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Maintenance of a large pericentric inversion generated by the *hobo* transposable element in a transgenic line of *Drosophila melanogaster*

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The impact of the *hobo* transposable element in the global reorganization of the *Drosophila melanogaster* genome has been investigated in transgenic lines generated by the injection of *hobo* elements into the Hikone strain, which lacked them previously. Extensive surveys of transgenic lines followed for 250 generations have identified 13 inversions with *hobo* inserts at most breakpoints. One of these inversions is pericentric on chromosome *2*. It has been maintained in the line where it was discovered and in several sublines at frequencies from 0.19 to 0.45, generating stable chromosomal polymorphisms, similar to cosmopolitan paracentric inversions in natural populations. Individuals homozygous for this inversion were viable and fertile, allowing the creation of a new homozygous strain. *Hobo* elements were still active after 250 generations.
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Introduction

Among the mobile elements in *Drosophila melanogaster*, three independent systems (*P*, *I* and *hobo*) produce aberrant germline events, such as high mutation rate, infertility and elevated levels of chromosome instability (Blackman and Gelbart, 1989). In recent years, transposable elements have been suggested to play an important role in genome evolution, as major players in genomic restructuring (McDonald, 1998). The contribution of *hobo* elements to chromosome instability was demonstrated by Lim and co-workers in several studies (Lim, 1988; Ho *et al*, 1993; Sheen *et al*, 1993; Eggleston *et al*, 1996). In order to trace the fate of *hobo* elements after their injection into a Hikone strain devoid of these elements, seven transgenic lines were followed over 250 generations (Ladevèze *et al*, 1994; Galindo *et al*, 1995; Ladevèze *et al*, 1998a, b, 2001). *Hobo* elements increased in each line, showing the autonomous amplification by transposition of this element. At the chromosomal level, *hobo* elements appeared to be actively involved in the formation of rearrangements. In all, 13 new inversions were observed among the different lines, most of them with a *hobo* insert at one or both inversion breakpoints. In the present study, we focus on a long-time survey of one of these lines (no. 11), in which a large *hobo*-mediated pericentric inversion on chromosome 2 was maintained after more than 120 unselected generations. The consequences of such a polymorphism on chromosomal restructuring of the *Drosophila* genome are discussed.

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Materials and methods

Basic strains and microinjection

Drosophila melanogaster embryos were transformed with pHFL1 using the standard microinjection technique (Spradling and Rubin, 1982). The microinjected plasmid pHFL1 (Blackman *et al*, 1989) contains one autonomous *hobo* element and some adjacent genomic DNA from cytogenetic locus *94E*, cloned in the pBLUESCRIPT KS plasmid. The strain used as a receptor was Hikone, an E-type strain in the *hobo* system that lacks *hobo* elements. It was also of the R and M types, that is, devoid of *I* and *P* transposable elements. In all, 30 independent lines were found by individually crossing one injected fly with one noninjected Hikone fly (Ladevèze *et al*, 1994). Subsequently, each line was bred *en masse*. Lines were analysed at generation 2 using the Southern blot technique to check for the presence of *hobo* elements. Seven *hobo*-positive lines were obtained. Each was maintained independently by mass matings (ca. 100 individuals) at 25° C in the following generations. Line 11, in which the large pericentric inversion was found, was duplicated at generation 145.

In situ hybridization

In every 10–15 generations, 10 larvae were screened for the presence of *hobo* element on their chromosomes in the replicates of line 11. *In situ* hybridization of biotinylated

probes (Boehringer kit) to salivary gland polytene chromosomes was adapted from Lemeunier and Aulard (2000). The pHFL1 plasmid used in the microinjection step was used as a probe for the *in situ* hybridizations. Under our experimental conditions, no hybridization site, including the *94E* locus, was observed when this pHFL1 probe was used on the parental Hikone strain chromosomes.

Drosophila stocks used in the analyses

All mutations and balancer chromosomes are described in Lindsley and Zimm (1992).

Cy^O/T(2–3): second chromosome balanced strain In(2-LR)O, dp^{lv1}, Cy, pr, cn^2/T(2–3) wg^-, cn, bw.

dp, b, cn: a strain homozygous for the second chromosome mutations *dumpy* (*dp* 2–13.0), *black* (*b* 2–48.5) and *cinnabar* (*cn* 2–57.5).

Isolation of the In(2LR) chromosome

In(2LR)27B;53C: the large *hobo*-mediated pericentric inversion on the second chromosome, observed in this study and denoted simply as *In(2LR)* in the rest of this paper.

