Characterization of natural populations of *Drosophila melanogaster* with regard to the *hobo* system: a new hypothesis on the invasion

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Summary

Until now, with regard to the *hobo* system of hybrid dysgenesis, natural populations of *Drosophila melanogaster* have been investigated using only two criteria: at the molecular level, the presence or absence of *Xho*I fragments 2·6 kb long or smaller; and/or at the genetic level, the ability to induce gonadal dysgenesis sterility in crosses A (females of an E reference strain crossed with males under test) and A* (females under test crossed with males of an H reference strain). Recently, analyses of laboratory strains using these criteria as well as the mobilization of two reporter genes, the male recombination and the number of 'TPE' repeats in the S region, revealed a lack of correlation between the different dysgenic parameters themselves, and also between these parameters and the molecular characteristics of the strains. Thirteen current strains derived from world populations were therefore investigated with regard to all these dysgenic traits, to determine discriminating criteria providing a robust method of classifying natural populations and deducing the dynamics of *hobo* elements in these populations. We show, as in laboratory strains, a lack of correlation between the parameters studied. Therefore, the significance of each of them as well as the nature of *hobo* hybrid dysgenesis are discussed, to propose an analysis method of the *hobo* system applicable to natural populations. According to the geographical distribution of *hobo* activities in world populations and to the variable polymorphism of the number of 'TPE' repeats, we propose a new scenario for the invasion of *D. melanogaster* by *hobo* elements.

1. Introduction

The *hobo* element is a member of the class II elements as defined by Finnegan (1989*a*). It transposes via a DNA intermediate and belongs to the *hobo*, *Ac*, *Tam* family (*hAT*) (Calvi *et al.*, 1991; Warren *et al.*, 1994). The *hobo* element was cloned, sequenced and characterized in *Drosophila melanogaster* by McGinnis *et al.* (1983) and Streck *et al.* (1986). The autonomous *hobo* element of reference *Hfl*1 (Blackman *et al.*, 1989) contains 2959 bp (Fig. 1). It possesses two inverted terminal repeats of 12 bp and generates an 8 bp duplication at its insertion site. The *hobo* elements present a polymorphic S region consisting of tandem repeats of a 9 bp sequence ('actccagaa', 'TPE' motif in the protein) (Blackman *et al.*, 1987; Calvi *et al.*, 1991; Bazin & Higuet, 1996). There are three perfect repeats in the *Hfl*1 element, when in other elements three, five, six, eight or ten repeats were observed.

At the molecular level, using the *Xho*I *hobo* restriction fragment (Streck *et al.*, 1986; Blackman *et al.*, 1989), three different types of *hobo* elements can be distinguished: full-size *hobo* elements (*Hfl*) characterized by a 2·6 kb fragment, internally deleted elements whose *Xho*I restriction fragment is less than 2·6 kb, and elements giving high molecular weight bands (more than 2·6 kb). The last are common to all strains and correspond to vestigial sequences of previously established *hobo* elements, which are localized in heterochromatin (Lim, 1988; Daniels *et al.*, 1990). These vestigial sequences are supposed to be inactive, and do not show 'TPE' repeats when tested by polymerase chain reaction (PCR) amplification (Bazin, unpublished data). Particular *hobo* deleted elements, the *Th* elements, give a 1·1 kb fragment and are described as potential regulatory elements (Periquet *et al.*, 1989*a*, 1990). Using these criteria, populations with full-size *hobo* elements are called H; they can also harbour internally deleted elements. Populations with deleted elements only are called DH,
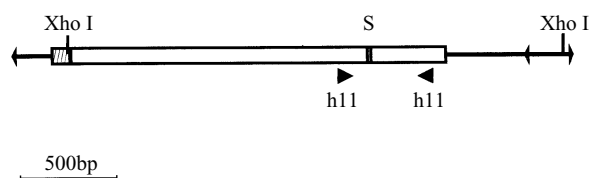
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Fig. 1. Map of the *hobo* transposable element *Hfl1* showing *Xho*I restriction sites, localization of primers used and major structural features. Each terminal repeat is represented by a black arrowhead and the S region by vertical lines. The ORF0 and ORF1 regions are shown by the hatched and the white horizontal bars respectively.

and populations devoid of any restriction fragment equal to or less than 2·6 kb in length are called E (empty) (McGinnis *et al.*, 1983; Yannopoulos *et al.*, 1987; Blackman *et al.*, 1987; Boussy & Daniels, 1991; Ho *et al.*, 1993; Calvi & Gelbart, 1994).

In the *hobo* system a dysgenic syndrome has been described (Yannopoulos *et al.*, 1987; Blackman *et al.*, 1987) including sterility by gonadal dysgenesis (GD) in F_1 individuals reared at 25 °C, chromosomal breaks and rearrangements, mutations and male recombination. This syndrome appears to occur in the germline of progenies from crosses between E females and H males (dysgenic cross). However, some dysgenic traits have been observed in the reciprocal cross ($\female H \times \male E$) (Blackman *et al.*, 1987; Bazin *et al.*, 1991). Recently, Bazin & Higuet (1996), describing the *hobo* dysgenic traits in laboratory strains, have shown the absence of correlation between the different dysgenic parameters themselves, and also between these parameters and the molecular characteristics of strains.

Until now, natural populations of *D. melanogaster* have been investigated using only two criteria: at the molecular level, the presence or absence of *Xho*I fragments equal to or less than 2·6 kb in length; and/or at the genetic level, the ability to induce GD sterility in crosses A (females of an E reference strain crossed with males under test) and A* (females under test crossed with males of an H reference strain). Temporal surveys revealed a clear difference between the oldest strains (generally E strains before the mid-1950s) and the most recently collected strains (generally H strains after 1966) (Periquet *et al.*, 1989*b*; Boussy & Daniels, 1991; Pascual & Periquet, 1991). With regard to the induced GD sterility, American and French natural populations have revealed a majority of populations with neither complete *hobo* repression potential nor *hobo* activity before 1969. Since then, the other populations analysed have demonstrated complete repression potential and no GD activity (Pascual & Periquet, 1991). This is consistent with the hypothesis of a world *hobo* element invasion of *D. melanogaster* during the past 50 years. Due to the absence of a large survey of contemporary populations, no clear evidence of the dynamics of the transposable element *hobo* through the natural populations of *D. melanogaster* has been proposed.

