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Closely related *Drosophila melanogaster* strains with altered fitness also show changes in their *hobo* element properties

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Summary – A set of *Drosophila melanogaster* strains of common origin, but with different fitness characteristics, established either spontaneously or after selection for the specific fitness parameters, appear to induce non-P-element-mediated gonadal atrophy in appropriate crosses to tester strains containing or lacking *hobo* elements (H and E strains). Using the gonadal dystrophy (GD) sterility assay, as well as genomic Southern blot hybridization and *in situ* hybridization on polytene chromosomes, it was found that all these strains possess *hobo* elements whose dysgenic activity, composition, copy number and cytogenetic locations appeared to be variable. In general, low fitness strains have moderate *hobo*-activity and *hobo*-repression potentials, while high fitness strains show no *hobo*-activity but also quite high *hobo*-repression potentials. Although distinct differences in the composition, copy number and location of the *hobo* elements in the genome were observed between these 2 groups of strains, these variations did not show any profound correlation with either *hobo*-related dysgenic potential or the fitness of the strains.

Drosophila melanogaster* / selection / fitness / transposable element / *hobo

Résumé – Des souches étroitement apparentées de *Drosophila melanogaster* avec des aptitudes reproductives modifiées manifestent aussi des changements dans les propriétés de leurs éléments *hobo*. *Différentes lignées de* Drosophila melanogaster, *issues d'une population naturelle russe, ont été sélectionnées sur des caractéristiques de* fitness. *Ces lignées, comme les lignées témoins, présentent des activités dysgéniques variables non reliées à l'élément transposable P. Nous montrons que la stérilité GD (*gonadal dystrophy*) qu'elles induisent est due à l'élément* hobo *et, de plus, que ces lignées ont des potentiels*

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dysgéniques différents. Les analyses moléculaires réalisées par buvardage de Southern et par hybridation in situ *sur les chromosomes polytènes font apparaître que ces lignées possèdent un nombre variable d'éléments* hobo, *qui ont des structures et des localisations cytogénétiques différentes. En général, les lignées ayant une* fitness *basse présentent une activité* hobo *et un potentiel de répression modérés, alors que les lignées ayant une* fitness *plus forte n'ont pas d'activité* hobo, *mais un potentiel de répression assez élevé. Bien que ces 2 groupes diffèrent par la structure, le nombre et la localisation cytogénétique de leurs éléments* hobo, *les différences mises en évidence ne montrent pas de corrélation directe, que ce soit avec le potentiel dysgénique ou avec la* fitness *des lignées analysées. Une relation entre les sélections réalisées pour établir les lignées et l'évolution de leurs éléments* hobo *est discutée.*

Drosophila melanogaster* / sélection / *fitness* / élément transposable / *hobo

INTRODUCTION

The evaluation of the genetic consequences of selection is one of the key purposes of population genetics, especially in the context of the theory of breeding (Hill and Caballero, 1992). To address this problem, a set of *Drosophila melanogaster* strains, originating from a natural population from Yessentuki (Russia), was established after close inbreeding and long-term selection for differences in male mating activity. Selection for low male mating activity also led to correlated changes in a number of morphological, physiological, behavioural, biochemical and genetic features which, altogether, greatly reduced the overall fitness of these strains, and thus made it possible to characterize them as low fitness or high fitness strains (reviewed in Kaidanov, 1980, 1990).

In spite of the close inbreeding, these strains appeared to possess a significant genetic load and a high rate of spontaneous mutability, including the occurrence of chromosomal rearrangements (Kaidanov, 1980, 1990; Kaidanov *et al*, 1991). Experiments directed to investigate the sources of this genetic instability showed that in the course of isogenization of the strains' 2nd chromosomes in order to evaluate their genetic loads and the rates of spontaneous mutability, the female progeny exhibited a high rate of gonadal dystrophy (GD). In these crosses, males from one of the low fitness strains, *LA*, were crossed with the females of a laboratory strain containing a balancer for the 2nd chromosome. This finding was highly reminiscent of hybrid dysgenesis, a syndrome attributed to the activation of the transposable elements *P* or *hobo* (Louis and Yannopoulos, 1988; Blackman and Gelbart, 1989; Engels, 1989). Since it was known that *LA* does not contain any *P* elements in its genome (Pasyukova *et al*, 1987), the probability that *hobo* elements were responsible for the GD sterility detected was checked by crosses to appropriate tester strains, containing or lacking *hobo* elements. These experiments revealed the presence of active *hobo* elements in *LA* (Kaidanov *et al*, 1991) thus raising the possibility that *hobo* may be the causative factor for the genetic instability observed. We extended our analysis to several strains which, though related, exhibited different fitness characteristics. Here, we report results on composition, copy number, cytogenetic location and dysgenic properties of their *hobo* elements.