In order to know if adults homozygous for the *In(2LR)* chromosome are viable and fertile, crosses have been performed involving 30 randomly chosen males from line 11 and 30 *dp, b, cn* females. In all, 61 F1 females were then individually testcrossed with three *dp, b, cn* males. The progeny of the 61 females were scored for the presence or absence of recombinants between the *b* and *cn* loci. As *In(2LR)* spans the *b–cn* interval, the absence of recombinant progeny indicated that the maternal F1 female was heterozygous for the *In(2LR)* chromosome. Six progeny of this type were observed with one half of genotypes *In(2LR)/dp, b, cn*. From each one of these progeny, a single [dp^+ b^+ cn^+] male was crossed with three *Cy^O/T(2–3)* females to isolate the *In(2LR)/Cy^O, dp, cn* adults in their descendants. These individuals were then crossed together and their progeny analysed to look for the presence of wild-type adults, corresponding to homozygous *In(2LR)/In(2LR)* if they were viable.

Genetics and molecular tests

The *hobo* status of line 11 was measured by crossing the line with Hikone and 23.5*/*Cy* and looking for the level of induced gonadal dysgenesis (% *GD*) in order to estimate the levels of *hobo*-activity and repression potential (Yannopoulos *et al*, 1987; Ladevèze *et al*, 1994). Standard techniques were used for DNA extraction, gel electrophoresis, blotting and hybridization (Maniatis *et al*, 1982). Genomic DNA of adult flies was digested by *Xho*I, which cuts near each end of the *hobo* sequence, yielding a 2.6 kb fragment from complete *hobo* elements. After electrophoresis on a 1% agarose gel, transfer and hybridization were performed on Nytran membranes (Schleicher and Schuell). The probe used was the 2.6 kb fragment generated by *Xho*I digestion of pHFL1. Hybridization was carried out overnight at 65°C in 0.25 M phosphate buffer pH 7.2, 1 mM ethylene diaminetetraacetate, 7% sodium dodecyl sulphate. The membranes were washed at 65°C for 20 min in 2 × standard saline citrate (SSC), 20 min in 1 × SSC and 20 min in 0.5 × SSC. Filters were then exposed to an X-ray film for 1 or 2 days.

Results

Observation of a pericentric inversion and its fate in the evolving population

At generation 130, we observed one individual heterozygous for a large pericentric inversion on the second chromosome. Breakpoints were at *27B* on the left arm and *53C* on the right arm of the chromosome (Figure 1). *Hobo* insertions were detected at both breakpoints. The frequency of the *In(2LR)* chromosome was a few percent as only one inverted chromosome was found among the 20 analysed.

As this inversion was also observed at generations 142 and 145, a duplication of line 11 was made at this last generation. The two sublines were maintained *en masse* independently under the same conditions and analysed during the following 100 generations. Figure 2 shows the maintenance of this chromosomal polymorphism during that time, with an average frequency of 0.28 and 0.29 for the *In(2LR)* chromosome in the two sublines.

In all, 14 isofemale sublines were initiated from line 11 at generation 145. Cytological analysis of their progeny identified four sublines in which *In(2LR)* was detected. These four sublines were maintained independently,

