Genetics and Molecular Biology, 29, 2, 384-390 (2006)

www.sbg.org.br

Research Article

Distribution and insertion numbers of transposable elements in species of the *Drosophila saltans* group

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Abstract

Information about the distribution and insertion numbers of many transposable elements is restricted to few species of *Drosophila*, although these elements are widely distributed throughout the genus. The aim of this work was to describe the distribution and insertion numbers of four retrotransposons (*copia*, *gypsy*, *micropia*, *I*) and four transposons (*hobo*, *mariner*, *Minos* and *Bari-1*) in the *saltans* group of *Drosophila.* Our data shows that, except for *mariner*, all the other elements are widespread within the *saltans* group and show variable insertion numbers of up to 24 copies.

Key words: Drosophila, genomic insertion, *saltans* group, retrotransposons, transposons.

Received: March 11, 2005; Accepted: September 20, 2005.

Introduction

Transposable elements are a major component of the genomes of most species and are widespread throughout the genus *Drosophila.* Most of our knowledge on transposable elements in *Drosophila* comes from studies carried out in natural populations of *Drosophila melanogaster* that provided information about the dynamics of transposable elements and the forces that maintain them in genomes and populations (*e*.g., Montgomery *et al*., 1987; Charlesworth and Langley, 1989; Charlesworth *et al*., 1992a,b; Eanes *et al*., 1992; Biémont *et al*., 1994; Sniegowski and Charlesworth, 1994; Nuzhdin and Mackay, 1994; Pimpinelli *et al*., 1995; Hoogland and Biémont, 1996; Nuzhdin *et al*., 1997; Charlesworth *et al*., 1997; Vieira *et al*., 1998; Junakovic *et al*., 1998; Maside *et al*., 2001; Ruiz and Carareto, 2003). Although it is well established that transposable elements have played a major role in evolution and may still be useful in maintaining the genetic variability of natural populations, the nature of the evolutionary forces that control their abundance are yet poorly understood. However, the sequence of *D. melanogaster* released by the *Drosophila* Genome Project (Celniker *et al*., 2003 Release 3) introduced a new perspective to understand the nature, number and location of the *D. melanogaster* transposable elements.

In brief, the published data have shown that the *Drosophila* euchromatic genome seems to be composed of a mixture of active and of ancient relic transposable elements, that their distribution along the chromosome results from natural selection and that the long terminal repeat (LTR) and non-LTR retrotransposons possess fewer divergent elements than transposons (Lerat *et al*., 2003). The elements are grouped into 96 families and can occur as a single copy or as many as 146 copies, with more than two-thirds of sequences being only partial (Kaminker *et al*., 2002). The data also show that transposable elements are not randomly distributed along the chromosomes but seem to be associated with reduced recombination rates (Bartolomé *et al*., 2002; Rizzon *et al*., 2002), however, this relationship depends on specific characteristics of the chromosomes, the transposable elements themselves and the species (Rizzon *et al*., 2002). These studies shed some light on the nature of the mechanisms involved in the control of transposable element abundance. However, comparative studies using species other than *D. melanogaster* are needed for a broad understanding of the evolutionary dynamics of *Drosophila* transposable elements.

Although a significant amount of data referring to transposable elements in *D. melanogaster* and other *Drosophila* species is available, much information is still missing about the occurrence and genomic distribution of many transposable elements in many *Drosophila* species. Two analyses (Martin *et al*., 1983; Stacey *et al*., 1986) and one review (Biémont and Cizeron, 1999) summarize the main knowledge accumulated so far regarding transposable elements in *Drosophila*. The first two studies concerned the elements *copia*, *412* and *297* in 32 species (Martin *et al*., 1983) and the elements *P*, *I*, *gypsy*, *copia* and *F* in 34 spe-

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cies (Stacey *et al.*, 1986), while the review includes data of individual studies on 228 *Drosophila* species and 43 transposable elements including *copia*, *gypsy*, *I* and *P* elements. These analyses showed that many transposable elements are present in many *Drosophila* species, but there are some major differences among them. For example, *copia*, *412* and *gypsy* have been detected in almost all species (Martin *et al*, 1983; Stacey *et al*., 1986), while the *I* retrotransposon is restricted to the *melanogaster* subgroup (de Frutos *et al*., 1992). In the *saltans* group, for example, only the *P* element has been studied in species of the five subgroups.