MATERIALS AND METHODS

The following *D melanogaster* strains (kept on standard corn-meal food at 25°C) were used for experiments:

LA: low activity strain, obtained from a natural population in Yessentuki (Russia) in 1965 as a result of inbreeding and long-term selection for low male mating activity. By the time of the beginning of the experiments described here, it had passed ~ 600 generations of selection.

HA and LA^+: high activity strains, obtained independently from *LA* at its 70th and 163rd generation of maintenance, respectively, by selection for high male mating activity and close inbreeding. These 3 strains (*LA*, *HA* and LA^+) were kept in the collection as families obtained from individual brother–sister matings and were described in detail by Kaidanov (1980, 1990).

$LA6^-$ and $LA6^+$: low activity and high activity strains, respectively, selected by close inbreeding from a high fitness strain (*LA6*) that arose spontaneously from one of the families of *LA*, and was subsequently lost from the collection. After the initial selection and close inbreeding, $LA6^-$ and $LA6^+$ are kept in the collection by mass mating without further selection.

LApas: low activity strain, established from one of the families of *LA* and reared by mass mating without further selection.

$23.5MRF/CyL^4$: a strain containing active P and *hobo* elements (PH-strain) and capable of weak induction of P–M hybrid dysgenesis and strong induction of H–E hybrid dysgenesis (Yannopoulos *et al*, 1987; Stamatis *et al*, 1989).

CyL/Pm: an ME strain containing a 2nd chromosome balancer and unable to suppress H–E hybrid dysgenesis. Female progeny resulting from crosses between this strain's females to $23.5MRF/CyL^4$ males exhibit high levels of gonadal atrophy (Kaidanov *et al*, 1991).

The test for male mating activity was performed as described by Kaidanov (1980, 1990). Single mature males from the strain to be tested were put in the same vial with 2–3 virgin-wild type females and the process of copulation was observed. Male mating activity was determined as the percentage of males succeeding in copulating with virgin females within 30 min. For each strain, 55–90 males were tested.

The GD sterility assay was performed according to the conditions described by Yannopoulos (1978). To determine the *hobo*-activity potential, the males of the strain under investigation were crossed with the *CyL/Pm* tester females, while to determine the *hobo*-repression potential, the females of the strain were crossed with $23.5MRF/CyL^4$ males. As a control, the GD sterility assay was also performed both within-strain and for the reciprocal crosses. All crosses were performed at 25°C to maximize the manifestation of H-E hybrid dysgenesis (Stamatis *et al*, 1989). Female progeny were collected every day and transferred to fresh vials for 3–4 d for maturation of the gonads, which were then dissected to analyze their morphology. The induction of GD-sterility was measured as the frequency of atrophic gonads. For each strain, 1–4 replicates of each cross were performed and 50–500 females were scored.

Genomic DNA for Southern-blot hybridization was extracted from 200 flies/strain as described by Ashburner (1989). Approximately 2 μg DNA was digested with *Xho* I restriction endonuclease, separated electrophoretically in 1.2% agarose

gels and blotted onto nylon membrane filters following standard protocols (Sambrook *et al*, 1989). Hybridization was performed according to Church and Gilbert (1984) using as a probe ^{32}P-labelled plasmid pHFL1 which contains a complete *hobo* element (Blackman *et al*, 1989).

In situ hybridization to salivary gland polytene chromosomes was performed as described by Ashburner (1989), using biotin-labelled plasmid pHcSac, containing a complete *hobo* element as a probe (Stamatis *et al*, 1989).

