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Research Article

Distribution of the *Bari-I* transposable element in stable hybrid strains between *Drosophila melanogaster* and *Drosophila simulans* and in Brazilian populations of these species

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Abstract

We analyzed the distribution of the *Bari-I* transposable element in *Drosophila melanogaster* (IN(1)AB), its sibling species *Drosophila simulans* (C167.4) and in eight hybrid strains derived from initial crosses involving *D. simulans* females and *D. melanogaster* males of the above cited strains as well as in Brazilian populations of these species. Polymerase chain reaction (PCR) data showed the presence of the *Bari-I* element among species populations and hybrid strains. Hybridization with a 703 bp probe homologous to the *Bari-I* sequence showed that the number of *Bari-I* copies in *D. melanogaster* IN(1)AB was higher than in *D. simulans* C167.4 strains. Hybrid strains presented *Bari-I* sequences related to both parental species. In addition some strains displayed a *Bari-I* sequence that came from *D. melanogaster*, suggesting introgression of *D. melanogaster* genetic material in the background of *D. simulans*. In contrast, some hybrids showed deletions of *D. simulans Bari-I* sequences.

Key words: Bari-I, Drosophila melanogaster, D. simulans, introgressive hybridization, transposable element.

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Introduction

The *Bari-I* element, initially found in *Drosophila melanogaster*, is a Class II transposable element with an open reading frame (ORF) able to encode for a polypeptide with 339 amino acids. The amino acids sequence of the putative protein in *Bari-I* is similar to the transposase of the *Tc-1* element of *Caernorhabditis elegans* (Rosenzweig *et al.*, 1983). While many heterochromatic elements exhibit rearrangements that result in a loss of coding capacity (O'Hare and Rubin, 1983; Di Nocera *et al.*, 1986; Streck *et al.*, 1986; Vaury *et al.*, 1989; Crozatier *et al.*, 1988;) the *Bari-I* element presents heterochromatic and euchromatic copies with very similar ORFs in structure and sequence. Moreover, all euchromatic copies of *Bari-I* are homogeneous in both size and sequence, suggesting that this element is in its active form (Caizzi *et al.*, 1993).

Studies regarding variability in the distribution of the *Bari-I* element conducted by Caggese *et al.* (1995) showed that this element occurs in *D. melanogaster* and *Drosophila simulans* populations. However, differences occur in the distribution patterns of the *Bari-I* element between these two sibling *Drosophila* species, the main differences being that *D. melanogaster* contains heterochromatic groups of the *Bari-I* element arranged in tandem on the second chromosome whereas in *D. simulans* there are just a few diffuse copies of this element spread throughout the genome.

Crosses between *D. melanogaster* and *D. simulans* occurs easily in laboratory (Sturtevant, 1920; Carracedo *et al.*, 1998) and in nature (Sperlich, 1962; Tracey *et al.*, 1973; Mensua and Perez, 1977; Kamping and Van Delden, 1988), but the offspring from these crosses frequently generate partially fertile offspring. Hybridization between these two species is the most studied system in the genus *Drosophila*, mainly in respect to the sterility observed in the hybrids the consequence of which is inhibited gene flow. Some studies have shown that crosses involving *D. simulans* females and *D. melanogaster* males can produce fertile descendants in some situations. For example, fertile hybrids females were observed in crosses between female *D. simulans* of strains C167.4, *Oxnard* and *vermilion* and male *D. melanogaster* of strains IN(1)AB or IN(1),f. (Davis *et al.*,1996), provid-

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ing a means to investigate the transference of DNA sequences between these two species.

We obtained stable hybrid strains from interspecific crossings between *D. simulans* C167.4 females and *D. melanogaster* IN(1)AB males. These strains contain sequences of the *D. melanogaster copia* retroelement introgressed into the genome of *D. simulans* (Ceron, Lisch and Kidwell, in preparation).

The study described in our present paper was undertaken to verify the presence and the distribution of the *Bari-I* element in *D. melanogaster* IN(1)AB and *D. simulans* C167.4 and analyze the distribution of this element in stable hybrids strains produced from crosses between these parental species. These analyses could help clarify the distribution of *Bari-I* in parental species as well as in hybrids. We also carried out a survey of the distribution of the *Bari-I* element in six *D. melanogaster* and five *D. simulans* populations collected at various locations throughout Brazil.