For *Drosophila* species in which the complete genomic DNA sequence is not available sampling of laboratory and natural strains for transposable elements by *in situ* hybridization and Southern blot analyses is still the best way to characterize their transposable elements and understand the evolutionary dynamics of these elements. The aim of our research was to provide more information on transposable element distribution and copy number in the *Drosophila saltans* group by describing the distribution and copy number of the *copia*, *gypsy*, *micropia* and *I* retrotransposons (class I elements) and the *hobo*, *mariner*, *Minos* and *Bari-1* transposons (class II elements) in members of the *Drosophila saltans* group of fruitflys.

Materials and Methods

Fruitfly stocks

All the *Drosophila* species and strains used in the present study are listed in Table 1 and were derived from a single, randomly selected female from a mass culture. Positive controls were *D. melanogaster* (Harwich strain) and *Drosophila mauritiana* (Tucson Stock Center: 14021-0241.1).

PCR and Southern blot analyses

For each *Drosophila* strain, total genomic DNA was prepared from 25-30 adult flies according to the method of Jowett (1986) and PCR reactions performed in 25 mL volumes using approximately 200 ng of template DNA, 100 mM of each primer, 200 mM of dNTPs, 1.5 mM of $MgCl_2$, 5% (v/v) of DMSO and 1 unit of Taq DNA Polymerase (GIBCO-BRL) in 1x Polymerase buffer. For amplification, we used an initial denaturation step of 5 min at 94 °C and an additional extension step of 10 min at 72 °C after the last cycle were performed. The amplification parameters varied as follows depending on the element: *Minos* = 29 cycles consisting of 1 min denaturation at 94 °C, 1 min annealing at 54 °C and 1 min extension at 72 °C; *micropia* = 40 cycles consisting of 1 min denaturation at 95 °C, 1 min annealing at 52 °C and 2 min extension at 72 °C; *Bari-1* = 35 cycles consisting of 1 min denaturation at 95 °C, 1 min annealing at 60 °C and 5 min extension at 72 °C; *gypsy* = 40 cycles consisting of 30 s denaturation at 94 °C, 30 s annealing at 55 °C and 30 s extension at 72 °C; *hobo*, *I* and *copia* = 30 cycles consisting of 30 s denaturation at 95 °C, 30 s annealing at 58 °C and 1 min extension at 72 °C. Despite trying various combinations of parameters *mariner* did not amplify by PCR.

To estimate the overall amount of each transposable element in the analyzed species using Southern blot, 10 mg of genomic DNA from each strain was digested with appropriate restriction enzymes (Table 2), submitted to electrophoresis on 0.8% (w/v) agarose gels and transferred to Hybond N+ nylon membranes (Amersham Biosciences).

Table 1 - Details of the *Drosophila saltans* species and strains used in this study.

Drosophila subgroup, species and strain	Location and year collected	Stock center number[1]
cordata		
D. neocordata NEO strain	Minas Gerais, Brazil 1959	(SC14041-0831.0)
elliptica		
D. emarginata EVC strain	Vera Cruz, Mexico 1962	(SC14042-0841.6)
parasaltans		
D. parasaltans PAT strain	Tapuruquara, Brazil 1962	Collected by H.Bicudo[2]
D. subsaltans SUB strain	Belém, Brazil 1959	(SC - 14044-0872.0)
sturtevanti		
D. milleri MEY strain	El Yunque, Puerto Rico 1962	(SC - 14043-0861.0)
D. dacunhai DAP strain	Pentionville, Haiti 1962	(SC - 14043-0854.0)
D. sturtevanti SMX strain	Matlapa, Mexico 1998	Collected by J.Silva[3]
saltans		
D. austrosaltans API strain	Pirassununga, Brazil 1959	(SC - 14045-0881.0)
D. saltans SAC strain	Chilpancingo, Mexico 1962	Collected by H. Bicudo[2]
D. prosaltans PTT strain	Sangre Grande, Trinidad Tobago 1962	Collected by H. Bicudo[2]

[1]Tucson Stock Center, University of Arizona, Tucson, AZ, USA. [2]UNESP-São José do Rio Preto, SP, Brazil. [3]University of Arizona, Tucson, AZ, USA.