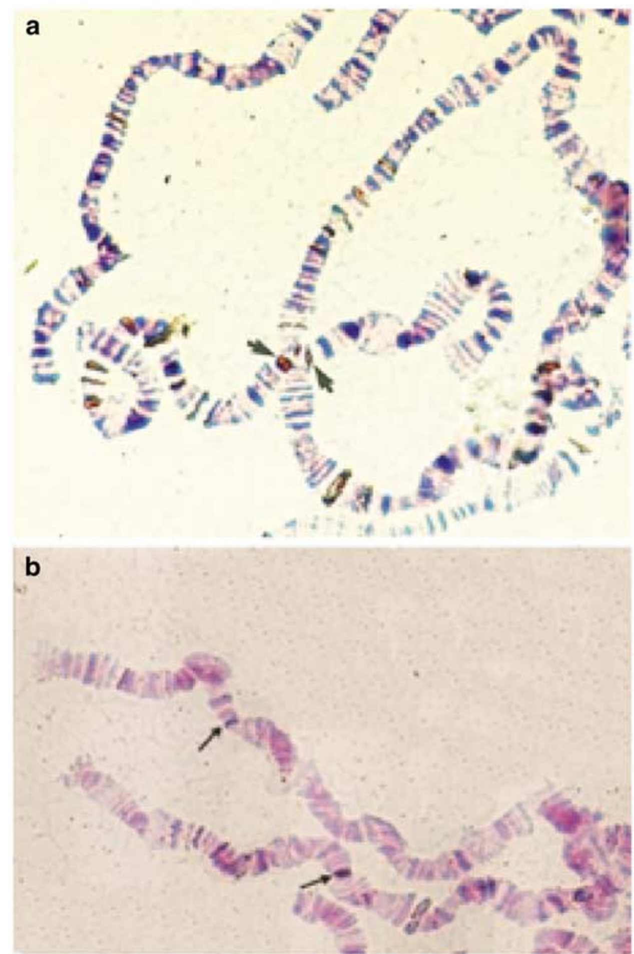

Figure 1 *In situ* hybridization with the biotinylated pHFL1 probe to inverted chromosome. (**a**) Heterokaryotype *In(2LR)27B-53C/St*. (**b**) Homokaryotype *In(2LR)27B-53C/In(2LR)27B-53C*. Arrows point to *hobo* inserts at the chromosomal breakpoints.

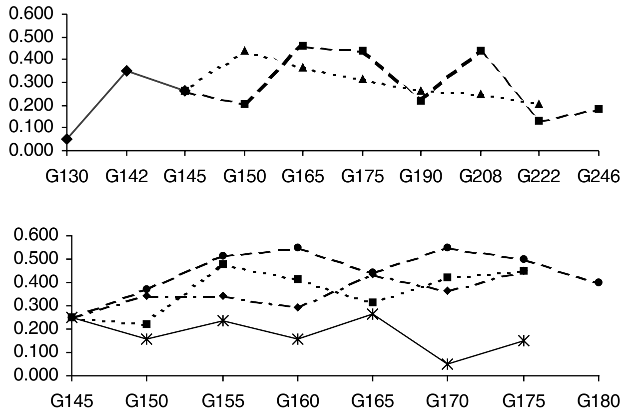

Figure 2 Maintenance of the pericentric inverted chromosome. *Top*: Two subpopulations founded by a duplication *en masse* of the original line 11. *Bottom*: Four subpopulations each founded by one inseminated female from the original line 11.

en masse, and analysed over 30–35 generations. The *In(2LR)* frequency was estimated to be 0.25 initially because it is most probable that only one of the two original parents was heterozygous, assuming the female mated only one time. Indeed, the first homozygous larva in the parental line 11 was detected at generation 150. Figure 2 shows the maintenance of the chromosomal polymorphism, with *In(2LR)* average frequencies from 0.19 to 0.45.

Viability and fertility of *In(2LR)* homozygous adults

The finding of larvae homozygous for *In(2LR)*, in line 11 and in the four isofemale sublines, demonstrates the viability of this genotype, at least at the third instar. In order to determine if homozygous adults are viable and fertile, appropriate crosses were performed (see Materials and methods). Each cross between females and *In(2LR)/Cy^{O}, dp, cn* males gave abundant progeny (ie 200 offspring per female over 3–4 days). Among progeny, two types of adults were observed: 2/3 of [*Cy*] phenotype corresponding to *In(2LR)/Cy^{O}, dp, cn* individuals and 1/3 of [wild type] corresponding to *In(2LR)/In(2LR)*. These results show that the *In(2LR)* chromosome is equally viable in the homozygous and the heterozygous states. Moreover, the homozygous individuals were fertile when intercrossed, producing abundant progeny and homozygous *In(2LR)* lines. Their homozygous karyotypes (Figure 1) were checked by observation of salivary gland polytene chromosomes.

Genetic and molecular analyses

The genetic tests show that from generation 130 onwards, line 11 has not acquired a strong *hobo*-activity potential (with values around 5–10% of the induced *GD* sterility) and has a low repression potential for *hobo* activity (79–86% of the induced *GD* sterility).

Southern blot analyses of line 11, from generation 63 onwards, show the presence of the 2.6 kb *Xho*I internal fragment expected for complete *hobo* HFL1 elements (Figure 3). Smaller restriction fragments that might have originated from internal deletions were observed after G63. Some bands were observed corresponding to deleted elements, but they were always very rare and none became fixed.