Material and Methods

Fruitfly stocks

The *D. melanogaster* IN(1)AB and *D. simulans* C167.4 strains used were derived from the stocks of Margaret G. Kidwell's laboratory at the University of Arizona, USA. Eight stable hybrid strains obtained from initial crossings between *D. simulans* females and *D. melanogaster* males of the above cited strains were also investigated. We cross-mated *D. simulans* C167.4 females with *D. melanogaster* IN(1)AB males, some parental females producing viable F_1 larvae which resulted in sterile males and fertile hybrid females. The hybrid females were individually retrocrossed with *D. simulans* males (crossing in the opposite direction were not viable) and just one produced fertile female F_2 offspring and, once again, sterile males. The F_2 females were retrocrossed once more with *D. simulans* males, this time producing F_3 fertile males and females, crosses between which produced all the hybrid strains used in this study. These strains were approximately in the 80^{th} generation from the initial parental crosses. We also used *D. melanogaster* and *D. simulans* strains of collected from several Brazilian localities (Table 1). Stocks were kept on banana-agar medium in the Institute of Biosciences, Letters and Exact Sciences, São Paulo State University (Instituto de Biociências, Letras e Ciências Exatas, *Universidade Estadual Paulista* (UNESP)), São José do Rio Preto, São Paulo, Brazil.

PCR and Southern blot analysis

We extracted DNA from about 50 flies using the chloroform phenol method (Jowett, 1986). The PCR reactions for amplification of the *Bari-I* sequences were performed in a final volume of 25 µL in 1X PCR buffer, containing 1 unit of *Taq* DNA polymerase (GIBCO), approximately 50 ng of DNA template, 5 pmol/µL of each primer, 800 µM dNTPs and 5 mM $MgCl_2$. Amplification used 35 reaction cycles, each cycle consisting of 1 min denaturation at 95 °C, 1 min annealing at 60 °C and 1 min elongation at 72 °C, followed by 5 min final extension at 72 °C. Two sets of oligonucleotide primers were designed, one amplifying the 1.7 kb complete sequence of the *Bari-I* element (sense 5'TTGTGAAAATACTTTTGCACACCT CTGT3', antisense 5'ACAGAGGTGGTCAAAAGTATT TTCACAA3') and another amplifying an internal 703 bp fragment complementary the positions 453 to 1196 of the

Table 1 - Brazilian *Drosophila* strains used in this study, geographic origin, latitude and year of collection.

Code	Geographic origin (town, state)	Latitude	Year collected	Collector, institution, state*
D. melanogaster				
TE	Teresina, Piauí (PI)	2°32' S	1996	Z.M. Silva, UEPI, PI
SL	São Luis, Maranhão (MA)	5°09' S	1995	S.R.P. Martins, UFMA, MA
SR	Santana do Riacho, Minas Gerais (MG)	18°20' S	1995	C.R. Vilela, USP, SP
NH	Novo Horizonte, São Paulo (SP)	21°29' S	1999	F.R. Torres, UNESP, SP
MA	Maringá, Paraná (PR)	23°25' S	1996	A.S. Lapenta, UEM, PR
SM	Santa Maria, Rio Grande do Sul (RS)	30°02' S	1995	V.L.S.V. Gaiesky, UFRGS, RS
D. simulans				
MN	Manaus, Amazonas (AM)	3°07' S	1997	I.R. Cabral, UFPA, PA
GO	Goiânia, Goiás (GO)	16°40' S	1988	D. C. Rigue, UNESP, SP
NH	Novo Horizonte, SP	21°29' S	1999	F. R. Torres, UNESP, SP
MA	Maringá, PR	23°25' S	1996	A.S. Lapenta, UEM, PR
BG	Bento Gonçalves, RS	29°10' S	1995	V.L.S.V. Gaiesky, UFRGS, RS

*All institutions were in Brazil. Key to abbreviations: UEPI = Universidade Estadual do Piauí; UFMA = Universidade Federal do Maranhão; USP = Universidade de São Paulo; UNESP = Universidade Estadual Paulista; UEM = Universidade Estadual de Maringá; UFRGS = Universidade Federal do Rio Grande do Sul ; UFPA = Universidade Federal do Pará.