Table 2 - Primers, probes and restriction enzymes used for the copy number detection of each transposable element in *saltans* group *Drosophila* species.

Element plasmid[1]	Primers	Annealing regions	Probe extension (bp)[2]	Restriction enzymes
copia	LTR-5'CTATTCAACCTACAA AAATAACG3'	33 to 56	439	Xho I
p77E4	PCS-5'ATTACGTTTAGCCTTGTCCAT3'	451 to 472		
gypsy	GM003-5'GTACTGAACATTATCAGAATC3'	2155 to 2176	542	Bam H1
pGGHS	GM004-5'TCTAAGGAGTCCTCTGCAAGG3'	2676 to 2697		
micropia	2813-5'TTAACTCCTAGAGTTCATCGCTGG3'	2813 to 2839	387	Bam HI
$dhMiF_2$	2814-5'CATGTACCTGGTTAACTACTGACC3'	3174 to 3198		
I	IF-5'CTCACACTCTGCTCTCCAAT3'	2178 to 2198	635	Eco RI
pI407	IR-5'TTGTGCGAATATGTTTAG CAA3'	2792 to 2813		
hobo	HA-5'CACCTCCAATTTATCCCGCC3'	651 to 671	963	Bam H1
pHX4	HB-5'GGATGGCAATACGAAGC3'	1597 to 1614		
mariner	MAR1-5'CCAGGTGTACAAGTAGG3'	1 to 15	800	Pst I
Mos1	MAR1286-5'GTATGAACATGTTGGACT3'	1286 to 1300	NheI/PvuII *	
Minos	M5-5'TATCGATAATTCACAATACAGCATG3'	1 to 26	1068	Xho I
pBCKSP	M3-5'ATCAAGCTTGAATTGTGTAACGTCGCC3'	1054 to 1068		
Bari-1	453-5'ATTCGTCGCAGGCTAAAAGA3'	453 to 1196	703	Eco RI
p28/47D	1196-5'TTGTAACACCACCTTTGGCA3'			

[1]Plasmid source: p77E4 and pHX4 = E. Loreto (UFSM, Santa Maria, RS, Brazil); pGGHS = D. Dorsett, Memorial Sloan-Kettering Cancer Center, USA; dhMiF2 = D.H Lankenau, University of Heidelberg, Heidelberg, Germany; pI407 = A. Bucheton, CNRS, Montpellier, France; Mos1 = D.L. Hartl, Harvard University, Cambridge, MA; pBCKSP = B. Arcà, University of Roma 'La Sapienza', Roma, Italy; and p28/47D = R. Caizzi, University of Bari, Bari, Italy. [2]TE sequences inserted into plasmids were derived from: *D. melanogaster* (p77E4, pGGHS, pI407, pHX4 and p28/47); *D. mauritiana* (Mos1); *D. mojavensis* (pBCKSP); and *D. hydei* ($dhMiF_2$). *Fragment.

The probes used were sequences amplified from plasmids containing sequences of each transposable element (Table 2). For hybridization and detection we used the chemioluminescent hybridization system Gene Images (Amersham Biosciences) at high stringency (58 °C - 60 °C) according to the manufacturer's instructions. Our analysis was based on the full transposable element sequences as described for *D. melanogaster* or the species from which a specific element was first sequenced (*copia:* X02599; *gypsy*: M12927*; micropia:* X13304; *I*: X78904; *hobo*: M69216, *mariner:* X78906; *Minos*: X61695 and *Bari-1*: X67681). In order to obtain a single band per element insertion, restriction enzymes that do not cut within the element sequence were selected for digestion of the genomic DNA (Table 2) so that different fragment lengths are assumed as product of variable genomic insertions.

