Original article

# Transposable elements in South American populations of *Drosophila simulans*

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**Abstract** – This study investigated the occurrence of four transposable elements (*mariner, gypsy, hobo* and *412*) in South American populations of *Drosophila simulans*. The genomic hybridization patterns of 12 different populations were determined by Southern blot analyses. Even though a low number of *mariner* copies was observed, each population presented a characteristic hybridization pattern, suggesting that the element is active. The number of *gypsy* copies was also low, but all populations bore a similar hybridization pattern. In this paper we describe the occurrence of an almost *gypsy*-free strain, which had not yet been found for *D. simulans* nor *D. melanogaster*. In the case of *hobo*, we did not detect the 1.1-kb-long deleted *hobo* element in any of the South American populations while this element is present in all strains originating from other geographical sites that were analysed up to now, suggesting that the *hobo* element may have very recently invaded the genome of South American *D. simulans* populations. The *412* element presented some population-specific band patterns, indicating that this element may have some transposition activity in the different populations. © Inra/Elsevier, Paris

*Drosophila simulans* / transposable element / *mariner* / *hobo* / *gypsy* / *412*

**Résumé – Éléments transposables dans des populations sud-américaines de *Drosophila simulans*.** Cette étude recherche la présence de quatre éléments transposables (*mariner, gypsy, hobo* et *412*) au sein de populations sud-américaines de *Drosophila simulans*. Le profil d'hybridation génomique de 12 populations différentes a été déterminé par Southern

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Blot. Bien que seul un petit nombre de copies de *mariner* ait été observé, chaque population présentait un profil d'hybridation caractéristique, ce qui suggère que l'élément était actif. Le nombre de copies de l'élément *gypsy* s'est avéré très petit ; avec un profil d'hybridation similaire dans toutes les lignées, ce qui indique que l'élément était inactif dans les populations étudiées. L'une des lignées ne comportait pratiquement pas d'élément *gypsy*, ce qui n'était encore jamais arrivé chez *D. simulans* ni *D. melanogaster*. L'élément *hobo* de 1,1 kb n'a été détecté dans aucune lignée d'Amérique du Sud alors qu'il existe dans toutes les lignées, originaires d'autres sites géographiques, analysées jusqu'à présent. Ces informations en accord avec les données de la littérature, suggèrent que l'élément *hobo* n'a peut-être que récemment envahi le génome des populations de *D. simulans* en Amérique du Sud. Pour l'élément *412*, les populations étudiées ont montré des bandes spécifiques, ce qui suggère que cet élément peut avoir des activités de transposition dans le génome de ces mouches. © Inra/Elsevier, Paris

**Drosophila simulans / élément transposable / mariner / hobo / gypsy**

## 1. INTRODUCTION

Transposable elements (TEs) have already been shown to compose a significant fraction of the genome of a wide variety of organisms [1]. Approximately 10 % of the *D. melanogaster* genome consists of 50 different families of TEs [13]. The amount of middle repetitive DNA sequences in *D. simulans* was estimated to be about one third that present in its sibling species *D. melanogaster* [10]. It is believed that a major portion of this repetitive DNA is composed of TEs [26]. Virtually all families of TEs that have been cloned from *D. melanogaster* are also represented in the genome of *D. simulans* [6]. The only exception to the similar TE composition between the two species is the presence of the *P* family, which is only found in *D. melanogaster* and *mariner* which is only found in *D. simulans*. Considering that virtually the same TE families are present in both species and that *D. simulans* has only one third the total repetitive DNA present in *D. melanogaster*, the mean number of element copies per family should be lower in *D. simulans* [17, 20, 23]. These last authors detected significant differences in copy number of TEs between those species by in situ hybridization.

Studies covering the whole distribution range of *D. simulans* should make it possible to better understand the differences in the number of copies of TEs between this species and *D. melanogaster*, in addition to providing important information on the evolutionary history of TEs. In an attempt to contribute to the resolution of this question, we investigated the occurrence of four different transposable element families (*mariner*, *hobo*, *gypsy* and *412*), by means of Southern blot analyses, in 12 *D. simulans* populations originating from different locations within the American continent.

