RESEARCH ARTICLE

Transposon display supports transpositional activity of *P* elements in species of the *saltans* group of *Drosophila*

NATHALIA DE SETTA[1], ANA PAULA PIMENTEL COSTA[2], FABRÍCIO RAMON LOPES[1], MARIE-ANNE VAN SLUYS[2] and CLÁUDIA MÁRCIA APARECIDA CARARETO[1]*

[1]*Universidade Estadual Paulista, Departamento de Biologia, São José do Rio Preto, SP, Brazil*
[2]*Universidade de São Paulo, Departamento de Botânica, São Paulo, SP, Brazil*

Abstract

Mobilization of two *P* element subfamilies (canonical and O-type) from *Drosophila sturtevanti* and *D. saltans* was evaluated for copy number and transposition activity using the transposon display (TD) technique. Pairwise distances between strains regarding the insertion polymorphism profile were estimated. Amplification of the *P* element based on copy number estimates was highly variable among the strains (*D. sturtevanti*, canonical 20.11, O-type 9.00; *D. saltans*, canonical 16.4, O-type 12.60 insertions, on average). The larger values obtained by TD compared to our previous data by Southern blotting support the higher sensitivity of TD over Southern analysis for estimating transposable element copy numbers. The higher numbers of the canonical *P* element and the greater divergence in its distribution within the genome of *D. sturtevanti* (24.8%) compared to the O-type (16.7%), as well as the greater divergence in the distribution of the canonical *P* element, between the *D. sturtevanti* (24.8%) and the *D. saltans* (18.3%) strains, suggest that the canonical element occupies more sites within the *D. sturtevanti* genome, most probably due to recent transposition activity. These data corroborate the hypothesis that the O-type is the oldest subfamily of *P* elements in the *saltans* group and suggest that the canonical *P* element is or has been transpositionally active until more recently in *D. sturtevanti*.

[de Setta N., Costa A. P. P., Lopes F. R., Van Sluys M.-A. and Carareto C. M. A. 2007 Transposon display supports transpositional activity of *P* elements in species of the *saltans* group of *Drosophila*. *J. Genet.* **86**, 37–43]

Introduction

The *P* element family has been one of the most studied families of transposable elements (TE) in *Drosophila* (e.g. Daniels and Strausbaugh 1986; Daniels *et al*. 1990; Clark *et al*. 1995; Clark and Kidwell 1997; Haring *et al*. 1998, 2000; Silva and Kidwell 2000; Loreto *et al*. 2001; Castro and Carareto 2004a,b,c; Silva and Kidwell 2004). In the *saltans* group (subgenus *Sophophora*), the analyses have focussed on the intraspecific and interspecific distribution of *P* element sequences (Daniels and Strausbaugh 1986; Almeida *et al*. 2003; Castro and Carareto 2004a), evolution (Silva and Kidwell 2000; Castro and Carareto 2004a), and transcriptional (Castro and Carareto 2004b) and transpositional (Silva and Kidwell 2004) activity. However, comparative studies to discriminate the number of genomic insertions and the capacity of mobilization between the canonical and O-type subfamilies of *P* elements within this group are not conclusive.

The *P*-element family can be grouped into up to 25 subfamilies, according to the identity of their sequences (Hagemann *et al*. 1994, 1996a,b; Clark and Kidwell 1997; Nouaud *et al*. 2003; Sarkar *et al*. 2003; Oliveira de Carvalho *et al*. 2004). Some of them are well characterized, such as the canonical and O-type subfamilies, which, in spite of diverging in about 30% of their nucleotide sequences, have the same basic structure with terminal and subterminal inverted repeats, four exons and three introns. The canonical subfamily occurs in all species of the *saltans* group, except in the *cordata* and *elliptica* subgroups (Daniels *et al*. 1990; Clark *et al*. 1995; Silva and Kidwell 2000). The canonical *P* element has been shown to be transcriptionally active in *D. saltans, D.*

*For correspondence. E-mail: carareto@ibilce.unesp.br.

Keywords. transposon mobilization; canonical *P* element; O-type *P* element; transposon display; *Drosophila saltans*; *Drosophila sturtevanti*.

prosaltans and *D. sturtevanti* (Castro and Carareto 2004b), but no information is available about its capacity of mobilization. The O-type subfamily was first identified in *D. bifasciata* (Hagemann *et al*. 1994). In the *saltans* group, this subfamily has been identified in *D. subsaltans, D. lusaltans, D. prosaltans* and *D. austrosaltans* (Haring *et al*. 2000) and in *D. milleri, D. sturtevanti* and *D. saltans* (N. de Setta and C. M. A. Carareto, unpublished data). Haring *et al*. (2000) suggested that, because there are small deletions observed along their sequences, and mutations in the transcription initiation codons, these sequences are old and inactive components of the *saltans* group genomes.