Results and Discussion

The status of the *saltans* group species in relation to the presence of the retrotransposable elements *copia*, *gypsy*, *micropia*, *I* and the transposable elements *hobo*, *mariner*, *Minos* and *Bari-1* is presented in Table 3 where all the available data on these elements is presented along with the data produced in the study described in this paper. The distribution of sequences homologous to these elements showed strong to weak or no hybridization signals among the *saltans* group species (Figures 1 and 2). All analyses were repeated several times but some of the blots still showed unsatisfactory hybridization signals (*e.g.* the *I* and *mariner* elements whose blots are not shown in the figures), underlining the difficulty in obtaining good hybridizations using probes for *D. melanogaster* elements. However, PCR amplifications using specific primers and the blotting of amplified sequences with the primers and probes described in Table 2 ensured that the weak signals were not due to nonspecific hybridization. The *mariner* transposon produced no PCR amplification products.

Hybridization to the *micropia*, *copia*, *gypsy* and *I* retrotransposon homologous sequences were observed in all species (Figure 1). Although m*icropia* sequences have previously been described for the *saltans* group (Almeida *et al*., 2001; Lankenau, 1993) the copy number has not previously been estimated. Despite the weak signals for *copia* and *I* elements (obtained after two hours of exposure, as compared with 30 minutes for the other sequences), our results indicate a broad distribution of both these retrotransposons among species of the *saltans* group. The same difficulty regarding *copia* hybridization blots in *obscura* group species was encountered by de Frutos *et al*. (1992). Previous reports have provided conflicting data regarding the distribution of *copia* homologous sequences in other *Drosophila* species. Brookfield *et al.* (1984) suggested that *copia* sequences are restricted to *D. melanogaster* and related species but a broader distribution within the *Sophophora* subgenus was subsequently observed by Stacey *et al*. (1986), who also reported that *gypsy* is widely distributed among Sophophoran species including the drosophilids *D. emarginata*, *D. sturtevanti*, *D. austrosaltans* and *D. prosaltans* but not *D. neocordata.* Our results support the hypothesis regarding the wide distribution of the retrotransposon *gypsy* in drosophilids and extend its presence to representatives of the five subgroups of the

Table 3 - Distribution of transposable elements in species of the *saltans* group.

Drosophila subgroup and species	Presence (+) or absence (-) of the element as determined by Southern blotting															
	Retrotransposons								Transposons							
	copia		*gypsy*		*micropia*		*I*		*hobo*		*mariner*		*Minos*		*Bari-1*	
	A	*	B	*	C	*	D	*	E	*	F	*	G	*	H	*
cordata																
D. cordata	+		-				-									
D. neocordata		+	-	+	+	+	-	+	-	+	-	+		+	+	+
elliptica																
D. elliptica	+		+				-									
D. emarginata		+	+	+	+	+	-	+	-	+		+		+	+	+
D. neoelliptica									-							
parasaltans																
D. parasaltans		+		+	+	+		+		+		+		+		+
D. subsaltans		+		+	+	+		+		+		+		+	+	+
sturtevanti																
D. sturtevanti	+	+	+	+	+	+	+	+	-	+	-	-		+	+	+
D. milleri		+		+	+	+		+	-	+		+		+		+
D. dacunhai		+		+	+	+		+		+		-		+		+
saltans																
D. saltans	+	+	+	+	+	+		+	-	+	-	-	+	+	+	+
D. lusaltans									-						+	
D. prosaltans	+	+	+	+	+	+	-	+	-	+		-		+		+
D. austrosaltans	+	+	+	+	+	+	-	+	-	+		+		+		+

References: A = Jordan and McDonald (1998), Martin *et al*. (1983), Stacey *et al*. (1986); B = Stacey *et al*. (1986); C = Almeida *et al*. (2001), Lankenau (1993); D = Bucheton *et al*. (1986), Stacey *et al*. (1986); E = Daniels *et al*. (1990); F = Brunet *et al*. (1994), Capy *et al*. (1992), Maruyama and Hartl (1991); G = Arcà Band Savakis (2000); and H = Moschetti *et al*. (1998). *Data produced in this study using the Southern blotting method.