The *t*-test (Sokal and Rohlf, 1969) was used to compare the means in our experiments.

RESULTS

Male mating activity

As some of the strains under investigation are kept without any further selection with respect to their male mating activity, we first performed a series of tests for all *LA* derivatives analyzed here. The data on their male mating activity are presented in table I. They confirm the status of *LA* and *LApas* as low activity strains (no matings within the first 30 min). The remaining strains showed a gradual increase in male mating activity, from 25.0 and 52.0% for the more recently established strains $LA6^-$ and $LA6^+$ to 76.6 and 94.0% for *HA* and LA^+ which had been established earlier.

Table I. Male mating activity, expressed as the percentage of successfully copulating males, GD sterility, expressed as the percentage of atrophic gonads in the females of the strain, and *hobo* activity and *hobo* repression potentials expressed as the percentage of atrophic gonads in the female progeny of test crosses using the strains under investigation*.

Strain	*Male mating activity*	*Intrastrain GD sterility*	hobo-*activity potential*		hobo-*repression potential*	
			Test cross	*Control*	*Test cross*	*Control*
LA	0	12.2 ± 3.8	32.6 ± 2.6	0	47.2 ± 2.2	0.4 ± 0.4
LApas	0	1.4 ± 1.4	36.5 ± 4.5	0	38.0 ± 3.5	1.4 ± 1.4
$LA6^-$	25.0 ± 5.6	0.6 ± 0.9	0.7 ± 0.5	0	25.0 ± 5.3	0
$LA6^+$	52.0 ± 6.4	0	0.8 ± 0.6	1.6 ± 1.8	23.0 ± 5.9	0
HA	76.6 ± 4.5	0	0	0	14.0 ± 2.1	0
LA^+	94.0 ± 2.4	2.5 ± 1.3	0	0	19.8 ± 2.3	1.2 ± 0.8

* The mean value and its standard error are given. The 'control' columns refer to the respective reciprocal crosses. For description of test and control crosses, as well as the GD sterility assay, see *Materials and methods*.

Activity and repression abilities of hobo *elements*

As the strains under investigation were previously shown not to contain any P elements (Pasyukova *et al*, 1987), we concentrated our analysis on the study of

hobo-mediated genetic instability. The *hobo*-activity potential of the strains was determined by the GD sterility assay using the females from the E strain *CyL/Pm* as tester strains, a high percentage of dystrophic gonads in the progeny of these crosses being indicative of a higher *hobo*-mediated dysgenic induction. The *hobo*-repression potential was determined in a similar way, this time crossing females from the *LA* derivatives with males of the H-strain *23.5MRF/CyL*^{4}. In this case, the higher the proportion of atrophic gonads among female progeny, the lower the potential of the strain under investigation to repress the action of *hobo*. In a test cross of *CyL/Pm* females to *23.MRF/CyL*^{4} males, the GD sterility among 150 female progeny reached 90%, confirming that the latter still retained its strong *hobo*-activity potential. As a control, intrastrain GD sterility assays were performed and, with the only exception of *LA*, where intrastrain sterility reached a moderate rate of about 12%, the proportion of atrophic gondas in all other strains did not exceed the background levels (0–2.5%, table I).

For both experiments, in control reciprocal crosses, the proportion of atrophic gonads was very low if any (0–1.6%, table I), thus implying that the *hobo* elements are the most probable causative factors of the GD sterility in experimental crosses. The data presented in table I show that low activity strains *LA* and *LApas* appeared to have similar moderate *hobo*-activity potential (32.6 and 36.5% of atrophic gonads, respectively; $t = 0.66$, $p > 0.05$), while the intermediate strains *LA6*$^-$ and *LA6*$^+$ and the high activity strains *HA* and *LA*$^+$ apparently lost their induction potential sometime after their establishment (GD sterility = 0–0.8%). The *hobo*-repression potential was lowest, though different, for *LA* and *LApas* (47.0 and 38.0% of atrophic gonads, respectively; $t = 2.18$, $p < 0.05$). Strains *LA6*$^-$ and *LA6*$^+$ showed similar *hobo*-repression potentials (GD sterility = 25.0 and 23.0%, respectively; $t = 0.25$, $p > 0.05$), which were significantly higher than that of *LApas* ($t = 2.06$, $p < 0.05$ and $t = 2.19$, $p < 0.05$, respectively). The strongest *hobo*-repression potentials were observed in *LA*$^+$ and *HA* (19.8 and 14.0% of atrophic gonads, respectively; $t = 1.63$, $p > 0.05$), values that are not significantly different from that observed for *LA6*$^+$ ($t = 0.63$, $p > 0.05$ and $t = 1.44$, $p > 0.05$, respectively).