Southern blot and *in situ* hybridization analyses of polytene chromosomes have usually been employed to evaluate TE copy numbers (Daniels and Strausbaugh 1986; Hagemann *et al*. 1992, 1994, 1996a; Almeida *et al*. 2003; Castro and Carareto 2004a). Transposon display (TD) is an alternative method for this type of analysis (Van den Broeck *et al*. 1998) that has proven to be a method of higher resolution for visualizing individual copies of TE. In short, it consists of digesting genomic DNA with an appropriate restriction endonuclease, ligating specific adapters at the extremities, and performing PCR, where one initiator anchors in the area of the adapter and the other, radioactively labelled, anchors in one of the extremities of the TE, 5′ or 3′ of the element. After PCR, the fragments produced are separated by polyacrylamide gel electrophoresis, and then visualized by autoradiography. The amplified fragments, which consist of the genomic sequences adjacent to the extremity of the TE plus part of the TE, vary in length according to the location of the nearest restriction site in the genomic DNA. Hence, the generated fingerprint indicates the occurrence of a specific copy inserted in a particular genomic site. One of the great advantages of this method is the use of specific primers, which allows detecting genomic insertions of different TE subfamilies that present low degrees of divergence and cannot be differentiated by Southern blot or *in situ* hybridization.

In this study, TD was used to evaluate the number of genomic insertions of the canonical and O-type *P* elements in strains of *D. saltans* and *D. sturtevanti* in an attempt to infer the history of dispersion of these TE in North, Central and South American populations. We further intended to demonstrate that this method is an advantageous alternative for evaluating the number of genomic insertions of TE in *Drosophila*.

Materials and methods

Strains

Nine strains of *D. sturtevanti* and five of *D. saltans* were analysed in this study (table 1).

Transposon display

Adapter and primer design: Total genomic DNA was isolated from pools of about 50 adult flies, as described by Jowett (1986). Two restriction endonucleases (*Pst*I and *Eco*RI) and

Table 1. Strains of *D. sturtevanti* and *D. saltans* used in this study, with geographic origins and dates of the collections.

Species	Strain	Location	Date
D. sturtevanti	Stu_Mexico	Matlapa, Mexico[1]	1998
	Stu_Costa Rica	Turrialba, Costa Rica (SC 14043-0871.00)	1960
	Stu_Colombia	Villavicencio, Colombia (SC H 193.3)	1960
	Stu_Brazil MG	Santana do Riacho (MG), Brazil[2]	1995
	Stu_Brazil RJ	Mangaratiba (RJ), Brazil[3]	1999
	Stu_Brazil SP1	Mirassol (SP), Brazil[4]	1971
	Stu_Brazil SP2	São José do Rio Preto (SP), Brazil[5]	1999
	Stu_Brazil RS	Maquiné (RS), Brazil[6]	1995
	Stu_Paraguay	Encarnación, Paraguay[5]	1999
D. saltans	Sal_Mexico1	Chilpancingo, Mexico[7]	1962
	Sal_Mexico2	Matlapa, Mexico[1]	1998
	Sal_Maxico3	Huichihuayan, Mexico[7]	1960
	Sal_El Salvador	San Salvador, El Salvador[7]	1960
	Sal_Guatemala	Guatemala, Guatemala[7]	1960

SC indicates Tucson *Drosophila* Stock Center ordering number. Collectors of other strains are: (1) J. C. Silva (University of Arizona, Tucson, USA), (2) C. R. Vilela (Universidade de São Paulo, São Paulo, SP, Brazil), (3) E. S. F. W Abdelhay (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil), (4) W. J. Tadei (Universidade Estadual Paulista, São José do Rio Preto, SP, Brazil), (5) L. M. Almeida (Universidade Estadual Paulista, São José do Rio Preto, SP, Brazil), (6) V. L. S. V. Gayeski (Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil), (7) H. E. M. C. Bicudo (Universidade Estadual Paulista, São José do Rio Preto, SP, Brazil).

primers that anneal to the 5′ and 3′ ends of the canonical and O-type *P* elements were tested. The best result was obtained with *Eco*RI (adapters 1: 5′-CTCGTAGACTGCGTACC-3′ and 2: 5′-AATTGGTACGCAGTCTAC-3′; primer E00: 5′-GTAGACTGCGTACCAATTC-3′) and with the primer that anneals to the 3′ end of the *P* element (C-IR3: 5′-AACAATCATATCGCTGTCTCACTC–3′ and O-IR3: 5′-TCAACAAGCACTTATTTTGCCATG-3′, respectively, for the canonical and the O-type *P* elements).