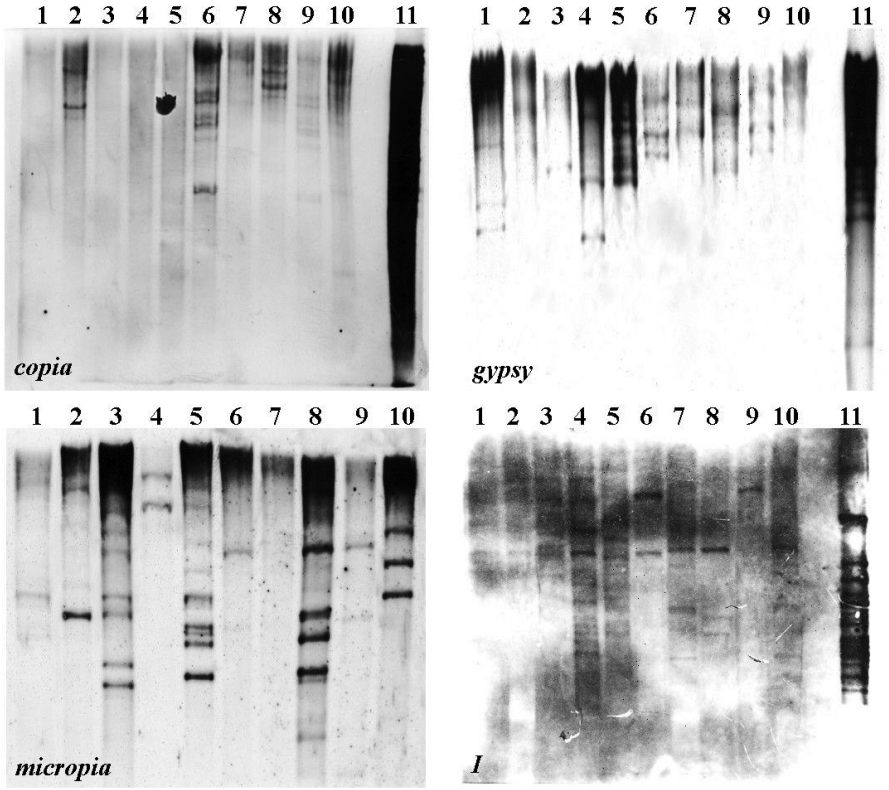

Figure 1 - Southern blot analysis of the retrotransposons *copia*, *gypsy*, *micropia* and *I* in *D. neocordata* (1); *D. emarginata* (2); *D. parasaltans* (3); *D. subsaltans* (4); *D. milleri* (5); *D. dacunhai* (6); *D. sturtevanti* (7); *D. austrosaltans* (8); *D. saltans* (9); *D. prosaltans* (10) and *D. melanogaster* (11).

Figure 2 - Southern blot analysis of the transposons *hobo*, *Minos* and *Bari-1* in *D. neocordata* (1); *D. emarginata* (2); *D. parasaltans* (3); *D. subsaltans* (4); *D. milleri* (5); *D. dacunhai* (6); *D. sturtevanti* (7); *D. austrosaltans* (8); *D. saltans* (9); *D. prosaltans* (10) and *D. melanogaster* (11).

saltans group, including *D. neocordata*. The fact that *gypsy* has been considered a retrovirus (Pélisson, 1994; Song *et al*., 1994) may explain its wide distribution. The wide distribution of the *I* element in the *saltans* group does not agree with the results reported by Stacey *et al.* (1986), who found *I* homologous sequences only in members of the *melanogaster* group, but partially agrees with Bucheton *et al.* (1986) who described a more widespread distribution (including *D. sturtevanti*) for this element. These inconsistent results could be accounted for by differences in the stringency conditions used by different investigators.

The distribution of transposons also conflicted with data previously reported by other investigators. With exception of *mariner*, for which only a few signals were observed by us in some species after the longer exposure time (three hours), we found that the other DNA elements were widely distributed in the *saltans* group (Figure 2). Daniels *et al.* (1990) carried out a wide distribution screening of *hobo* transposable elements in the genus *Drosophila* and found homologous sequences only in the *melanogaster* group, with Loreto *et al.* (1998) subsequently reporting the presence of *hobo* homologous sequences in *D. willistoni.* Our results extend the presence of *hobo* homologous sequences to the *saltans* group. Previous surveys have reported the absence of hybridization signals for *mariner* elements in the *saltans* group (Brunet *et al*., 1994) and outside the *melanogaster* species group (Maruyama and Hartl, 1991). For our part, we found no sequences homologous to *mariner* in the drosophilids *D. dacunhai*, *D. sturtevanti*, *D. saltans* and *D. prosaltans*, and only faint *mariner* hybridization signals in the other species investigated by us. However, this does not mean that this element is absent from these species but could simply reflect a high level of divergence between *mariner* elements in different species. This proposal is supported by the difficulty of hybridization between the *saltans* group sequences and the *D. mauritiana* probe. The *Minos* (Arcà and Savakis, 2000; Almeida and Carareto, 2005) and *Bari-1* (Moschetti *et al*., 1998) sequences have been reported in some species of the *saltans* group, and our study shows that these sequences are also present in representatives of the five subgroups of the *saltans* group.