Composition of hobo *elements*

Genomic DNAs of the strains were digested with *Xho* I restriction endonuclease, which has recognition sites close to both ends of the complete *hobo* element, yielding a characteristic fragment of 2.6 kb after Southern-blot hybridization with a *hobo* probe. In addition, *Xho* I digests also produce fragments of smaller molecular weight, corresponding to different internal deletion derivatives of *hobo* (Streck *et al*, 1986). As a control, *23.5MRF/CyL*^{4} flies were also analyzed (fig 1A), showing their characteristic pattern of hybridization (Stamatis *et al*, 1989), while as expected, no hybridization corresponding to either complete or deleted *hobo* elements was seen in the corresponding digests of the *CyL/Pm* strain (fig 1A), confirming that it is a *bona fide* E strain (Kaidanov *et al*, 1991).

All tested *LA* derivatives appeared to possess the 2.6 kb fragment indicating the presence of full-length *hobo* elements in their genome. On the other hand, the pattern of *hobo*-deletion derivatives was qualitatively and quantitatively different for all strains (fig 1B). In *LA*, the most intense band has a length of 1.7 kb with some weaker bands also being present, while the predominant band in *LApas* is

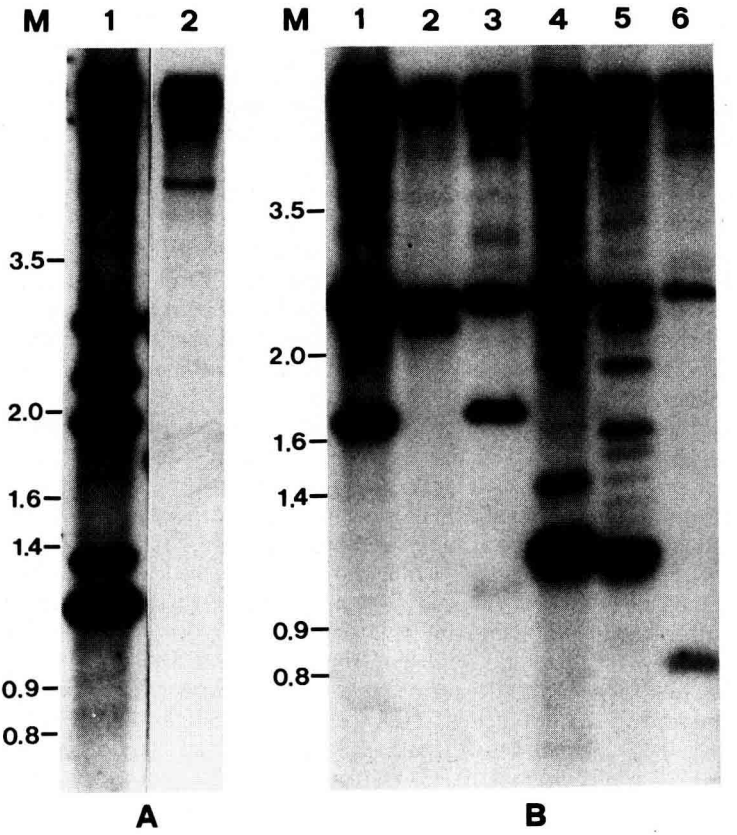

Fig 1. Southern-blot analysis of *Xho* I-digested genomic DNAs isolated from different strains using *pHFL1*, a plasmid containing a complete *hobo* element, as a probe. **A**: 1-*23.5MRF/CyL*^{4}; 2-*CyL/Pm*; **B**: 1-*LA*; 2-*LApas*; 3-*LA6*$^-$; 4-*HA*; 5-*LA*$^+$; 6-*LA6*$^+$; M-molecular size markers (in kb).