Bari-I element (sense 5'ATTCGTCGCAGGCTAAAAG A3', antisense 5'TTGTAACACCACCTTTGGCA3').

To estimate the *Bari-I* copy number we submitted *Bari-I* genomic DNA to overnight digestion with the *Hind III* endonuclease (GIBCO), for which there is only one cleavage in *Bari-I* sequence. DNA fragments were separated by electrophoresis on 1% (w/v) agarose gels and transferred to nylon membranes (Hybond N+/Amersham-Pharmacia). Hybridization was performed using the ECL Direct Nucleic Acid Labeling and Detection Systems chemoluminescent hybridization system (Amersham Life Sciences) using 42 °C as the hybridization and washing temperatures. The probe used was the *D. melanogaster Bari-I* element 703 bp internal fragment cloned into the pUC8 plasmid (provided by R. Caizzi Bari University, Bari, Italy).

Results

Identification of the *Bari-I* element

The PCR analyses verified the presence of the *Bari-I* element in *D. melanogaster* IN(1)AB and *D. simulans* C167.4 using the primers described above which amplify the complete 1.7 kb sequence of the *Bari-I* element (Figure 1), but these primers produced only very faint amplified bands in the hybrids strains. However, the primers for the 703 bp *Bari-I* element internal fragment produced bands with better definition in all parental and hybrids strains (Figure 2).

The *Bari-I* copy numbers

The number of *Bari-I* copies in *D. melanogaster* IN(1)AB, *D. simulans* C167.4 and eight derived hybrid strains is shown in Figure 3. We can see that strain IN(1)AB presents the highest *Bari-I* copy number, as highlighting by the *in tandem* arrangement indicated by the thick 1.7 kb band, while strain C167.4 showed a smaller number of copies and an absence of the 1.7 kb band. These results are in agreement with those obtained by Caggese *et al.* (1995) regarding the distribution of the *Bari-I* element transposable in *D. melanogaster* and *D. simulans*. In our study, all hybrid strains showed some bands originating from one, or both, parental species. In addition, besides bands that were present in the parental *D. simulans* and *D. melanogaster* strains, the hybrid strain SM_2 displayed a band originally not found in either of the parent strains, while strains SM_3 and SM_4 showed a band originally found in *D. melanogaster* but not

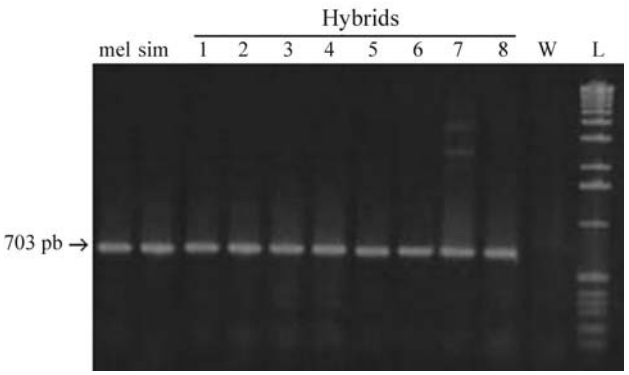

Figure 2 - The *Bari-I* transposable element PCR products corresponding to the internal segment with 703 bp in *D. simulans*, *D. melanogaster* and hybrid strains visualized in 1% agarose gel. Lanes are as follows: mel, *D. melanogaster* IN(1)AB; sim, *D. simulans* C167.4; 1-8: SM_1 to SM_8 hybrid strains; W, water; L, 1 kb DNA ladder size marker (GIBCO-BRL).

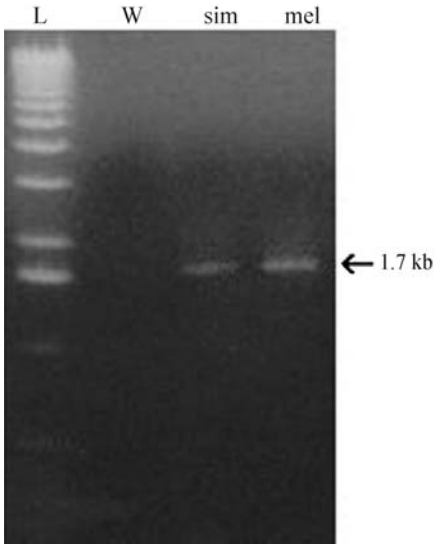

Figure 1 - The *Bari-I* transposable element PCR products corresponding to the complete sequence in *D. simulans* and *D. melanogaster* strains visualized in 1% agarose gel. Lanes are as follows: L, 1 kb DNA ladder size marker (GIBCO-BRL); W, water; sim, *D. simulans* C167.4; mel, *D. melanogaster* IN(1)AB.