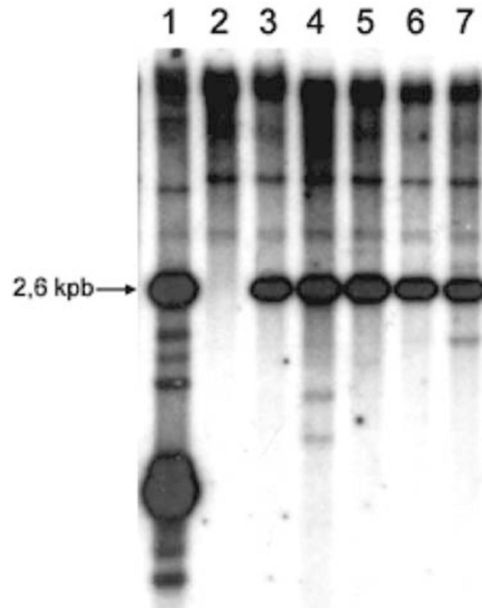

Figure 3 Southern blot analysis of *hobo* sequences in line 11 at different generations. The probe used was the 2.6 kb fragment generated by *Xho*I digestion of pHFL1. Lane 1 corresponds to Tours82 strain as a positive control, lane 2 to Hikone strain as negative control, lanes 3–7 to line 11 at G63, G71, G110, G125 and G208, respectively.

Distribution of hobo inserts

At G8, an average of 1.7 *hobo* inserts per individual was detected by *in situ* hybridization in line 11. The number of insertion sites increased slowly until G81 (seven inserts). At G130, this number was three times higher (21) and increased regularly during the 80 following generations. By generation 208, the number reached 31. However, the increase in inserts was not similar for all chromosomes. Since G130, we observed that the number of insertion sites was always higher on both arms of chromosome *2* compared to chromosomes *X* and *3* (Figure 4). The total number of *hobo* inserts detected during these 208 generations is 20 for the *X*, 77 and 87 for the left and right arms of chromosome *2*, respectively, and 37 and 36 for those of chromosome *3*. As in the other transgenic lines, no hybridization was observed on chromosome *4* or in the chromocentre.

Discussion

From the seven transgenic lines obtained, 13 chromosomal inversions were observed (Ladevèze *et al*, 1994, 1998a, b and unpublished data): nine paracentric (two on the *X*, one on the second and six on the third chromosomes) and four pericentric (two on each of the large autosomes). Most were detected in a single larva. Two paracentric inversions on *3L* were observed twice, at different generations. The pericentric inversion *In(2LR)27B;53C* is the only one retained for many generations. Over about 120 generations, it was present at stable frequencies (ca. 0.28–0.29) in the different subpopulations, showing the unselected maintenance of a chromosomal polymorphism generated by *hobo* elements.

Heterozygotes for pericentric inversions are expected to be semisterile because recombination within the

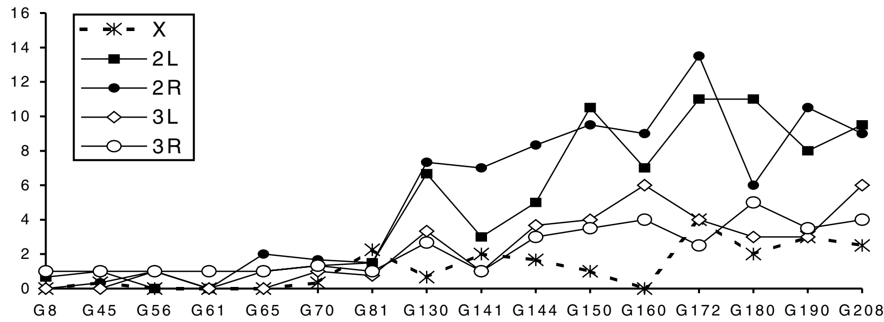

Figure 4 Mean number of insertion sites per individual, for each chromosome, at different generations.

inverted region produces aneuploid gametes. Owing to this underdominance, these arrangements should quickly be eliminated from populations when they first arise. This would explain the quasi-absence of polymorphism for this kind of inversion in nature. The only example of such a polymorphism in *D. melanogaster* was recorded by Aulard (1990), Coyne *et al* (1991) and Aulard *et al* (2002). Coyne *et al* (1993) found that fertility was not reduced in many cases, for laboratory-induced pericentric inversions, due to suppressed crossingover in heterokaryotypic individuals. This lack of underdominance depends on the position of breakpoints on the chromosome. These 'sensitive sites', described on chromosomes *2* and *3*, appear to reduce recombination in a heterozygous inversion whose breakpoints are nearby. However, these sites do not correspond to our *In(2LR)* breakpoints.