## 2.MATERIALS AND METHODS

### 2.1. Fly stocks

The following strains of *D. simulans* were employed in the present study: 1) Peru (obtained from Bowling Green Center – no. 14021-0251-5) – originally collected in

Lima, Peru; 2) Salvador – collected in Salvador, Bahia – Brazil, in 1995; 3) RJ – collected in the National Park of Tijuca, Rio de Janeiro – Brazil, in 1995; 4) SBC – collected in São Bernardo do Campo, São Paulo – Brazil, in 1993; 5) MGS – collected in Eldorado, Mato Grosso do Sul – Brazil, in 1994; 6) Maquiné – collected in Maquiné, Rio Grande do Sul – Brazil, in 1994; 7) Goethe – collected in Porto Alegre, Rio Grande do Sul – Brazil, in 1991; 8) dpp-like – derived from a spontaneous mutant originating from in a hypermutable wild strain of *D. simulans* collected in Porto Alegre, Rio Grande do Sul – Brazil, in 1990; 9) yellow – derived from a spontaneous mutant of the yellow locus encountered in a population sample from Itapuã, Rio Grande do Sul – Brazil, in 1982; 10) Camobi – collected in Santa Maria, Rio Grande do Sul – Brazil, in 1994; 11) Montevideo – collected in Montevideo, Uruguay, in 1991; 12) 1093 (obtained from Caltech Center) – collected in Islamorada, Florida – USA.

## 2.2. Southern blots

Genomic DNA was prepared from 40–50 adult flies, according to Jowett [16]. DNA samples (approximately 5 µg from each strain) were completely digested with restriction endonucleases, submitted to electrophoresis on 0.8 % agarose gels, transferred to nylon membranes and hybridized to nick-translated DNA probes (labeled with $^{32}$P-$\alpha$-dATP) in the presence of 50 % formamide at 42 °C. Each filter was washed three times with 0.2X SSC and 0.5 % SDS for 20 min at 42 °C.

A 0.9-kb fragment obtained by *Nhe*I/*Pvu*II digestion of Mos 1 plasmid DNA [19] was used as a probe for the analysis of the *mariner* TE family. As a probe for the *gypsy* element, we employed the pGGHS plasmid, which contains a complete *gypsy* element [9]. Members of the *hobo* family were probed with the pHX4 plasmid, which contains a *Xho*I 2.6-kb fragment of *hobo* element of *D. melanogaster*. The 2.6-kb fragment was removed from a complete element contained in the pHLF1 plasmid [4] and subcloned into the Bluescript plasmid. The probe used to detect *412*, was an element four *Hind*III-*Eco*RI fragment from cDm*412* with a total length of 4.4-kb was used [27].

## 3. RESULTS

### 3.1. Southern blot analyses

#### 3.1.1. Mariner *family*

When the DNAs from *D. simulans* populations of diverse geographical origins were hybridized to the *mariner* element probe, we observed hybridization patterns that were characteristic to each one of them. The mean number of hybridizing bands varied according to the restriction enzyme used. For instance, when *D. simulans* DNA was digested with *Sal*I, we saw, on average, $9.0 \pm 4.1$ hybridizing bands (see *figure 1A*). The *yellow* strain presented the largest number of hybridization bands (16 bands); the Montevideo population presented the smallest number (one band). Since the *mariner* element possesses a single site for *Sal*I, we expected each copy of the element to produce two hybridization bands when the genomic DNA was

digested with this enzyme. The number of copies per genome should have been equal to approximately half the number of observed bands. Enzymes that do not have internal sites in the *mariner* element sequence would yield a smaller number of bands, which should correspond to the approximate number of copies of the element. When DNA was digested with *Xho*I, for example, the number of hybridizing bands was on average $4.4 \pm 1.5$. Once again, the *yellow* strain was the one with the largest number of bands (a total of nine) which were not seen in this Southern, but have been observed in other experiments (data not shown). Maquiné and 1093 were the ones showing the smallest number (only three hybridization bands) (*figure 1B*).
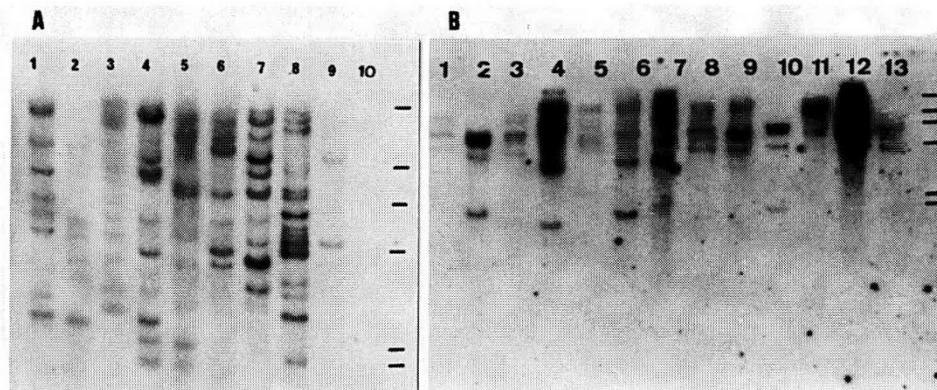