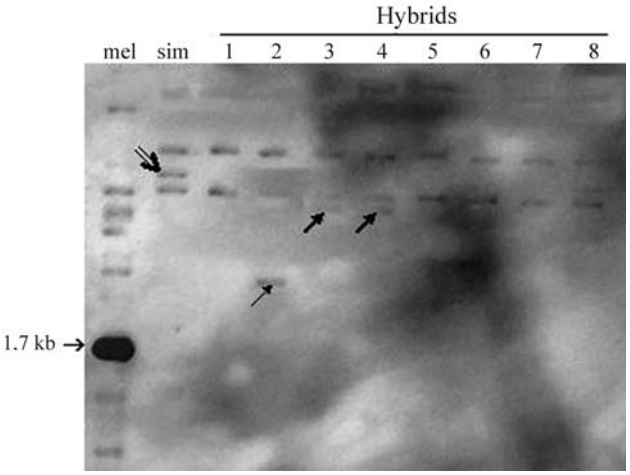

Figure 3 - Hybridization of 703 bp probe homologous to *Bari-I* internal sequence with genomic DNA of *Drosophila melanogaster*, *D. simulans* and hybrids strains after cleavage with *Hind III* restriction endonuclease. Lanes are as follows: mel, *D. melanogaster* IN(1)AB; sim, *D. simulans* C167.4; 1-8, SM_1 to SM_8, respectively. Thin arrow show band not found in *D. melanogaster* or *D. simulans* but present in SM_2 hybrid strain. Thicker arrows show bands in SM_3 and SM_4 hybrid strains and in *D. melanogaster* but no in *D. simulans*. Double arrow shows band found in *D. simulans* but absent in SM_1 to SM_7 hybrid strains.

in *D. simulans*. Furthermore, all the hybrid strains except SM_8 showed the loss of one band initially present in *D. simulans.*

For the Brazilian *D. melanogaster* and *D. simulans* strains different patterns occurred for both species (Figure 4). In general, the *Bari-I* copy number was higher in *D. melanogaster* strains than in *D. simulans* strains and, disconsidering the *in tandem* array, the *Bari-I* copy number for *D. melanogaster* strains varied between three for strain SM to nine for strain (NH), while the *Bari-I* copy for *D. simulans* varied from one in strains BG and NH to seven in strain MN. There was no association between the *Bari-I* copy number and geographic location for the *D. melanogaster* strains but for the *D. simulans* strains the MN and GO strains from northern Brazil had higher copy numbers than the other strains from further south. This suggests a possible relationship between copy number and geographic localization, although more strains need to be studied to allow any firm conclusions about such relationships.

Discussion

The analysis of Bari I element copy number in *D. melanogaster* and *D. simulans* Brazilian populations are in agreement with Caggese *et al.* (1995) that showed a higher *Bari-I* copy number and the occurrence of a tandem arrangement in *D. melanogaster* not presented in *D. simulans*. These results are probably related to the fact that the *D. melanogaster* genome has many more insertion sites for many transposable elements than the *D. simulans* genome (Bièmont and Cizeron, 1999).