Before such a study, it is necessary to verify whether the lack of correlation between dysgenic traits observed in laboratory strains (Bazin & Higuet, 1996) is also detected in natural populations. In this case, through the necessity to take into account all the components of the dysgenic syndrome to analyse world populations, it would be essential to determine discriminating criteria providing a robust method of classifying populations in the *hobo* system. This paper reports the results of molecular and genetic analyses of 13 strains derived from world populations collected since 1992. GD sterility, *hobo* mobilization using two reporter genes and male recombination have been investigated for each strain. At the molecular level, full-size and defective *hobo* elements, as well as the number of 'TPE' repeats in *hobo* elements, have been searched for.

Our results show a lack of correlation between the parameters studied. Therefore the significance of each of them is discussed to propose an analysis method applicable to natural populations regarding the *hobo* system. The particular character of this system of hybrid dysgenesis is also pointed out. Moreover, it appears that the invasion by *hobo* elements has been continuing in recent years. Indeed, all current natural populations seem to have a greater number of full-size and *Th* elements than populations previously studied. Finally, according to the geographical distribution of *hobo* activities in world populations and to the variable polymorphism of the number of 'TPE' repeats, we envisage a new scenario for *hobo* element invasion in *D. melanogaster*.

2. Experimental procedure

(i) *Strains*

The *Drosophila melanogaster* strains used were kept at 25 °C under standard laboratory conditions on a cornmeal–sugar–yeast–agar medium.

Natural populations: These were studied using strains derived from a large sample (>10 individuals) collected since 1992. Each strain was maintained as isofemale lines, from which mass cultures were established just before the study. A mass culture is a proportional mixture of isofemale lines. Such lines are interesting since they allow a part of the initial polymorphism to be maintained. The mass cultures were analysed simultaneously between March 1995 and August 1996, after being kept at 25 °C for at least two generations before testing. Thirteen wild strains derived from diverse localities around the world were investigated in this study: Canada – Montreal; West Indies – St Martin; Kenya; Madagascar; France S – Sabarros; France C – Le Chatelet; England – Nottingham; Poland – Warsaw; Greece – Lambini; Russia – Moscow; Daghestan; India – Chandigarh; Vanuatu – Tanna.

Canton-*y* strain: This E strain is a subline of the Canton-S-M strain (Kidwell *et al.*, 1977) that bears a

recessive mutation at the *yellow* (*y*) locus that arose spontaneously.

23·5 strain: This strain, 23·5*/Cy MRF, comes from Yannopoulos' laboratory. This is a classical H strain established from 23·5Δ/CyL4 MRF through the replacement of all its chromosomes, except the 23·5 MRF second chromosome, by those of the Cy/Sp E laboratory strain (Yannopoulos *et al.*, 1987). This strain is an M′ strain in the P–M system.

haw strain: This is a *yellow white* E strain which carries two *h*(*w*$^+$) composite elements on the X chromosome (Calvi & Gelbart, 1994).

vg^{al}* strain: The second chromosome, multimarked *cinnabar* (*cn*) *vestigial* (*vg^{al}*) *brown* (*bw*), derives from the AL strain (Bazin & Higuet, 1996). The *vg^{al}* scalloped-wing phenotype corresponds to the *vg^{np}* phenotype. The *vg^{al}* mutation results from the insertion of a deleted *hobo* element into a *vg* intron (Bazin *et al.*, 1993). For mutant phenotype descriptions see Lindsley & Zimm (1992). This train is an M′ strain in the P–M system (Bazin *et al.*, 1991).

CyHBL1 strain: This strain contains only a *Hfl*1 truncated element (HBL1) located on the balancer Cy° second chromosome.

(ii) *Molecular analysis*

(a) *Southern blot*

The Southern blot method was used to characterize the complete or defective *hobo* elements. DNA was extracted from 50 females per strain using the method described by Junakovic *et al.* (1984). Restriction enzyme digestion of about 3 μg of DNA was performed according to the manufacturers' instructions, and Southern blots were performed using standard techniques (Sambrook *et al.*, 1989). Filters were prehybridized at 65 °C for 2 h with hybridization mixture (6× SSC, 5× FPG, 0·5% SDS), to which salmon testis DNA was added to 100 μg/ml after denaturation at 100 °C for 10 min. Hybridization was carried out overnight at 65 °C in a similar mixture, which contained 80 μg/ml salmon testis DNA and ^{32}P-labelled p*Hfl*1 plasmid as probe (Blackman *et al.*, 1989). After two post-hybridization washes of 10 min in 2× SSC, 0·1% SDS at 65 °C, the filters were wrapped in plastic film and exposed to X-ray film.

(b) *Scanning densitometry*

The Southern blot was scanned with a scanning densitometer (Phosphor Imager) to measure density of individual bands. First, a hybridization with a *white* probe was used to assess the relative amount of DNA between lanes. Secondly, the Southern blot was rehybridized with the *hobo* probe. Then the volume of each band corresponding to full-size elements (2·6 kb *Xho*I fragments) or to *Th* elements (1·1 kb fragments) was measured on the printout and normalized to the volume of the *white* (*w*) bands previously estimated. The quantification of the *hobo* full-size elements was performed using the CyHBL1 strain as a reference (one *Hfl* copy per diploid genome). We could then estimate the number of these elements present in the different strains. The ratio between full-size (*Hfl*) and *Th* elements was calculated using a correction corresponding to the size of each sequence (2·36 = 2·6/1·1). Then the number of *Th* elements could also be estimated.