**Figure 1.** Southern blots of *D. simulans* populations hybridized to a 0.9-kb fragment obtained by *Nhe*I/*Pvu*II digestion of Mos1 plasmid used as a *mariner* probe. A) Genomic DNA digested with *Sal*I. The samples are as follows: 1) Peru, 2) Salvador, 3) RJ, 4) SBC, 5) MGS, 6) Camobi, 7) Maquiné, 8) *yellow*, 9) Goethe, 10) Montevideo. B) Genomic DNA digested with *Xho*I. The populations are as follows: 1) H134-18, 2) 1093, 3) Peru, 4) Salvador, 5) RJ, 6) SBC, 7) MGS, 8) Maquiné, 9) Camobi, 10) *dpp*-like, 11) Goethe, 12) *yellow*, 13) Montevideo. Bars on the right represent the λ *Hind*III fragments (24, 9.5, 6.8, 4.3, 2.3 and 2.0 kb).

### 3.1.2. Hobo *family*

The cleavage sites for *Xho*I in the *hobo* element are close to the inverted terminal repeats, and digestion with this enzyme yields a 2.6-kb-long fragment when a complete *hobo* copy is present, and smaller bands if deleted elements are present. All strains analysed possessed the 2.6-kb band, indicating the occurrence of complete elements (*figure 2*). Boussy and Daniels [5] have shown the occurrence of a 0.7-kb band (corresponding to a deleted element very common in *D. simulans*) in the majority of the strains they analysed. In the present study, we detected this band in the North American strains (1093), but we did not find it in any of the South American populations.

Some strains, such as *dpp* and *yellow* presented a very weak signal for the 2.6-kb band, indicating that possibly only a few copies of the complete element were present in these strains.

Bands greater than 2.6 kb, as seen in *figure 2*, were also encountered in other species of the *melanogaster* subgroup. According to Boussy and Daniels [5] the occurrence of such bands can be explained by the presence of other TEs which carry sequences related to *hobo*. *Hobo* elements bearing a deleted or altered *Xho*I site are also interpreted as old sequences of *hobo* localized in heterochromatin.
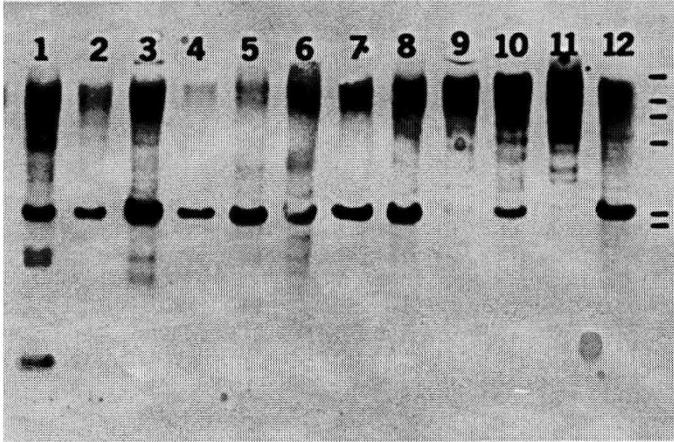


**Figure 2.** Screen for potentially complete *hobo* elements of *D. simulans* populations Genomic DNA was digested with *Xho*I and probed with pHX4. Samples are as follows: 1) 1093, 2) Peru, 3) Salvador, 4) RJ, 5) SBC, 6) MGS, 7) Maquiné, 8) Camobi, 9) *dpp*-like, 10) Goethe, 11) *yellow*, 12) Montevideo. Bars on the right represent the 1 *Hind*III fragments (24, 9.5, 6.8, 4.3, 2.3 and 2.0 kb).