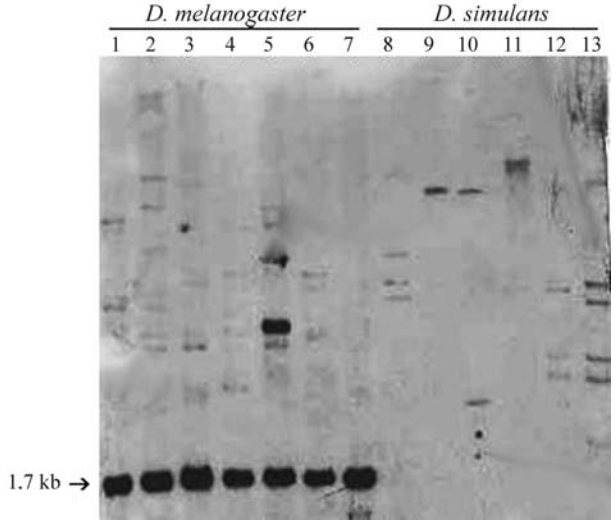

Figure 4 - Hybridization of 703 bp probe homologous to *Bari-I* internal sequence with genomic DNA of *Drosophila melanogaster* and *D. simulans* Brazilian populations after cleavage with *Hind III* restriction endonuclease. Lanes are as follows: 1-7: 1, *D. melanogaster* IN(1)AB; 2, TE (Teresina, PI); 3, SL (São Luís, MA); 4, SR (Santana do Riacho, MG); 5, NH (Novo Horizonte, SP); 6, MA (Maringá, PR); 7, SM (Santa Maria, RS); 8-13: *D. simulans*; 8, *D. simulans* C167.4; 9, BG (Bento Gonçalves, RS); 10, MA (Maringá, PR); 11, NH (Novo Horizonte, SP); 12, GO (Goiânia, GO); 13, MN (Manaus, AM).

One of the aims of our study was to investigate the possible transference of *Bari-I* sequences from *D. melanogaster* to the *D. simulans* genome that could have occurred during the establishment of the hybrid strains. In a previous study by one of the authors of the present paper the same hybrid strains were found to contain seven copies of the *D. melanogaster copia* retrotransposable element introgressed in *D. simulans* background genome (data not shown, unpublished results by C. R. Ceron). In the study described in the present paper, we observed introgression of *Bari-I* sequences, originally only found in *D. melanogaster*, into the hybrid strains, suggesting the concomitant introgression of these two TEs. In fact, the hybrid strains SM_3 and SM_4 presented *Bari-I* sequences from *D. melanogaster* (Figure 3, thick arrows), indicating that hybridization and introgression of the genetic material had occurred. Sawamura *et al.* (2000) also demonstrated introgression of genetic material carrying a complex of six sterility genes from the *D. simulans* into the *D. melanogaster* genome. Capy *et al.* (1998) has stated that although horizontal transfer is frequently invoked as a transfer mechanism, in the transfer of genetic material between sexually isolated species at least two mechanisms can operate: transfer by vectors and/or transfer by fertile hybrids retrocrossed with one of the parental species, our results supporting the latter mechanism.

As mentioned above, the *Bari-I* element has euchromatic and heterochromatic copies that are identical in size and sequence, which suggests that they are active elements in both portions of the genome (Caizzi *et al.*, 1993). Although it was not possible to determine conclusively whether the transference of *Bari-I* to the *D. simulans* genome occurred by transposition or recombination in the hybrids, our data provides some hints. For example, the bands in the SM_3 and SM_4 hybrids corresponding to those normally present only in *D. melanogaster* were probably the result of introgression whereas the bands in these hybrids that were not found in *D. simulans* or in *D. melanogaster* could be due to transposition (as observed in the SM2 hybrid) or ancestral polymorphism present in some strains of one or both species. We also observed that in some hybrid strains (SM_1 to SM_7) some of the copies that were initially presents in the *D. simulans* genome were deleted. In these cases, these sequences might have been deleted by recombination events or even by excision, although an alternative explanation is that only the DNA sequences of *D. melanogaster*, which did not harbor *Bari-I* insertions, were maintained within the hybrid genomes.

Our proposition of introgression of the *Bari-I* sequence from the *D. melanogaster* genome to the *D. simulans* genome is supported by work concerning the transposable elements common in *D. melanogaster* and the two related species *D. simulans* and *Drosophila yakuba* (Sanchez-Gracia *et al.*, 2005). In nine out of the eleven families studied by Sanchez-Gracia *et al.* (2005) the divergence

between *D. melanogaster* and *D. simulans* elements was much lower than that observed for nuclear genes, these workers properly interpreting this as an unexpectedly increased rate of horizontal transfer of transposable elements between these species and that variation of the divergence values across transposable element families suggest that the horizontal transfers might have occurred in multiple independent steps rather than being the result of a single episode of genomic admixture between two species. Even though we partially agree with Sanchez-Gracia *et al.* (2005), it is difficult to imagine that such an increased rate could be the result of horizontal transfer only. Our results suggest that hybridization may also have played a significant role in the evolutionary history of transposable elements in *D. melanogaster* and *D. simulans*.

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