(c) *PCR amplification*

PCR amplification was performed using internal primers *h11* and *h6* (Fig. 1) of *Hfl*1 described in Bazin & Higuet (1996). About 50 ng of DNA was used in 25 μl of total reaction volume containing 1 unit of *Taq* polymerase (Promega). Amplifications were performed on a MJ Research Minicycler for 30 cycles. Cycling conditions were 95 °C for 45 s, 65 °C for 2 min and 72 °C for 2 min.

(iii) *GD sterility assays*

Gonadal dysgenesis (GD) at 25 °C was used to assay *hobo* activity in the A reference crosses (♀ Canton-*y* × ♂ under test) and *hobo* repression potential in the A* reference crosses (♀ under test × ♂ 23·5). Virgin females were held for 2–5 d after emergence before crossing. Each cross was made at 25 °C with 50 pairs of flies in half-pint bottles. After 2 d, parents were discarded. Approximately 3 d after the onset of emergence, F$_1$ flies were transferred to fresh medium at 25 °C and allowed to mature for 2 d. About 50 females were taken at random for dissection to assay GD. GD sterility is calculated using the percentage of dystrophic ovaries: (number dystrophic/number dissected) × 100.

(iv) Hobo *mobilization*

hobo transposase tests involving the mobilization of the *hvg^{al}* element at the *vestigial* locus and the mobilization of *h*(*w*$^+$) from the X chromosome were performed according to Bazin & Higuet (1996). As *hobo* mobilization occurs at a low rate and generates clusters in the F$_1$ germlines, it is necessary to estimate this rate in individual F$_1$ germlines. All rates were estimated as the percentage of F$_1$ individuals with at least one mobilization even in their progenies (i.e. all the descendants obtained during the 8 days after the onset of emergence: approximately 100 individuals).

(a) hvg^{al} *mobilization test*

The *hvg^{al}* insertion is stable in the vg^{al}* strain but it can be mobilized *in trans* by active *hobo* elements. Such events can generate different alleles dominant on the *vg^{al}* allele, giving two kinds of phenotypes: *vg$^+$* or

vg^e (Bazin *et al.*, 1993). To perform the hvg^{al} mobilization test, hybrid males from the cross ♀vg^{al}* × ♂ under test, or from the reciprocal cross, were singly backcrossed with vg^{al}* females. The progenies were then scored for exceptional phenotypes: $cn\ vg^+$ bw or $cn\ vg^e\ bw$. Each exceptional progeny was crossed with vg^{al}* flies to ensure that the vg^+ or vg^e phenotypes were due to genetic events and not to vg penetrance or to somatic events.

(b) $h(w^+)$ transposition test

To perform the $h(w^+)$ transposition test, two initial crosses were used: ♀ haw × ♂ under test and ♀ under test × ♂ haw. Then at least 24 pairs were performed using one F_1 female crossed with one F_1 male. The progenies were scored for the appearance of exceptional individuals with reference to their eye colour phenotype.

(v) *Male recombination*

Male recombination is one of the phenomena linked to the *hobo* hybrid dysgenic system (Yannopoulos *et al.*, 1987). The tests were performed during the hvg^{al} mobilization (see above). The $cn\ vg^{al}\ bw$ flies have white eyes because of the $cn\ bw$ association on the second chromosome. Thus a recombination event gives a bright red phenotype (cn) or a brown phenotype (bw). Accordingly, the progenies were scored for recombinant phenotypes: $cn\ vg^+$, $bw\ vg^+$, $cn\ vg^{al}$ or bw vg^{al}. The percentage of male recombination was calculated as the number of F_1 males that give at least one recombination event in their progenies.

3. Results

(i) *Molecular analysis*

Fig. 2 shows the restriction patterns of genomic DNAs digested by *Xho*I and probed with the p*Hfl*1 plasmid. All 13 strains are H since they harbour full-size *hobo* elements (*Hfl*, 2·6 kb *Xho*I fragment). They also harbour deleted elements, including *Th* elements (1·1 kb *Xho*I fragment) present in numerous copies in the majority of the populations. Variations in the number of both full-size and *Th* elements were observed between strains (Table 1). With regard to full-size elements the extremes – England (13 copies) and Daghestan (2 copies) – differ by a factor 6. However, among the 13 strains studied, 6 present an intermediate number of full-size elements (6–8 copies). The strains are more variable with regard to the *Th* elements, numbers differing by a factor 10 between France C (39 copies) and Kenya (4 copies). Variations may be also expressed by the ratio of full-size elements to *Th* elements (Table 1). Only one strain (Kenya) presents an excess of *hobo* full-size elements (*Hfl/Th* = 1·76). Two strains (England and Vanuatu) have the
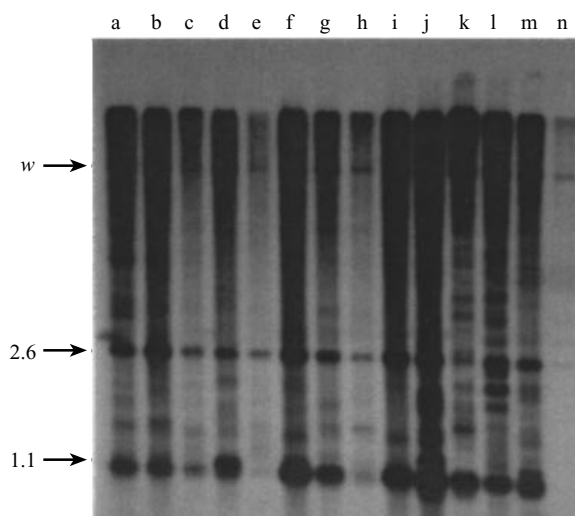

Fig. 2. *Xho*I digests of genomic DNA from the 13 strains of *D. melanogaster* and from the laboratory strain CyHBL1, probed with a *white* probe (*w*) and the p*Hfl*1 plasmid. The expected 2·6 kb internal *Xho*I fragment from full-sized *hobo* elements is indicated, as is the 1·1 kb fragment corresponding to the *Th* elements (Periquet *et al.*, 1989*a*). Strain names are as follows: lane (a) West Indies; (b) England; (c) Canada; (d) India; (e) Kenya; (f) France C; (g) Russia; (h) Vanuatu; (i) France S; (j) Poland; (k) Daghestan; (l) Greece; (m) Madagascar; (n) CyHBL1.

same amount of each element, while the others have a majority of *Th* elements, especially Daghestan with 6 times fewer full-size than *Th* elements (0·16).