### 3.1.3. Gypsy *family*

Hybridizing the membranes to the *gypsy* element probe resulted in a small number of hybridization bands, with an average of $7.8 \pm 2.8$ bands. A highly conserved hybridization pattern was observed throughout the *D. simulans* populations. When the fly DNA was digested with *Sal*I or *Eco*RI, the majority of the bands was common to almost all populations – few bands were specific to each population (*figure 3*). Based on the known sequence of *D. melanogaster*'s *gypsy*, this element should have a single cleavage site for *Eco*RI and none for *Sal*I. Therefore, if we assume the same element structure for *gypsy* of *D. simulans*, we might have expected to see different hybridization bands among the different studied populations in the case of the occurrence of insertion site polymorphism.

The *yellow* strain was an important exception. It seemed to bear very few copies of *gypsy* in its genome, as can be seen in *figure 3A* (lane 8). It should be noted that the Southern membrane shown in *figure 3A* is the same as the one used in *figure 1A*, where yellow was one of the strains showing the largest number of hybridized bands with the *mariner* element.
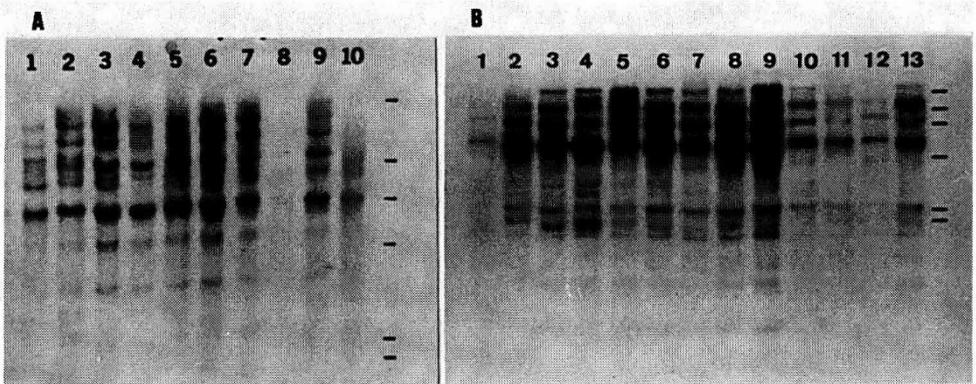
**Figure 3.** Southern blots of DNA from *D. simulans* strains hybridized to the *gypsy* element. A) Genomic DNA of *D. simulans* populations digested with *Sal*I. The populations are as follows: 1) Peru, 2) Salvador, 3) RJ, 4) SBC, 5) MGS, 6) Camobi, 7) Maquiné, 8) *yellow*, 9) Goethe, 10) Montevideo; B) Genomic DNA of *D. simulans* populations digested with *Eco*RI. The populations are as follows: 1) yellow, 2) Montevideo, 3) Camobi, 4) *dpp*-like, 5) Goethe, 6) Maquiné, 7) SBC, 8) MGS, 9) RJ, 10) Salvador, 11) Peru, 12) H143-18, 13) 1093. Bars on the right represent the λ *Hind*III fragments (24, 9.5, 6.8, 4.3, 2.3 and and 2.0 kb).

### 3.1.4. 412 family

The *412* element of *D. melanogaster* carries four *Xho*I restriction sites [27]. We thus expected to obtain bands corresponding to 2.6, 2.1 and 0.9-kb in size in the presence of a complete copy of *412*. As shown in *figure 4A*, all the analysed populations carried the expected bands, in addition to greater-sized bands, some of which were specific for each population.

The 1.4- and 0.6-kb fragments expected with an EcoRI digestion were found in all populations, in addition to greater-sized bands, some of which were specific for each population (*figure 4B*).

## 4. DISCUSSION

Capy et al. [8], Maruyama and Hartl [18] and Giraud and Capy [14] have analysed several *D. simulans* populations by Southern Blot, and their results showed that the number of *mariner* copies per genome varies from 0 to 15. Our present findings on the number of *mariner* copies occurring in the genome of *D. simulans*, as inferred from the number of bands encountered in the Southern blot assays, were very much in agreement with values found in previous studies about the occurrence of *mariner* in strains of this species from other origins.