Template preparation and PCR conditions: TD was performed as described in Ellis *et al*. (1998), but with the following specifics: genomic DNAs (500 ng) were digested with *Eco*RI in a 25-μl volume of 1× RL buffer (10 mM Tris-acetate pH 7.5, 10 mM magnesium acetate, 50 mM potassium acetate, 5 mM DTT) and incubated at 37°C for 15 h. To this digest, 25 μl of a solution containing 0.2 mM ATP, 1 pM of *Eco*RI adapters 1 and 2, and 1 U T4 DNA ligase (Promega) in 1× RL buffer were added, and the final volume of 50 μl was incubated at 37°C for 4 h. Then the 50 μl volume of digested–ligated DNA was diluted by adding 150 μl of stabilization buffer (Tris-HCl 10 mM, EDTA 0.1 mM pH 8) and stored at −20°C.

PCR was performed with a total volume of 25 μl for each *P* element (O-type and canonical), using 5 μl of digested–ligated DNA, 50 ng *Eco*RI primer, 50 ng [γ-^{33}P]dATP-labelled *P* element primer C-IR3 or O-IR3, 0.24 mM dNTP, 3 mM $MgCl_2$, 1.5 U of *Taq* polymerase in 1× PCR buffer. The *P* element primers (50 ng each) were end-labelled with 0.5 U T4 DNA kinase and 1 μl of 1 μCi [γ-^{33}P]ATP in a final volume of 10 μl, and incubation for 1 h at 37°C. Kinase was inactivated by raising the reaction mixture temperature to 70°C for 15 min. The solutions were heated to 94°C for 5 min, followed by one cycle of 30 s at 94°C, a 30-s step at 65°C and a 2-min step at 72°C. The annealing temperature was lowered by 0.7°C at each cycle for 13 cycles, and the solutions were then kept at 56°C for another 25 cycles. After the last cycle, an additional extension step of 7 min at 72°C was performed. The PCR products were mixed with 20 μl of formamide dye (98% formamide, 0.005% bromophenol blue, 0.005% xylene cyanol, 10 mM EDTA) and stored at −20°C.

The amplified fragments were separated in 6% polyacrylamide gel (7 M urea, 0.1% TEMED, 0.05% ammonium persulphate in 1× TBE). Before loading 5 μl of the 50-μl total volume, the samples were heated for 5 min at 94°C and quickly cooled on ice to denature the DNA chains. The gel was run using 1× TBE at constant power, to give constant heat development (about 40–50 V cm^{-1}). After the migration the gel was vacuum dried at 80°C for 1 h, and standard X-ray film was exposed to the gel (usually for seven days) at room temperature, without the use of intensifying screens.

Statistics and data analysis: The fingerprints were analysed directly on the X-ray films for the presence (1) or absence (0) of a particular fragment, and a binary matrix was built for calculating *p* distances using PAUP v.4.0b10 software (Swofford 2002). Student's *t*-test was used to evaluate the differences between the average numbers of genomic insertions within species for each *P* element subfamily, as well as to evaluate the average pairwise distances (p) between species in the diversity of genomic insertions for each *P* element subfamily ($P < 0.01$).

Results

TD provides insertional profiling of a particular transposon to estimate the copy number and the polymorphism

Table 2. Numbers of genomic insertions observed for the O-type and the canonical *P* elements in *D. sturtevanti* and *D. saltans.*

Species	Strain	O-type	Canonical
D. sturtevanti	Stu_Mexico	18	35
	Stu_Costa Rica	17	24
	Stu_Colombia	13	19
	Stu_Brazil MG	3	13
	Stu_Brazil RJ	3	16
	Stu_Brazil SP1	12	24
	Stu_Brazil SP2	7	23
	Stu_Brazil RS	5	18
	Stu_Paraguay	3	9
Mean ± SE		9.00 ± 6.10	20.11 ± 7.56
D. saltans	Sal_Mexico1	4	16
	Sal_Mexico2	9	11
	Sal_Mexico3	7	16
	Sal_El Salvador	15	16
	Sal_Guatemala	28	23
Mean ± SE		12.60 ± 9.50	16.40 ± 4.28