The number of 'TPE' repeats in the S region was investigated using primers (*h11*, *h6*) that surround it. The PCR amplification results (Fig. 3, Table 1) reveal a polymorphism within strains and a variability between them which can not be detected by our Southern analysis. The number of PCR products varies from 1 (e.g. Canada, Daghestan) to 3 (France C & S, Greece) (Table 1). All strains have elements with three repeats; some also have compatible elements with five repeats (Kenya, France C & S, Greece, Madagascar) and a few strains harbour other kinds of elements (France C & S: 6 repeats; Greece: 7 repeats). These numbers of 'TPE' repeats are estimated in accordance with the length of the PCR products compared with the sequenced control fragments (HBL1 with 3 'TPE'; vg^{al} with 5 'TPE'; 341 with 6, 8 or 10 'TPE', Bazin, unpublished data). The differences observed in the intensity of the signals within a strain may express differences in the ratio between the numbers of elements of each type (Brunet *et al.*, 1996). Considering these differences, it appears that the two African strains (Kenya, Madagascar) have a majority of elements with three 'TPE' repeats while the two French strains harbour a majority of elements with five or six repeats. Finally the fifth polymorph strain, Greece, presents a majority of three 'TPE' repeats and an undescribed element compatible with seven repeats.

Table 1. *Molecular characteristics of 13 strains of* D. melanogaster

Strain	Band densities			Copy number		No. of 'TPE' repeats[b]
	2·6 kb	1·1 kb	Hfl/Th^a	Hfl	Th	
West Indies	752	651	0·49	5	10	3
England	2021	895	0·96	13	14	3
Canada	919	804	0·48	6	12	3
India	700	1038	0·29	4	16	3
Kenya	1224	294	1·76	8	4	<u>3</u>, 5
France C	1797	2581	0·30	12	39	3, <u>5</u>, <u>6</u>
Russia	976	1159	0·36	6	18	3
Vanuatu	1045	597	0·74	7	9	3
France S	1537	2110	0·31	10	32	3, <u>5</u>, <u>6</u>
Poland	1356	1737	0·33	9	26	3
Daghestan	305	822	0·16	2	12	3
Greece	1066	1019	0·44	7	15	<u>3</u>, 5, 7
Madagascar	1019	1294	0·33	7	20	<u>3</u>, 5
CyHBL1	156			1		<u>3</u>

Relative densities of the *hobo* full-size element bands (2·6 kb *Xho*I fragment) and of the *Th* element bands (1·1 kb *Xho*I fragment) are normalized with respect to the relative densities of a *white* probe. The Hfl/Th ratio is estimated directly in each lane. The number of copies is estimated relative to the CyHBL1 strain
[a] Hfl: Full-size *hobo* elements. A correction corresponding to the size of each sequence (2·36 = 2·6/1·1) was used.
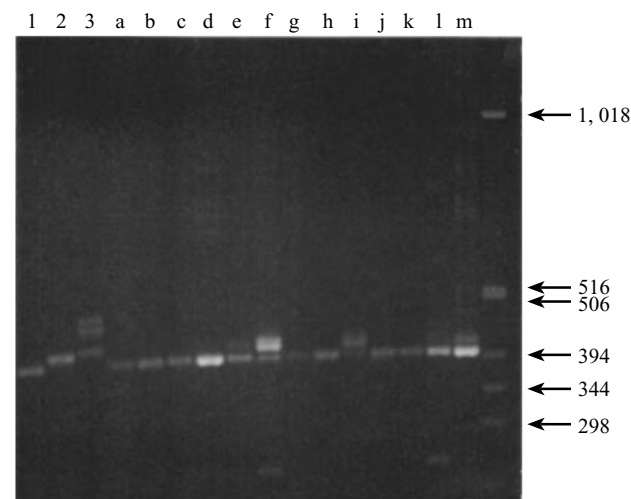[b] Majority elements are underlined.

Fig. 3. PCR amplification product using *h11* and *h6* primers (Bazin & Higuet, 1996). Lanes (1) to (3) represent the control strains and lanes (a) to (m) the strains from natural populations. The lanes are as follows: lane (1) CyHBL1 (413 bp: 3 'TPE' repeats); (2) vg^{al}* (431 bp: 5 'TPE' repeats); (3) 341 (440, 458 and 476 bp: 6, 8 and 10 'TPE' repeats); (a) West Indies; (b) England; (c) Canada; (d) India; (e) Kenya; (f) France C; (g) Russia; (h) Vanuatu; (i) France S; (j) Poland; (k) Daghestan; (l) Greece; (m) Madagascar.

(ii) *Gonadal dysgenic sterility analysis*

Table 2 summarizes the induced GD sterility detected in the F_1 females of A, A* and Intra crosses at 25 °C. For all strains, GD sterility in the Intra cross is less than 5%. These results indicate that the strains are stable with regard to the *hobo* system; populations

with active *hobo* elements seem to develop at the same time a regulation system. In the A* cross (♀ under test × ♂ 23·5), the females were classified according to their Cy phenotype because the 23·5 *hobo* active elements are located on the homologous MRF second chromosome. The percentage of MRF *hobo* regulation potential with regard to 23·5 MRF is estimated as the difference between the percentage obtained with [+] females (bearing the MRF chromosome) and that obtained with [Cy] females as they share all other genomic constituents.