Maruyama and Hartl [18] have detected hybridization bands that are specific for each strain, indicating the existence of insertion site polymorphism for the *mariner* element. Nevertheless, these authors have also observed some genomic sites common
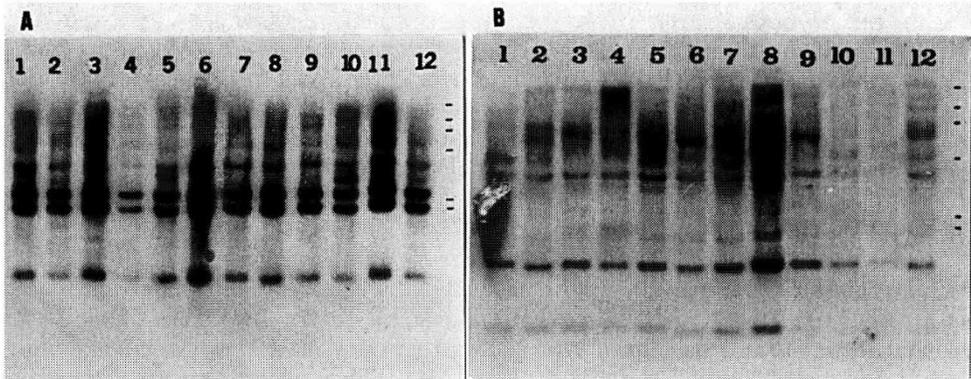
**Figure 4.** Southern blots of *D. simulans* populations probed with four *Hind*III-*Eco*RI fragments from a cDm412 plasmid (412 element). A; Genomic DNA of *D. simulans* populations digested with *Xho*I. The samples are as follows: 1) 1093, 2) Peru, 3) Salvador, 4) RJ, 5) SBC, 6) MGS, 7) Maquiné, 8) Camobi, 9) *dpp*-like, 10) Goethe, 11) *yellow*, 12) Montevideo. B; Genomic DNA digested with *Eco*RI. The samples are as follows: 1) Montevideo, 2) Camobi, 3) *dpp*-like, 4) Goethe, 5) Maquiné, 6) SBC, 7) MGS, 8) RJ, 9) Salvador, 10) Peru, 11) H134-18, 12) 1093. Bars on the right represent the λ *Hind*III fragments (24, 9.5, 6.8, 4.3, 2.3 and 2.0-kb).

to strains from diverse geographical origins, indicating that at least some of them are long-existing insertion sites and may be fixed within the species. Giraud and Capy [14] described three banding patterns in *mariner* Southern blot analysis: i) populations from different geographical origins which share the same three bands, these populations having few bands; ii) populations with more bands, some of these common to all the lines of a population; iii) populations with few bands different among populations. In the present study, we did not find any hybridization bands common to the different populations; each population showed a characteristic hybridization profile, in agreement with Giraud and Capy's pattern 3.

Laboratory populations show greater variability in *mariner* copy number than natural populations [15]. This fact might explain the high copy number occurring in our yellow strain, as this population has been kept in the laboratory since 1982. All South American strains employed in the present study were recently collected in the wild, except for the yellow and *dpp*-like strains, and none of them were *mariner*-free. This apparent non-existence of *mariner*-free populations among recently collected ones is also supported by the findings of Capy et al. [8] and Giraud and Capy [14].

Periquet et al. [21] recorded the fact that almost all natural populations of *D. melanogaster* possess copies of a 1.5-kb-long internally deleted *hobo* element. They called this element *Th*. These authors suggest that the accumulation of these *Th* elements in the genome might have some regulatory role in *hobo* transposition. Such a role has been suggested for the KP element of the *P* family of *D. melanogaster* [3]. Boussy and Daniels [5] analysed 19 *D. melanogaster* and 31 *D. simulans* strains by Southern blot. E (empty of *hobo*) and H (with *hobo*) strains

were found in both species. In several *D. melanogaster* strains they observed the occurrence of the 1.5-kb (*Th*) element. In *D. simulans*, only four strains (one from Peru; one from Colombia; and one of unknown origin) did not have the hybridization band that indicates the presence of a complete element. The three South American strains also did not bear the 0.7-kb band that corresponds to an internally deleted element of 1.1 kb. All other strains, originating from North America, Australia and South Africa, as well as the one with unknown origin, showed a very strong hybridization signal corresponding to the 0.7-kb band.

