed. Virtually all the recently derived laboratory populations are H° strains with very weak or no *hobo* activity potential (as measured by the gonadal dysgenesis criterion) but able to completely repress the introduction of active *hobo* elements. In this sense they are analogous to the Q type, as defined in the P-M system. The mechanism of this regulation remains to be understood and is probably complex (Blackman et al. 1989; Stamatis et al. 1989). Specific deleted derivative *hobo* elements presenting an internal deficiency have been found in the Tours population (as *Th* elements) and in almost all current natural populations (Periquet

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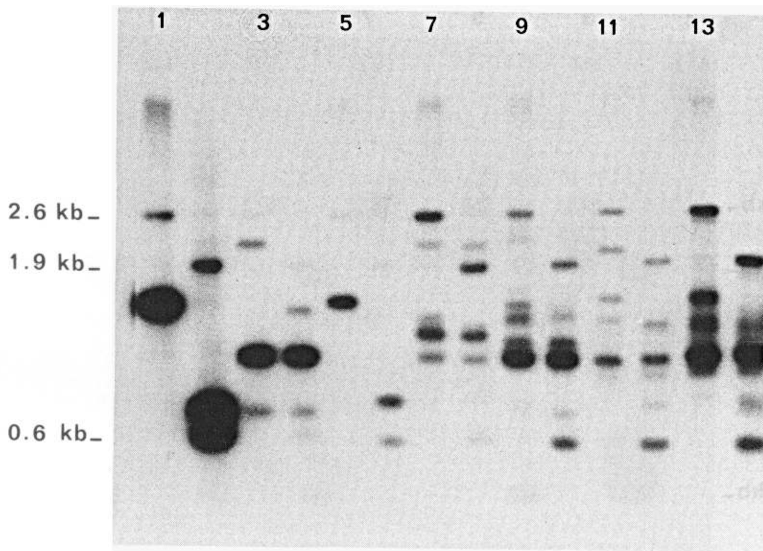

FIG. 7.—Southern blot analysis of presence of *Pvu*II site in *hobo* element of *Drosophila melanogaster* French strains. DNA samples were digested with either *Xho*I (odd numbered lanes) or *Xho*I+*Pvu*II (even numbered lanes). Strains are as follows: Oregon R^s 1925 (lanes 1 and 2); Marseillan 1965 (lanes 3 and 4); Tautavel 1967 (lanes 5 and 6); Le Chatelet 1970 (lanes 7 and 8); Mèze 1978 (lanes 9 and 10); Tautavel 1983 (lanes 11 and 12); Tours 1988 (lanes 13 and 14).

et al. 1989*a*). Two subfamilies, *Th1* and *Th2*, are present in most strains from different continents (Periquet et al., accepted). Their presence in so many strains at such high numbers per genome might contribute to the regulation mechanisms of the *hobo* system, as is the case for the specific deleted derivative *P* element found in the Krasnodar population (as the *KP* element) in the P-M system (Black et al. 1987; Jackson et al. 1988).

The existence of H strains that behave like H$^-$ strains in genetic analysis can be explained if we assume that the *hobo* sequences present in these strains are defective and have no regulatory function (like, e.g., M′ strains in the P-M system). It has already been noted that the presence of full-sized *hobo* elements does not necessarily mean that they are functional (Blackman and Gelbart 1988; Stamatis et al. 1989).

The existence of H$^-$ strains shows that divergent sublines can be obtained with relative ease in the *hobo* system. Such divergence, although not due to artificial selection but merely to genetic drift, is well illustrated by the Harwich^Y strain (H$^-$) derived from the original Harwich (United States) strain, which is an H° strain.

At the population level, the genetical and molecular analyses show that the majority of strains derived from natural populations in the Americas and in France before the mid-1950s contain few *hobo*-hybridizable sequences. In contrast, almost all recently captured strains carry numerous *hobo* elements. This is in agreement with results obtained for other areas such as the USSR, China, Japan, and Africa (Periquet et al. 1989*b*, and unpublished data) and now correlated with gonadal dysgenesis potential.

In the I-R and P-M systems the striking difference between old and more recently collected strains may be interpreted either by a stochastic loss of elements in strains maintained for several decades in laboratory conditions or by a recent introduction of elements into *D. melanogaster* (Kidwell 1983; reviewed in Engels 1988; Finnegan

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1988). For both systems the invasion hypothesis currently prevails, given that (1) the oldest stocks are either devoid of any element (as in the P-M system) or present identical molecular patterns (as in the I-R system) and (2) numerous studies at the population and phylogenetic levels plausibly suggest an origin of these elements in various related species (reviewed in Bucheton et al. 1986; Anxolabéhère and Periquet 1987; Daniels et al. 1990*b*).

In the *hobo* system the situation appears more complicated. The oldest stocks harbor different patterns of restriction fragments with probably deleted and rearranged *hobo* sequences and some rare potentially full-sized *hobo* elements. No (E;P), (E;Q), or (E;M′) strains have been found in *D. melanogaster*. Moreover, *hobo* sequences have been detected in sibling species of the *melanogaster* subgroup and in some species of the *montium* subgroup (Streck et al. 1986; Daniels et al. 1990*b;* Periquet et al., accepted).