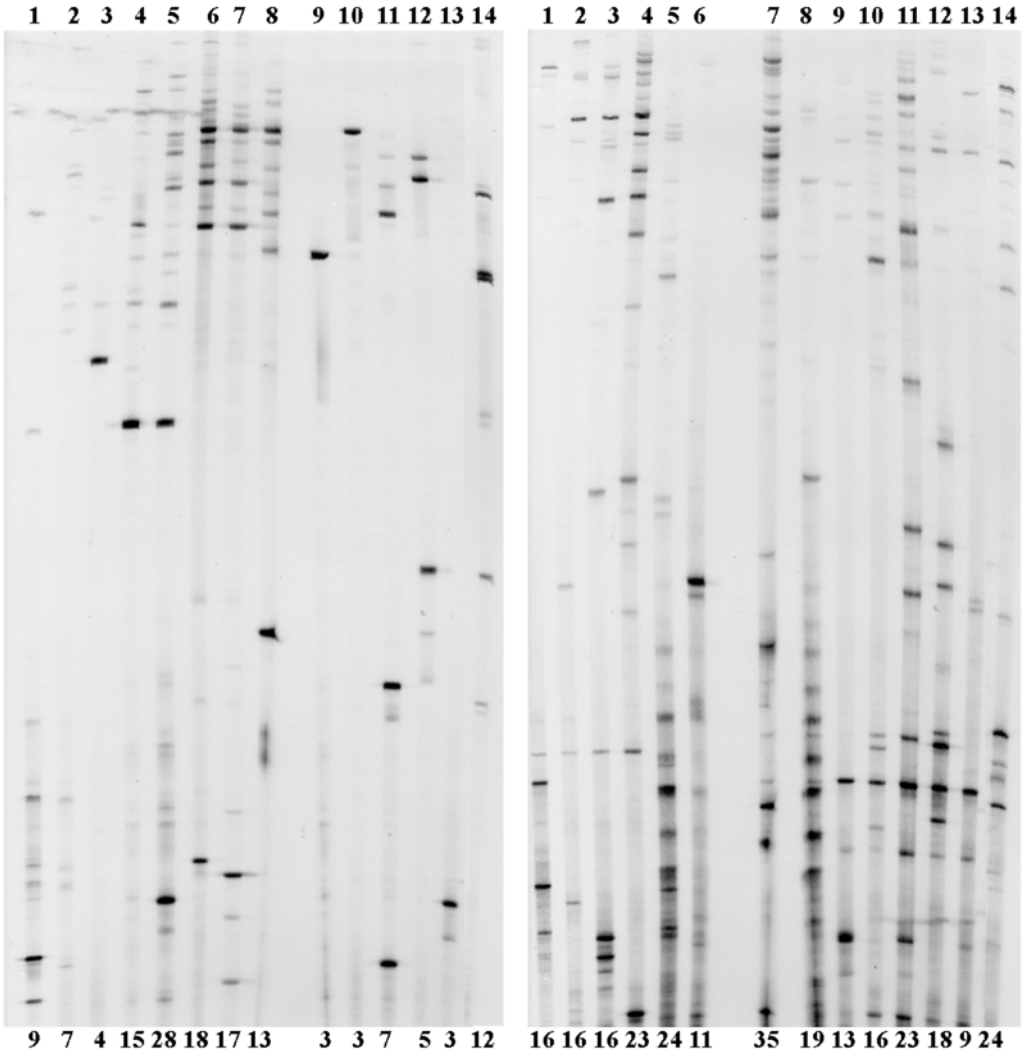

Figure 1. Transposon display exhibiting the number of genomic insertions of O-type (left) and canonical (right) *P* elements in geographically distinct strains of *D. saltans* and *D. sturtevanti*. The bands in the autoradiogram indicate copies inserted at a particular genomic site. Numbers at top indicate the analysed strain (O-type: 1, Sal_Mexico2; 2, Sal_Mexico3; 3, Sal_Mexico1; 4, Sal_El Salvador; 5, Sal_Guatemala; 6, Stu_Mexico; 7, Stu_Costa Rica; 8, Stu_Colombia; 9, Stu_Brazil MG; 10, Stu_Brazil RJ; 11, Stu_Brazil SP2; 12, Stu_Brazil RS; 13, Stu_Paraguay; 14, Stu_Brazil SP1. Canonical: 1, Sal_Mexico3; 2, Sal_Mexico1; 3, Sal_El Salvador; 4, Sal_Guatemala; 5, Stu_Costa Rica; 6, Sal_Mexico2; 7, Stu_Mexico; 8, Stu_Colombia; 9, Stu_Brazil MG; 10, Stu_Brazil RJ; 11, Stu_Brazil SP2; 12, Stu_Brazil RS; 13, Stu_Paraguay; 14, Stu_Brazil SP1). Numbers at bottom indicate the copy number of each strain.