In the present study, we found two *D. simulans* strains with very weak hybridization signals for the 2.6-kb band (the existence of which represents the occurrence of a complete element), indicating that these strains carry few copies of the *hobo* element. Such strains have been kept in the laboratory for a longer period of time (14 and 6 years) than all the other populations, which were recently collected in nature and have strong hybridization signals for the 2.6-kb band. Furthermore, the 0.7-kb band (corresponding to an internally deleted element) was encountered only in the two North American populations; all the South American populations are devoid of this element. Considering our findings of few *hobo* sequences in older fly stocks and the results obtained by Boussy and Daniels [5], as a whole, we may suggest that *hobo* has been recently scattered in the genome of South American populations of *D. simulans*. Another argument in favor of this possible event is the reduced number of copies of deleted elements in the South American populations, most of all, the absence of the 1.1-kb elements which are so common to populations of other geographical origins. If the deleted elements do accumulate in the genome as a function of time after invasion, and if they are truly involved in the regulation of *hobo* activity [11], then we might suppose that *hobo* is active in the South American populations of *D. simulans* but not enough time has elapsed for a sufficient accumulation of deleted *hobo* copies in their genomes.

All *D. melanogaster* strains contain inactive copies of *gypsy* located at the same positions in the pericentromeric heterochromatin [22]. Even non-related strains, when compared by Southern blot analysis, show very similar hybridization patterns, suggesting that the *gypsy* element has invaded the genome of *D. melanogaster* very early in the species evolutionary history [7]. Nevertheless, a low copy number of putative active *gypsy* with no fixed sites were detected by in situ hydridization in *D. melanogaster* chromosomes arms [2, 23] and in *D. simulans* [23].

The South American populations of *D. simulans* presently examined bear very similar hybridization patterns, in the same way as *D. melanogaster* strains carrying few *gypsy* copies do. According to our estimates, as judged by the number of Southern hybridization bands, South American populations of *D. simulans* also bear a reduced number of *gypsy* copies. However, this Southern pattern may represent the ancient inactive copies of *gypsy* in the heterochromatin. If there exist active copies of *gypsy* in a low number in polymorphic insertions sites, these can be related to the weak bands specifically detected in some populations or these copies were not able to be detected by the Southern technique, since every individual presents one specific band.

In *D. melanogaster*, there is no evidence of a strain not presenting a hybridization signal when probed by *gypsy* sequences. Our finding of a *D. simulans* strain almost devoid of *gypsy* copies is most interesting. This strain has been kept in the laboratory

for 14 years. Stochastic loss [12] may be an explanation for the extremely low number of *gypsy* sequences it carries. This hypothesis is supported by the fact that all other studied strains that were derived from recently collected population samples do possess the *gypsy* element.

The *412* element is a retrotransposon, 7.6 kb in length, with 481-pb-long LTRs [27]. This element, similar to other retrotransposons, has apparently been inhabiting the genome of drosophilids for a very long period of time. Vieira and Biémont [24, 25] determined, by in situ hybridization to polytene chromosomes, the number of copies of the *412* element occurring in natural populations of *D. simulans*. A gradient in copy number was observed, varying from 20 in Europe, to 3–9 in Africa, with the same tendencies between North and South America. Some transposition bursts were observed within certain local populations that possess a high copy number of *412*. These authors suggest this element has recently invaded the genome of *D. simulans* populations.

In the present study we observed that all the studied populations carried copies of an apparently complete *412* element, and that the *Eco*RI and *Xho*I sites were conserved in relation to the *D. melanogaster 412* element. The occurrence of a few bands that were characteristic of each strain may suggest that this element is active in South American *D. simulans* populations.

## ACKNOWLEDGEMENTS

## REFERENCES

[1] Berg D.E., Howe M.M., Mobile DNA, Am. Soc. Microbiol, Washington, DC, 1989.

[2] Biémont C., Lemeunier F., Garcia Guerreiro M.P., Brookfield J.F., Gautier C., Aulard S., Pisyukova E.G., Population dynamics of the copia, mdg1, mdg3, gypsy and P transposable elements in a natural population of *Drosophila melanogaster*, Genet. Res. 63 (1994) 197–212.

[3] Black D.M., Jackson M.S., Kidwell M.G., Dover G.A., KP elements repress P-induced hybrid dysgenesis in *D. melanogaster*, EMBO 6 (1987) 4125–4135.

[4] Blackman R.K., Macy M., Koehler D., Grimaila R., Gelbart, W.M., Identification of a fully-functional hobo transposable element and its use for germ-line transformation of Drosophila, EMBO 8 (1989) 211–217.

[5] Boussy I.A., Daniels S.B., Hobo transposable elements in Drosophila melanogaster and *D. simulans*, Genet. Res. 58 (1991) 27–34.