The *hobo* elements are very variable in laboratory stocks and can be lost. Strains kept under laboratory conditions for many years may harbor active *hobo* elements that can be mobilized or deleted, and they may be subject to recombination leading to different derivative strains (Streck et al. 1986), as has also been observed in different H^+ and H° Oregon populations. H° and H^- Harwich populations and different H^- Canton-S populations show different patterns of restriction fragments by Southern blot analysis. But if some of these strains may have lost their active elements, it is more difficult to conceive how they could have rid themselves of the remaining non-autonomous deleted elements in a few decades. It would be necessary to assume some hitherto unknown mechanisms of recurrent internal deletions of these elements, leading to the presence of relictual sequences scattered throughout the genome, giving rise to the different patterns seen in old E strains. Until experimental evidence resolves this question, we need to consider the alternative hypothesis.

The absence of E;P strains appears not to be due to the fact that a strain must possess *hobo* elements in order to integrate *P* elements. This is shown by the injection of *P* elements into the Gruta stock (R;M strain), which produces an R;P strain (Anxolabéhère et al. 1987)—which is also an E;P strain, given that Gruta is an E strain. In long-established stocks *hobo* sequences appear to be more frequent that *P* sequences, and, given that P strains are known to be able to conserve their *P* elements under laboratory conditions, E;P strains should be more easily detected if *hobo* elements have disappeared in the laboratory.

The first populations that show complete *P* and *hobo* elements (H;P strains) date from captures carried out in the Americas around the mid-1950s. They have been kept under laboratory conditions for >30 years and do not seem to have produced E;P strains. This, along with the existence of rare H;M strains, suggests the hypothesis that in the Americas the *D. melanogaster* genome was invaded by *hobo* elements before the arrival of *P* elements. The fact that codon usage in open reading frame 1 of *hobo* differs from that of *Drosophila* genes (Streck et al. 1986) might also argue in favor of the introduction of this element into the *Drosophila* genome. But was this introduction ancient or recent?

The present-day distribution of *hobo*-hybridizable sequences, which are only found in the *melanogaster* and *montium* subgroups (Daniels et al. 1990*a*), could be explained either by a single introduction of *hobo* elements into the *melanogaster*-species-group lineage at some point prior to the divergence of the two subgroups or by two independent invasions: (1) an initial introduction into the *melanogaster-montium* species group prior to the separation of these subgroups and (2) a more recent reintroduction

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into the progenitor of the *melanogaster* complex, perhaps by horizontal transmission. This second introduction would account for the apparent difference between the *hobo* elements in the *melanogaster* complex and those in the rest of the *hobo*-bearing species of the *melanogaster* species group (Daniels et al. 1990*a*).

Whatever the correct hypothesis, the model must account for the existence of E strains in *D. melanogaster* and in other species of the two subgroups. It could be proposed that the elements have been lost in some lineages during the course of their evolution, in which case a unique introduction followed by such a "derivation and loss" process is sufficient to account for the patchy distribution of *hobo*-hybridizing sequences in the *melanogaster* and *montium* subgroups.

In this model the E strains of *D. melanogaster* represent an advanced evolutionary state with the presence of rare and probably nonfunctional relictual *hobo* sequences. In the first half of the 20th century an active *hobo* element able to transpose might have originated either from *D. melanogaster* itself or by recombination-reactivation from an active element harbored in a related species, a scenario reminiscent of those proposed for the T-R and P-M systems. This would be possible if *D. melanogaster,* in its expansion, encountered either the donor species from which it got these elements or a new vector that could have effected the necessary transfer. In this hypothesis *D. willistoni* or a related species appears to have been a plausible donor for the *P* element (Daniels et al. 1990*b*) but not for the *I* and *hobo* elements, which have not been found in the *willistoni* group. For these latter elements *D. simulans* would be the best donor candidate, considering the sequence similarity found at the restriction-map level (Bucheton et al. 1986; Periquet et al., accepted). In this light, the assumption of horizontal transfer by an exceptionally rare but fertile interspecific cross between *D. simulans* and *D. melanogaster* might be taken into consideration.

Whatever their origins, the presence of these elements poses the problem of three successive (*I*, *hobo,* and *P*) but virtually simultaneous element invasions in *D. melanogaster* during the same century. Even if we consider that *I* and *hobo* were transferred from *D. simulans* in a unique event, it remains that the success of two element invasions in a century seems highly unprobable (Engels 1988). One solution may be that transfers of new elements into a species are not really very rare but can only succeed under appropriate conditions in the recipient genome. Such conditions, similar to a genetic revolution (Mayr 1970, p. 309), may be transient and occur in response to environmental changes. Did American populations of *D. melanogaster* encounter such changes during the first part of the 20th century, thus leading to genetic modifications allowing the success of reactivation or transfer of transposable elements? The characterization of *hobo* sequences in *D. melanogaster* sibling species, together with knowledge of mechanisms of transposition, will enable us to clarify the evolutionary relationships of transposable elements in this complex of species.

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