From these results, three types of populations can be distinguished: (1) Most of the strains (8 of 13) are unable to induce dysgenic ovaries in A cross progenies (♀ Canton-*y* × ♂ under test) and can repress 23·5 MRF *hobo* activity in an A* cross (GD below 5% in both cases). Such strains were already uniformly found in America and France after the mid-1950s (Pascual & Periquet, 1991). (2) However, this study reveals some strains showing *hobo* dysgenic activity in A crosses: England, Canada and India with a low level of GD (13–16%) and Kenya with a higher GD (47%). These strains can also repress 23·5 MRF *hobo* activity in A* crosses with an induced GD sterility below 5%. (3) The West Indies, at least, represents a third type of strain with *hobo* activity (18% in cross A) and partial MRF *hobo* repression potential in an A* cross (13% of GD).

(iii) Hobo *mobilization*

The results regarding *hobo* transposase activity using *hobo* reporter gene mobilization (with *h(w⁺)* and *hvg^{al}*

Table 2. Hobo *activity, repression potential and stability measured by the percentage of induced GD sterility at 25°C*

Strain	*hobo* activity cross A	*hobo* repression potential cross A*			Cross Intra
		+	Cy	MRF[a]	
West Indies	18 (45)	13 (66)	0 (45)	13	0 (50)
England	13 (50)	12 (100)	7 (100)	5	2 (50)
Canada	13 (50)	8 (50)	5 (50)	3	1 (50)
India	16 (37)	3 (50)	2 (50)	1	0 (50)
Kenya	47 (100)	2 (50)	3 (50)	0	4 (50)
France C	0 (50)	0 (50)	2 (50)	0	0 (50)
Russia	1 (50)	4 (50)	1 (50)	3	2 (50)
Vanuatu	3 (50)	4 (79)	1 (71)	3	0 (50)
France S	1 (66)	0 (50)	5 (50)	0	0 (50)
Poland	5 (78)	3 (50)	0 (50)	3	2 (50)
Daghestan	1 (62)	8 (50)	2 (50)	6	0 (50)
Greece	0 (50)	1 (50)	3 (35)	0	1 (50)
Madagascar	0 (50)	0 (50)	0 (50)	0	0 (50)

Figures in brackets are the number of dissected females.
[a] *hobo* repression potential with regard to 23·5 MRF is estimated as the difference between the percentage obtain with [+] and [Cy] females; it was considered as null when this difference is negative.

Table 3. *Detection of* hobo *activities measured by the frequencies of* F_1 *individuals with at least one mobilization or recombination event in their progenies*

Strain	*hobo* reporter gene mobilization			Male recombination[a]	
	$h(w^+)$ ♀ haw (D)	*hvg*[al]		(D) ♀ vg[al]*	(ND) ♂ vg[al]*
		♀ vg[al]* (D)	♂ vg[al]* (ND)		
West Indies	0·0 (24)	5·7 (53)	6·9 (29)	17·0	0·0
England	0·0 (24)	0·0 (29)	0·0 (28)	0·0	0·0
Canada	0·0 (24)	0·0 (24)	0·0 (30)	12·5	0·0
India	0·0 (24)	0·0 (30)	0·0 (28)	0·0	3·4
Kenya	4·2 (24)	2·2 (46)	0·0 (28)	17·4	0·0
France C	0·0 (24)	0·0 (50)	0·0 (29)	20·0	10·4
Russia	8·3 (24)	0·0 (52)	0·0 (26)	0·0	0·0
Vanuatu	8·3 (24)	0·0 (41)	0·0 (25)	9·7	4·0
France S	0·0 (24)	2·6 (38)	0·0 (31)	2·6	3·3
Poland	0·0 (24)	0·0 (26)	0·0 (29)	0·0	0·0
Daghestan	0·0 (24)	0·0 (28)	0·0 (29)	0·0	3·4
Greece	0·0 (24)	0·0 (27)	0·0 (28)	0·0	17·2
Madagascar	0·0 (24)	0·0 (30)	0·0 (27)	3·3	11·1

The initial crosses involved 25 individuals from a strain derived from a natural population and 25 individuals from one of the strains given in reference. To perform the $h(w^+)$ mobilization test, F_1 males were singly mated to one F_1 female. To perform the *hvg*[al] mobilization and male recombination tests, F_1 males were singly mated to three virgin vg[al]* females. The number of F_1 individuals studied is in brackets.
(D), dysgenic cross; (ND), non-dysgenic cross.
[a] Report of *hvg*[al] mobilization for the number of F_1 males studied.

reporter genes) are shown in Table 3. The percentages of hybrid F_1 males or pair (♀ F_1 × ♂ F_1) which give at least one mobilization event in their progenies have been calculated. Since single crossing-over between *cn*-vg and vg-*bw* on the vg[al]* strain were detected only at very low rate (see below), the probability of a double crossing-over event is very low. Therefore all observed events have been considered as mobilization events.

In the $h(w^+)$ test, mobilization occurs exclusively in

the dysgenic cross (♀ haw × ♂ under test) and not in the reciprocal cross (results not shown). Mobilization events are rare: among the 13 strains only three (Kenya, Russia and Vanuatu) show transposase activity in this test, and this at a low level (from 4·2% to 8·3%, i.e. one or two pairs giving mobilization events in their progenies out of 24 tested; Table 3).

In the hvg^{al} test, mobilization can occur in the two reciprocal crosses. In the dysgenic cross, three strains (West Indies, Kenya and France S) again show transposase activity at a low level, from 2·2% to 5·7% (i.e. 1 or 2 males with mobilization events in their progenies; Table 3). In the reciprocal cross, only the West Indies strain manifests mobilization events (6·9%). This result confirms that dysgenic traits may appear in the two directions of a cross.