2.2 kb long. In the *LA6*$^-$ the most prominent band also corresponds to a length of 1.7 kb and there is a very weak band of 1 kb. The most abundant fragment in the 2 high activity strains (*HA* and *LA*$^+$) has a length of 1.15 kb with an additional intense band of 1.4 kb appearing in digest of *HA* as well as numerous weaker bands. Finally, the only intense band observed in *LA6*$^+$ is 0.9 kb long.

hobo *elements copy number and their distribution on the polytene chromosomes*

To determine the copy number of the *hobo* elements and their location in the genome of the strains under investigation we performed an *in situ* hybridization of a labelled *hobo* element to polytene chromosomes. Squashes were prepared from salivary glands of several larvae, taken either from one vial for each strain kept by

mass matings, or from 2 vials (families) for each of the strains kept in families. The copy number of *hobo* elements and the sites of their cytological location were analysed for each individual larva. The data from this experiment are presented in table II. Both intrastrain and interstrain variation in copy number and their location are characteristic for all strains. In low activity strains *LA* and *LApas* the number of *hobo* elements were between 12 to 15 and 10 to 22 respectively, while in the intermediate activity strains $LA6^-$ and $LA6^+$ they were from 8 to 11 and from 13 to 15, respectively. Both high activity strains, *HA* and LA^+, showed a considerably higher copy number (40–42 and 36–37, respectively).

The proportion of polymorphic sites (expressed as the ratio of the number of sites varying among individuals from one strain, to the total number of sites revealed within the same strain) also appeared to be different between the strains (table II). Quite low polymorphism was observed in strains kept as individual families with selection. *HA* had 12% polymorphic sites, the corresponding number for LA^+ was 24%, and a higher value (47%) was observed for LA^-. On the other hand, the strains kept by mass matings without selection (*LApas*, $LA6^-$ and $LA6^+$) had even higher values (69, 56 and 63% of polymorphic sites, respectively).

Analysis of cytological locations of *hobo* elements revealed that, in the course of selection and subsequent maintenance of the strains, their *hobo* elements underwent massive transpositions. Only *LA* and *LApas* share 9 common sites among a total of 46 sites for both strains (or 20%), while all other pair-wise comparisons revealed only 2–5 common sites among a total of 33–84 sites, or 3–12% (table III). Another strain with decreased activity, $LA6^-$, shared only 2 (6.5%) and 4 (8.9%) sites with *LA* and *LApas*, respectively. Only 2 sites at 17E and 96E were common to all 3. The high activity strains *HA*, LA^+, and $LA6^+$ had 2–5 sites in common (3.0–7.7%), and there were no sites that were shared by all 3. The most frequent sites among all strains analyzed were 17A, 38C, 67A and 96E.

DISCUSSION

In a previous paper (Kaidanov *et al*, 1991) we showed that the *D melanogaster* strain *LA*, established from a natural population by close inbreeding and selection for low male mating activity, harboured active *hobo* elements in its genome. We extended this analysis to encompass *LA*-derivative strains with altered male mating activity, and in this report we present evidence that the *hobo* elements in these strains underwent considerable changes in their dysgenic properties, composition and copy number, accompanied by extensive transpositions.

Both low activity strains, *LA* and *LApas*, preserved dysgenically active *hobo* elements resulting in similar intermediate *hobo*-activity and *hobo*-repression potentials, as revealed by the GD sterility assays. They also appear to possess quite similar copy numbers of *hobo* elements, and the differences observed in the composition of *hobo* elements (notably the presence of the prominent band of 1.7 kb in *LA*) and in their locations in the genomes seem to have no direct effect on dysgenic properties of both strains.

In the strains with intermediate ($LA6^-$ and $LA6^+$) and high male mating activity (*HA* and LA^+) we have found the full absence of *hobo*-activity and a considerable increase of *hobo*-repression potentials. These changes, especially evident in the

Table II. The number of *hobo* elements on polytene chromosome arms, as well as the total number of sites (n), of polymorphic sites (n PS) and the percentage of polymorphic sites (in parenthesis) is given for several individuals from the 6 strains investigated, as determined by *in situ* hybridization of a *hobo* probe.