For the retrotransposons studied by us the copy number varied between two for the *micropia* element in *D. dacunhai* and 24 for the *I* element in *Drosophila parasaltans*, while for transposons the copy number varied from two for the *Minos* element in *Drosophila milleri* and 15 for *Bari-1* in *D. parasaltans*. Except for the *I* element, the mean copy number was seven to nine different insertions per species (Table 4). However, the copy numbers were very variable among different species, probably due to drift due to the fact that the strains studied had been maintained in the laboratory for a long time.

For high copy number transposable element families Southern blotting, as compared to *in situ* hybridization, is known to underestimate the abundance of transposable elements, although for intermediate and low copy number families the abundance estimates produced by these two techniques show good agreement (Maside *et al*., 2001). Since our results showed intermediate and low copy numbers, we assumed that our methodology was appropriate for estimating the copy number of the *saltans* group transposable elements investigated. However, we should point out that the *saltans* species transposable element sequences homologous to most of the *D. melanogaster* elements studied did not, in general, show close sequence similarity to their homologues since the hybridization signals generated were not very strong, although it is still possible to hypothesize that all eight elements studied in this work were present in the ancestral *saltans* group.

Except for occasional examples of lateral transfer between species of the *saltans* and *repleta* groups (Almeida and Carareto, 2005) and based on the transposable element life cycle (Kaplan *et al*., 1985; Pinsker *et al*., 2001) it is to be expected that most of the transposable element se-

Table 4 - Number of insertions (copy number) of each transposable element found in *saltans* group species.

	Copy number						
	Retrotransposons				Transposons		
Saltans group *Drosophila* species	*copia*	*gypsy*	*micropia*	*I*	*hobo*	*Minos*	*Bari-1*
D. neocordata	3	10	7	10	8	5	10
D. emarginata	5	7	9	14	12	9	6
D. parasaltans	5	6	14	24	8	4	15
D. subsaltans	8	9	2	19	11	5	8
D. milleri	13	10	12	17	11	2	5
D. dacunhai	12	8	2	7	7	14	5
D. sturtevanti	6	9	4	15	8	5	7
D. austrosaltans	9	9	12	5	7	8	12
D. saltans	10	7	4	13	10	13	9
D. prosaltans	9	7	6	16	11	6	7
Mean number of insertions ± SD[1]	8.0 ± 3.2	8.2 ± 1.4	7.2 ± 4.4	14.0 ± 5.6	9.3 ± 1.9	7.1 ± 3.9	8.5 ± 3.2

[1]Standard deviation.

quences detected in our study are highly divergent compared to those found in *D. melanogaster*, since species from the *melanogaster* and *saltans*/*willistoni* groups, as well as species belonging to the subgenus *Drosophila* (*Drosophila hydei* and *Drosophila mojavensis*) and *Sophophora* (*saltans* species), are phylogenetically separated by about 40 million years (Russo *et al*., 1995).

Given the possibility of copy number underestimation by Southern blot and the high divergence between probes generated from transposable element sequences of species outside the *saltans* group our negative results should not be taken as definitive because it is known that the more divergent the sequence the more difficult is to detect using canonical sequences as a probe. Our study indicates the need for more complete information about the occurrence and molecular characteristics of transposable elements among different *Drosophila* species groups in order to understand the evolutionary history of these and other transposable elements.

Acknowledgements

This investigation was supported by the Brazilian agencies FAPESP (Grants n. 95/7192-2, 98/08734-1, 00/11313-0 and a fellowship to J.P.C.) and CNPq.

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Editor: Fábio de Melo Sene