Using both these criteria, four types of populations can be distinguished: (1) most of the strains (8 of 13) do not show any mobilization event; (2) three strains show *hobo* activity in only one of the tests (Russia and Vanuatu for $h(w^+)$; France S for hvg^{al}); finally a particular status is observed for (3) West Indies, which shows hvg^{al} mobilization in both crosses, and for (4) Kenya, which is the only strain mobilizing the two *hobo* reporter elements. These results confirm the differences between $h(w^+)$ and hvg^{al} mobilizations described in Bazin & Higuet (1996), and reveal the independence between the abilities of a natural populations to induce one or the other.

(iv) *Male recombination*

The third phenomenon linked to *hobo* hybrid dysgenesis that we have studied is male recombination. Contrary to *hobo* mobilization activity, male recombination can be due to the P-M system (Kidwell *et al.*, 1977). Thus, it could be considered as a population characteristic taking into account P and *hobo* together. As in the mobilization tests, the percentages of F_1 males giving at least one recombination event in their progenies are exposed in Table 3. In each of the two initial crosses about half the strains present variable recombination rates: from 2·6% to 20·0% in crosses involving $vg^{al}*$ females and from 3·3% to 17·2% in crosses involving $vg^{al}*$ males. Fisher's exact test was used to verify the null hypothesis of the absence of differences between strains. Relative to crosses involving $vg^{al}*$ males the differences observed are statistically significant ($P < 0·01$), but the strains presenting male recombination are homogeneous ($P = 0·361$). Relative to the reciprocal crosses the test could not be carried out due to the marginal value being too high. Considering the preceding result we then distinguished strains with no male recombination from strains showing male recombination. The latter also appear homogeneous ($P = 0·07$). Four types of strains can therefore be distinguished according to whether or not they demonstrate recombination events

in each initial cross: (1) some show male recombination in both crosses (France C & S, Vanuatu, Madagascar); (2) others only in the dysgenic cross (West Indies, Canada, Kenya) or (3) only in the non-dysgenic cross (India, Daghestan, Greece); and finally (4) yet others do not show recombination events (England, Russia, Poland). Those results reveal the independence between the abilities of natural populations to induce male recombination in the two reciprocal crosses.

4. Discussion

In this study we wanted to characterize natural populations of *D. melanogaster* with regard to the *hobo* system to investigate the dynamics of *hobo* elements in these populations. For these reasons, 13 strains were analysed both at the molecular level (nature of elements present, number of 'TPE' repeats) and with regard to their genetic properties (induced GD sterility, mobilization of two reporter genes and male recombination). All the strains analysed are H; they have both full-size and *Th hobo* elements. These strains show a variability with regard to the number of 'TPE' repeats; although all of them possess elements with three repeats, some present a polymorphism including other types of elements. The populations are also variable with regard to each of the dysgenic criteria, since they do or not demonstrate events linked to the different dysgenic symptoms. Furthermore no evident correlation between the dysgenic criteria was revealed in this study.

Before considering the entirety of the components of the *hobo* system, the respective information they provide can be discussed. Since the I-R system does not present either any GD sterility or male recombination (Finnegan, 1989 *b*; Chaboissier *et al.*, 1995), it can not be involved in our genetic results. The same is not true regarding hybrid dysgenesis due to *P* elements, where these two traits have indeed been described by Kidwell *et al.* (1977). Thus the 13 strains have been characterized in the P-M system (West Indies, Canada, Kenya and Vanuatu are P strains; France C & S are Q strains; the other strains are M′).

With regard to male recombination, it appears that in the dysgenic cross (♀ $vg^{al}* × ♂$ pop) all the populations giving male recombination are P or Q. Thus the P-M system could be involved, alone or in combination with the *hobo* system, in these results. Therefore, in this dysgenic cross, male recombination can not be taken into account in a study of the *hobo* dysgenic system. In the reciprocal cross the populations with recombinants are Q or M′. In non-dysgenic crosses, Kidwell (1977) and Engels (1979) found that *P* elements can also give male recombination at a very low rate. However, it is also possible that the male recombination they observed was in fact the result of *hobo* activity. Thus we suggest that in

most cases male recombination has to be considered as the result of the combined action of these two dysgenic systems. Accordingly, this criterion could be conserved as a discriminating one in the *hobo* dysgenic system but only for the M′ strains.

The P-M system could also be implicated in the induced GD sterility in cross A. However, the highest P activity measured in this study is in the West Indies strain, with only 31 % of GD sterility at 29 °C. Since such weak P strains would not show any GD sterility at 25 °C (Higuet, 1991), the GD sterility we observed at 25 °C may be fully attributed to *hobo* activity. Previous studies of the *hobo* system using this criterion (Pascual & Periquet, 1991), which were limited to American and French natural populations, have revealed a majority of populations without complete repression potential and no *hobo* activity before 1969. All the analyses on populations sampled since that time show a complete repression potential with regard to 23·5 MRF and no GD activity. Our results reveal the existence today of numerous natural populations exhibiting *hobo* GD activity (Table 2). Thus world populations are more variable than supposed. The absence of such strains in previous studies may be due to the fact that strains kept under laboratory conditions lost their activity by genetic drift and/or *hobo* element rearrangement. Nevertheless, this absence may be due to the geographical distribution of the populations previously studied. With regard to GD repression potential, only one population, West Indies, shows induced GD sterility in the A* cross (Table 2). Thus induced GD sterility appears to be a discriminating *hobo* criterion.

Hobo reporter gene mobilization can also be a discriminating criterion in natural populations. Even if the $h(w^+)$ mobilization test in the non-dysgenic cross (♀ under test × ♂ haw) did not lead to mobilization in any of the strains, the other tests ($h(w^+)$ in the dysgenic cross and hvg^{al} mobilization) allow discrimination of the different strains (Table 3). Therefore these three tests are discriminant ones. For a same strain there could be differences between the capacities to mobilize hvg^{al} or $h(w^+)$ elements. These differences in behaviour could be partly explained by the nature and/or the location of the two reporter genes: $h(w^+)$ is a composite element on the X chromosome whereas hvg^{al} is a naturally deleted element on the second chromosome; genomic factors of reference strains may also interfere. However, these differences are more likely due to the fact that different kinds of events are implied in the two tests: $h(w^+)$ mobilization is a transposition test (Calvi & Gelbart, 1994; Bazin & Higuet, 1996) when hvg^{al} mobilization corresponds to local rearrangements (Bazin *et al.*, 1993). Our hypotheses do not leave out the difficulties linked to the fact that mobilization events occur only at a very low level. It is therefore difficult to determine whether the absence of mobilization results from a genuine inability of strains to give such events or from a sampling effect.