Strain	*No of larvae*	X	2L	2R	3L	3R	*Total*
LA	1	6	2	3	1	3	15
	2	4	1	3	1	3	12
	3	4	2	2	1	3	12
	4	4	2	2	1	3	12
	5	4	2	2	1	3	12
	n sites	6	2	5	1	3	17
	n PS (%)	3(50)	1(50)	4(80)	0(0)	0(0)	8(47)
LApas	1	5	5	1	5	3	19
	2	7	3	2	7	3	22
	3	4	2	1	1	2	10
	4	5	3	1	1	3	13
	n sites	7	5	2	9	6	29
	n PS (%)	3(43)	3(60)	1(50)	8(89)	5(83)	20(69)
$LA6^-$	1	1	3	1	0	4	9
	2	1	3	1	0	3	8
	3	1	5	1	0	4	11
	4	1	5	1	0	4	11
	5	1	4	1	0	3	9
	n sites	2	7	1	0	6	16
	n PS (%)	2(100)	4(57)	0(0)	0(0)	3(50)	9(56)
$LA6^+$	1	3	1	3	3	4	14
	2	4	2	1	2	4	13
	3	4	1	3	3	4	15
	4	4	1	1	3	6	15
	5	5	2	2	2	4	15
	n sites	7	3	3	4	7	24
	n PS (%)	4(57)	2(67)	2(67)	3(75)	4(57)	15(63)
HA	1	8	5	9	9	11	42
	2	7	4	9	9	11	40
	3	7	4	10	9	10	40
	4	8	4	10	9	11	42
	n sites	8	5	10	9	11	43
	n PS (%)	1(13)	2(40)	1(10)	0(0)	1(9)	5(12)
LA^+	1	4	7	6	8	11	36
	2	4	8	5	10	10	37
	3	4	7	6	10	9	36
	n sites	4	8	6	12	11	41
	n PS (%)	0(0)	1(13)	1(17)	5(42)	3(27)	10(24)

strains $LA6^+$, *HA* and LA^+, were accompanied by a drastic recomposition of *hobo*-deletion derivatives, which appeared to be completely different from the complement of *LA* and *LApas*. Nevertheless, our results do not imply any correlation between the

Table III. Pair-wise comparison of the cytogenetic localization of *hobo* elements of the strains under investigation.

Strains	LA	LApas	LA6$^-$	LA6$^+$	HA	LA$^+$
Total No of sites	17	29	16	24	43	41
LA		9(19.5%)	2(6.5%)	5(12.2%)	3(5.0%)	4(6.9%)
		3D 6A 17A 25F 42D 67A 85B 96E 99A	*17A 96E*	*10B 38C 67A 96E 99A*	*42D 57B 96E*	*17A 57B 67A 85B*
LApas			4(8.9%)	4(7.5%)	4(5.6%)	5(7.1%)
			17A 38B 38C 96E	*10A 38C 67A 96E*	*7C 10A 42D 71C*	*12E 17A 67A 70C 74A*
LA6$^-$						
				2(5.0%)	2(3.4%)	4(7.0%)
				38C 96E	*85D 91B*	*17A 30C 91B 92E*
LA6$^+$						
					2(3.0%)	5(7.7%)
					10A 43D	*11C 64B 67A 97C*
HA						
						3(3.6%)
						57A 57B 84B
LA$^+$						–

The numbers indicate the sites which are shared by a pair of strains (the percentage is indicated in parentheses) as well as their cytological location in italics.

presence or absence of a deletion derivative and the dysgenic properties and fitness of the strains under investigation. For example, *LA* and *LA*$^-$ have quite similar composition of *hobo* elements, yet they differ markedly in both characteristics. Similarly, *LA*$^+$ and *LA6*$^+$ have similar dysgenic properties and fitness but exhibit strong differences in the restriction patterns of the *hobo* elements. More experiments are clearly needed to assess the impact of different members of the *hobo* element family in the dysgenic properties of a given strain.