The maintenance of the *In(2LR)* may result either from a selection effect (for example, an advantage of the heterozygote) or from a stochastic event (a transient phase before extinction or fixation by genetic drift). As the size of the experimental populations is always limited, the role of genetic drift must be taken into consideration. The question is whether maintenance over 120 generations is very probable in our experimental populations. Kimura (1971) showed that for a population of size N, the probability for a neutral nonrecurrent mutation to be lost is $(1-1/2N)$. In our case, N was about 100 and probability of loss of the inversion was about 0.995. Moreover, taking t_o as the number of generations until loss, Kimura shows that its mean is given by: $2(N_e/N)\log_e(2N)$ and its variance by: $(16N_e^2/N-(2N_e/N)\log_e 2N)^2$. If we consider that the effective size (N_e) of *Drosophila* populations is about $0.7N$ (Crow and Morton, 1955), we obtain a mean value of 7.4 generations (with a standard deviation of 27.0) for the time to loss of a chromosomal inversion if neutral. Therefore, in more than 99% of such cases, a neutral inversion will not persist more than 50–60 generations. Compared with the 120 generations of maintenance observed for the *In(2LR)* chromosome in both subpopulations and the 4×40 generations in the four isofemale replicates, our results reject the genetic drift hypothesis and point to a very likely role of selection in the maintenance of this chromosomal rearrangement.

In this case, a possible explanation resides in the advantage of the chromosomal heterozygote *In(2LR)/Standard*. Simulation analyses fitting the model of selection with overdominance were performed on our observed data. They allowed us to estimate the selective values of each genotype. The fitness estimates $w_1=0.94$ for *Std/Std*, $w_2=1$ for *In(2LR)/Std* and $w_3=0.85$ for *In(2LR)/In(2LR)* are compatible with a stable polymorphism and an equilibrium frequency of 0.28 for *In(2LR)*, as observed in our experiments. The frequency of the *In(2LR)* chromosome will increase from 0.05 (initial estimate at its first observation) to around 0.25 in about 30–40 generations, in a way similar to our experimental populations.

At the evolutionary level, such an inversion could be maintained for many generations, producing a chromosomal polymorphism similar to the ones encountered in natural populations for paracentric cosmopolitan inversions of *D. melanogaster* (review in Lemeunier and Aulard, 1992) and many other *Drosophila* species (review in Sperlich and Pfriem, 1986). Moreover, that the *In(2LR)* inversion is viable and fertile shows that a *hobo* element can induce inversions that become fixed in small populations leading to new homokaryotypic populations.

In accordance with these results, several observations suggest a possible impact of *hobo* element in the generation of chromosomal inversions in natural populations. In a natural population of *D. melanogaster* in the Hawaiian islands, four endemic inversions, with *hobo* elements at one or both breakpoints, have been described, but the cosmopolitan inversions on the same chromosome do not show this association (Lyttle and Haymer, 1992). One of the individuals in this population carried an unstable second chromosome, as revealed by laboratory backcrosses, with 12 *hobo* elements. About 1% of the progeny of this fly had new inversions, duplications or transpositions in the second chromosome. Many of the breakpoints of these rearrangements were sites occupied by *hobo* elements in a way similar to those generated in our experimental populations (Ladevèze *et al*, 1998a).

In a Greek natural population of *D. melanogaster*, an analysis extending over three seasons showed that three out of the five cosmopolitan inversions analysed and two of the three endemics were found to have *hobo* inserts at or very near one of the two breakpoints. A comparative analysis of the chromosomal distribution of the *P* element demonstrated that two out of the five cosmopolitan inversions and one of the three endemics had a *P* insertion at or very near one of the two breakpoints. None of the inversions detected had *P* or *hobo* insertions at both breakpoints (Zabalou *et al*, 1994).

Inversions in which the breakpoints lack *hobo* elements may represent cases in which *hobo* excised after causing

the rearrangement, or may have deleted copies too small to be detected by *in situ* hybridization. Another explanation is that some inversions are generated by transposable elements other than *hobo* or by agents other than transposable elements. However, in line 11, 11 other transposable elements (*Bel, copia, DocA, gypsy, mdg1, mdg3, P, roo, Stalker, 412, 1731*) have been regularly checked by *in situ* hybridization and in no cases have been found at an inversion breakpoint. This points to a major role of *hobo* in our experiments due to their introduction as active elements in the initial strain, but does not rule out the role of other elements in other lines (Lim and Simmons, 1994). The *hobo* elements were still active after 250 generations. No *GD* was observed in the Hikone strain, devoid of *hobo* elements. The *GD* status of the transgenic lines showed a global evolution towards a low *hobo*-activity potential with a variable level of repression potential. Southern blots revealed the presence of many full-length elements, the activity of which was confirmed by continued mobilization after 250 generations. Many new sites of transposition were observed until the last generations, showing that the *hobo* element can always participate in the genesis of genetic variability (genic and chromosomal).

These observations show that *hobo* elements are actively involved in the formation of rearrangements that generate important chromosomal polymorphisms. In nature, we can therefore consider the transposable elements as a reservoir of mutagenic agents, which help to reshape the structure of the *Drosophila* genome.

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