[6] Brookfield J.F.Y., Montgomery E., Langley C., Apparent absence of transposable elements related to the P elements of *D. melanogaster* in other species of *Drosophila*, Nature 310 (1984) 330–332.

[7] Bucheton A., The relationship between the flamenco gene and gypsy in *Drosophila*: how to tame a retrovirus, Trends Genet. 11 (1995) 349–353.

[8] Capy P, David J.R., Hartl D.L., Evolution of the transposable element mariner in the *Drosophila melanogaster* species group, Genetica 86 (1992) 37–46.

[9] Dorsett D., Viglianti G.A., Rutledge B.J., Meselson M., Alteration of hsp82 gene expression by the gypsy transposon and supressor genes in *D. melanogaster*, Genes Dev. 3 (1989) 454–468.

[10] Dowsett A.P., Young M.W., Differing levels of dispersed repetitive DNA among closely related species of *Drosophila*, Proc. Nat. Acad. Sci. USA 79 (1982) 4570–4574.

[11] Engels W.R., Hybrid dysgenesis in *Drosophila* and the stochastic loss hypothesis, Cold Spring Harbor Symp. Quant. Biol. 45 (1981) 561–565.

[12] Engels W.R., *P* element in *Drosophila melanogaster*, in: Berg D.E., Howe M.M., (Eds.), Mobile DNA, Am. Soc. Microbiol., Washington, DC, 1989, pp. 437–484.

[13] Finnegan D.J., Transposable elements, in: Lindsley D.L., Zinn G.G. (Eds.), The Genome of *Drosophila melanogaster*, Acad. Press, San Diego, 1992, pp. 1096–1107.

[14] Giraud T., Capy P., Somatic activity of the mariner transposable element in natural population of *Drosophila simulans*, Proc. R. Soc. Lond. B 263 (1996) 1481–1486.

[15] Hartl D.L., Transposable element mariner in *Drosophila* species, Berg D.E., Howe M.M. (Eds.), Mobile DNA Am. Soc. Microbiol., Washington, DC, 1989, pp. 531–536.

[16] Jowett T., Preparation of nucleic acids, in: Roberts D.B. (Ed.), *Drosophila: a Practical Approach*, IRL Press, Washington, DC, 1986, pp. 275–286.

[17] Leibovitch B.A., Glushkova E.G., Pasyukova E.G., Belyaeva E.S., Gvozdev V.A., Comparative analysis of retrotransposon localization and mobility in sibling species *Drosophila simulans* and *Drosophila melanogaster*, Genetika 28 (1992) 85–97.

[18] Maruyama K., Hartl D.L., Evolution of the transposable element mariner in *Drosophila* species, Genetics 128 (1991) 319–329.

[19] Medhora M., Maruyama K., Hartl D.L., Molecular and functional analysis of the mariner mutator element Mos 1 in *Drosophila*, Genetics 128 (1991) 311–318.

[20] Nuzhdin S.V., The distribution of transposable elements on X chromosomes from a natural population of *Drosophila simulans*, Genet. Res. 66 (1995) 159–166.

[21] Periquet G., Hamelin M.H., Bigot Y., Hu K., Presence of the deleted hobo element Th in Eurasian populations of *Drosophila melanogaster*, Genet. Sel. Evol. 21 (1989) 107–111.

[22] Vaury C, Bucheton A., Pélisson A., The b-heterochromatic sequences flanking the I elements are themselves defective transposable elements, Chromosoma 98 (1989) 215–224.

[23] Vieira C, Biémont C., Selection against transposable elements in *D. simulans* and *D. melanogaster*, Genet. Res. 68 (1996) 9–15.

[24] Vieira C, Biémont C., Geographical variation in insertion site number of retrotransposons 412 in *Drosophila simulans*, J. Mol. Evol. 42 (1996) 443–451.

[25] Vieira C, Biémont C., Transposition rate of the 412 retrotransposable element is independent of copy number in natural populations of *Drosophila simulans*, Mol. Biol. Evol. 14 (1997) 185–188.

[26] Young M.W., Schwartz H.E., Nomadic gene families in *Drosophila*, Cold Spring Harbor Symp. Quant. Biol. 45 (1981) 629–640.

[27] Yuki L., Inouye S., Ishimaru S., Saigo K., Nucleotide sequence characterization of a *Drosophila* retrotransposon 412, Eur. J. Biochem. 158 (1986) 403–410.