While *LA6*$^-$ and *LA6*$^+$ did not differ considerably from *LA* and *LApas* in terms of the copy number of *hobo* elements, in *HA* and *LA*$^+$ we observed an amplification of the members of this family. Not unexpectedly, all these processes were accompanied by massive transpositions of *hobo* elements. Although the *in situ* hybridization data are limited, we could not detect any specific transpositions of *hobo* elements that would be coupled to changes in the dysgenic properties or the fitness of the strains. Unlike the case of *copia*-like elements, which transpose from/to specific sites during

selection for high and low fitness (Pasyukova *et al*, 1986), we observed an almost complete reshuffling in the cytological localization of *hobo* elements. Our data may imply that the process of change in male mating activity of the strains also changed some genetic mechanisms regulating the activity of *hobo* elements.

In spite of the fact that *hobo* elements have been studied for almost a decade, little is known about the mechanisms regulating their activity in the genome. Unlike active P elements, which are fully suppressed by a P cytotype, and are inactive in the germline and can be mobilized under normal conditions (*ie* without considering strains containing engineered P elements) only in dysgenic crosses (see Engels, 1989), *hobo* elements can transpose within the strains known to contain active *hobo* elements without a need for outcrosses (Blackmann *et al*, 1987; Hatzopoulos *et al*, 1987; Lim, 1988) and they seem to also be active in somatic cells (Kim and Belyaeva, 1991). The fact that *hobo* may play a significant role in population biology is also exemplified by the finding that these elements have been found to be located on the breakpoints of naturally occurring inversions (Lyttle and Haymer, 1992). It is not surprising that most of the *D melanogaster* natural populations, although possessing both complete and deleted *hobo* elements in their genome, have completely lost the ability to activate them in dysgenic crosses (no *hobo*-activity potential) while strongly suppressing the activity of foreign *hobo* elements (high *hobo*-repression potential) (Pascual and Périquet, 1991). This may imply that some genetic mechanisms develope to suppress the activity of *hobo* elements and thus prevent deleterious consequences of their transposition (lethal mutations and chromosomal rearrangements). These mechanisms may involve the effects of some specific *hobo*-deletion derivatives that are wide spread in natural populations (Périquet *et al*, 1989; 1990) but also other genetic factors, acting in both maternal and zygotic fashion (Ho *et al*, 1993). Some recent findings also imply that these mechanisms can be flexible, as the *hobo*-activity and *hobo*-repression potentials in natural populations can vary seasonally and change rapidly when wild strains are brought into a laboratory environment (Zabalou *et al*, 1991).

Considering this, we can assume that the selection for low fitness may have somehow prevented the full development of the mechanisms suppressing the activity of *hobo* elements, as is the case in *LA*. This would have resulted in the transposition of *hobo* elements within the strains, revealed as heterogeneity in their locations in the genome of several individuals (as a possible source of genetic variability under the conditions of highly unfavourable direction of selection) and their activation in dysgenic crosses. Selection for the high fitness (or its spontaneous increase) eventually derepressed the formation of genomic mechanisms controlling the *hobo* elements' activity. The fact that all *LA*-derived strains with increased fitness have completely different composition, copy number and genomic distribution of *hobo* elements and at the same time exhibit an absence of *hobo*-activity and increased *hobo*-repression potentials point to the predominance of these non-*hobo*-mediated regulatory mechanisms over the element's own contribution. The considerable increase in copy number, classes of deletion derivatives and intrastrain site polymorphism of *hobo* elements that accompanied the formation of repression mechanisms in *HA* and LA^+ is, at first glance, unexpected. Some preliminary data indicate that during the initial steps of selection of *LA* from low to high fitness the activity of its *hobo* elements increased considerably, as revealed by the GD

sterility assay (AP Galkin and LZ Kaidanov, unpublished observations). This may have created the diversity in the content of *hobo* elements observed in the *HA* and LA^+ strains. Experiments are in progress to monitor the changes in *hobo* properties during the course of selection of *LA* from low to high male mating activity and the impact of different chromosomes in this process.

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