Considering the whole of the discriminating dysgenic criteria, the West Indies strain is the only one to demonstrate *hobo* activity in the non-dysgenic cross (Table 3) and also the only one to show induced GD sterility in the A* cross (Table 2). Moreover no GD sterility was observed in the Intra cross although this strain presents *hobo* GD activity. Thus the induced GD sterility in the A* cross may be interpreted as the occurrence of *hobo* activity also, and not only as an absence of *hobo* repression potential. Except for this particular relation, no correlation was detected between the measures of *hobo* activity through GD sterility and the mobilization of *hobo* reporter genes. Indeed, of the five strains with *hobo* GD activity, only two (West Indies and Kenya) also show mobilization activity, but not in the same test: West Indies mobilizes hvg^{al} in the two reciprocal crosses, whereas Kenya mobilizes the two reporter genes but only in the dysgenic crosses. Conversely, strains such as Russia, Vanuatu or France S show mobilization activity but no GD activity (Table 4). The lack of correlation already observed in laboratory strains (Bazin & Higuet, 1996) is thus also detected in natural populations.

To determine the causes of the different behaviours observed, we have tried to link the variability of the strains to their molecular characteristics. With regard to the 'TPE' repeats there was no clear evidence of a relationship between the S polymorphism observed and the *hobo* elements' behaviour. For example, two populations with the same 'TPE' polymorphism can have, respectively, a high *hobo* activity (Kenya) or no *hobo* activity (Madagascar). However, these populations show a very different *Hfl/Th* ratio. If *Th* elements have a role in the regulation of the *hobo* system, as has been suggested (Periquet *et al.*, 1989 *a*), populations with a high *Hfl/Th* ratio should exhibit several activities. Conversely, populations with a low *Hfl/Th* ratio should show low or not activity. The more active strain, Kenya, which has very few *Th* elements and a weak ratio, may support this possible role of *Th* elements. Nevertheless India and France S, which have a weak ratio, show *hobo* activity. Therefore the potential role of *Th* elements in the regulation of the *hobo* system could not be demonstrated and no correlation could be detected between genetic properties and molecular characteristics, regarding the number of 'TPE' repeats and/or the *Hfl/Th* ratio.

According to the results obtained, it appears that *hobo* is a particular system of hybrid dysgenesis according to the Kidwell & Kidwell definition (1976). Various dysgenic traits such as GD, male recombination or hypermutability are observed in hybrids between certain mutually interacting strains. Nevertheless the *hobo* system is intrinsically different from the P-M and I-R systems previously described in *D. melanogaster*. First, our results, as well as those obtained by Blackman *et al.* (1987) and Bazin *et al.* (1993), show that the *hobo* system does not have a

Table 4. *Synthesis of the molecular and phenotypic characteristics of the*
13 D. melanogaster *strains studied*

Strain	Hfl/Th	No. of 'TPE' repeats[a]	GD sterility		hobo reporter gene mobilization		
					$h(w^+)$ ♀ haw (D)	hvg^{al} ♀ vg^{al*} (D)	♂ vg^{al*} (ND)
			Cross A	Cross A*			
West Indies	0·49	3	18	13	0	5·7	6·9
England	0·96	3	13	5	0	0	0
Canada	0·48	3	13	3	0	0	0
India	0·29	3	16	1	0	0	0
Kenya	1·76	3, 5	47	0	4·2	2·2	0
France C	0·30	3, 5, 6	0	0	0	0	0
Russia	0·36	3	1	4	8·3	0	0
Vanuatu	0·74	3	3	3	8·3	0	0
France S	0·31	3, 5, 6	1	0	0	2·6	0
Poland	0·33	3	5	3	0	0	0
Daghestan	0·16	3	1	6	0	0	0
Greece	0·44	3, 5, 7	0	0	0	0	0
Madagascar	0·33	3, 5	0	0	0	0	0

(D), dysgenic cross; (ND), non-dysgenic cross.
[a] Majority elements are underlined.

strict non-reciprocal character. Indeed, if dysgenic traits usually appear in one direction only, that direction is not necessarily the same according to the strain or the trait considered. Thus, the designation of the initial cross as 'dysgenic' or 'non-dysgenic' seems now to be obsolete. Secondly, our results, strengthening those of Bazin & Higuet (1996), point out the lack of correlation between dysgenic traits in the *hobo* system. This lack of correlation could result from the *hobo* elements and/or the regulation system implied, bearing in mind that host factors can also influence the appearance of *hobo* activities within strain (Ho *et al.*, 1993; Sheen *et al.*, 1993).

Trying to investigate the dynamics of *hobo* elements in natural populations of *D. melanogaster* the geographical distribution of *hobo* activity, including GD activity, the hvg^{al} and $h(w^+)$ mobilization activity in the dysgenic crosses, was represented on a map (Fig. 4). The different types of activities are unequally distributed and no geographical gradient or pattern appears. Therefore, according to the hypothesis of a world invasion during the past 50 years (Periquet *et al.*, 1989*b*; Pascual & Periquet, 1991; Boussy & Daniels, 1991), the current distribution observed implies that the dynamics of *hobo* elements in natural populations is quite different from that of the *P* elements. This difference may be due to particular behaviours of *hobo* elements themselves, in keeping with the characteristics of the *hobo* system, and/or to different components of the invasion.

Considering the behaviour of *hobo* elements, Southern blot results show that all the strains have several full-size and *Th hobo* elements. Comparing those results with previous observations of 1980s populations (Periquet *et al.*, 1989*a*, *b*; Boussy &

Daniels, 1991), we suspect that the number of full-size and *Th hobo* elements is still increasing in current populations. Even if populations appear to be stable because no GD sterility was observed in an Intra cross, the other tests show that transposition events are still possible and so *hobo* elements could still invade natural populations. Moreover if we suppose that *hobo* elements have a weak dynamics within a genome in *D. melanogaster*, then drift effects could be strong enough to partially explain the heterogeneous phenotypic distribution observed.

Considering the different components of the invasion, the interpretation of all our results seems not to support an American origin of *hobo* elements in the populations of *D. melanogaster* as suggested by Periquet *et al.* (1989*b*). Our study with regard to the number of 'TPE' repeats reveals a variability between natural populations. If *hobo* elements with three repeats are common to all strains, the other elements allow characterization of the populations (Fig. 4). Then it appears that (1) the two French strains have PCR amplification products which are specific both qualitatively and also in the relative intensities of the different bands, with a majority of five or six repeat elements; (2) the two African populations share the same polymorphism, with a minority of five repeat elements; (3) all the polymorph populations are localized in the same area including Africa and the Mediterranean. According to the independence observed between the 'TPE' polymorphism and the genetical properties studied, 'TPE' repeats could be useful neutral markers of *hobo*'s spread throughout natural populations. Invasion of *D. melanogaster* by *hobo* elements could therefore have started in Western Europe, where the maximum of polymorphism is
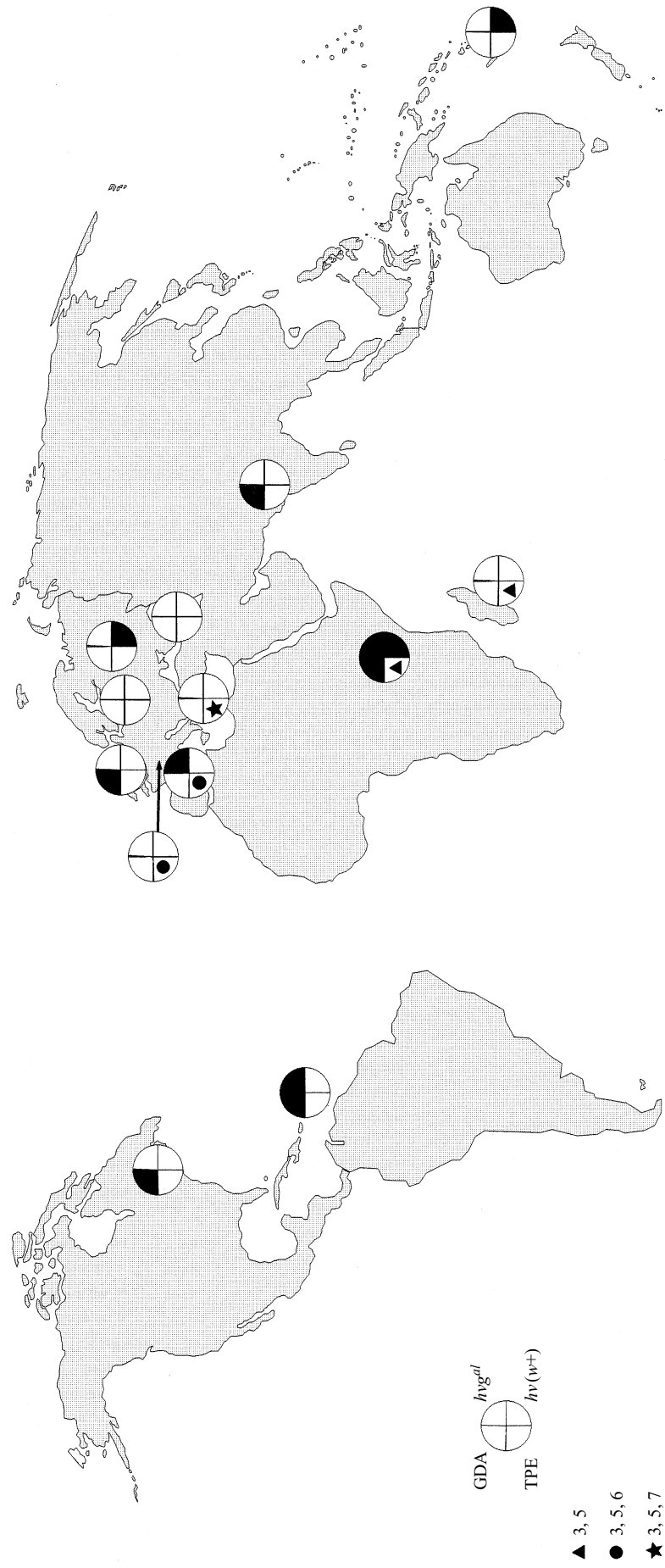
Fig. 4. Geographical distribution of the different components of *hobo* activity in natural populations of *D. melanogaster* and of their characteristics with regard to the number of 'TPE' repeats.

found. On the other hand, the most active population is Kenya, which can be described as 'completely' active. Except for the West Indies, which has two kinds of activity (GD sterility and hvg^{al} mobilization), the other populations show no or only one kind of activity. If the number of *hobo* activities depends on the time spent by *hobo* in a population, two hypotheses can be considered: (1) the spread of *hobo* elements in *D. melanogaster* could have occurred from Africa, where populations are 'completely' active (Fig. 4). Under this hypothesis, some of these activities may have been randomly lost during the invasion depending on migration and on the establishment of regulation mechanisms. (2) On the contrary, these activities could reflect a more recent invasion of African populations, in which regulation systems are not still established.

To discriminate between these hypotheses it is necessary to study more of the current populations, particularly African and European ones. The study of *hobo* dynamics also requires data on old strains, with regard to the 'TPE' repeat polymorphism, which could provide new information on the history of *hobo* in *D. melanogaster*.

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