associated with genomic insertions, based on the amplification of the DNA sequences adjacent to the insertion. The numbers of *P* element insertions of the canonical and O-type subfamilies were highly variable (table 2, figure 1). Comparison of the average copy numbers demonstrates that the canonical *P* element is significantly more abundant than the O-type in *D. sturtevanti* ($t = 2.93$; $P = 0.0036$), but not in *D. saltans* ($t = 4.33$; $P = 0.8550$).

Pairwise distances between the strains in the location of the genomic insertions of the canonical and the O-type *P* elements were estimated using the 0–1 matrix derived from the pattern of insertions in each strain of *D. sturtevanti* and *D. saltans*. The diversity among the populations (table 3) was used to infer the activity of the families, based on two kinds of comparisons: (i) between the average pairwise distances of the canonical and O-type subfamilies within the same species, and (ii) between the average pairwise distances between strains of each species for the same *P* element subfamily (table 4).

The first comparison was based on the argument that populations that possess lower pairwise distances must share several insertions of a certain element at the same place in their genomes. Sharing of genomic insertions suggests that these copies have been immobile since the geographical

Table 3. Pairwise distances between genomic insertions of O-type (above diagonal) and canonical (below) *P* elements in strains of *D. saltans* and *D. sturtevanti*.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Sal_Mexico1	-	0.112	0.092	0.306	0.173	0.204	0.194	0.173	0.071	0.071	0.133	0.112	0.092	0.071
2	Sal_Mexico2	0.159	-	0.061	0.316	0.204	0.276	0.244	0.224	0.122	0.122	0.224	0.143	0.122	0.102
3	Sal_Mexico3	0.188	0.174	-	0.296	0.184	0.255	0.224	0.184	0.102	0.102	0.204	0.122	0.122	0.102
4	Sal_Guatemala	0.196	0.167	0.239	-	0.255	0.388	0.398	0.337	0.276	0.296	0.337	0.316	0.316	0.316
5	Sal_El Salvador	0.174	0.159	0.159	0.210	-	0.316	0.327	0.224	0.163	0.184	0.224	0.204	0.184	0.184
6	Stu_Mexico	0.326	0.341	0.283	0.362	0.341	-	0.255	0.214	0.214	0.153	0.276	0.255	0.235	0.214
7	Stu_Costa Rica	0.246	0.188	0.188	0.239	0.232	0.384	-	0.245	0.184	0.163	0.265	0.245	0.224	0.204
8	Stu_Colombia	0.210	0.196	0.239	0.232	0.210	0.362	0.196	-	0.143	0.122	0.245	0.184	0.163	0.163
9	Stu_BrazilMG	0.145	0.116	0.174	0.210	0.116	0.297	0.188	0.181	-	0.061	0.163	0.102	0.082	0.061
10	Stu_BrazilRJ	0.196	0.167	0.210	0.246	0.167	0.275	0.254	0.217	0.152	-	0.163	0.102	0.082	0.061
11	Stu_BrazilSP1	0.239	0.210	0.225	0.246	0.225	0.362	0.268	0.261	0.225	0.232	-	0.184	0.163	0.163
12	Stu_BrazilSP2	0.246	0.203	0.246	0.268	0.246	0.312	0.319	0.297	0.217	0.254	0.297	-	0.102	0.102
13	Stu_BrazilRS	0.188	0.174	0.188	0.239	0.203	0.341	0.246	0.268	0.203	0.210	0.283	0.261	-	0.061
14	Stu_Paraguay	0.152	0.109	0.167	0.203	0.152	0.319	0.210	0.188	0.138	0.145	0.188	0.225	0.167	-

Table 4. Mean pairwise distances and standard deviations with regard to insertion sites of canonical and O-type elements in *D. sturtevanti* and *D. saltans*.

P element	*D. sturtevanti*	*D. saltans*	*t*
Canonical	0.248 ± 0.064	0.183 ± 0.026	4.88**
O-type	0.167 ± 0.066	0.200 ± 0.092	1.27
t	2.65**	3.14	

t, For differences between means; **, $P < 0.01$.

isolation of these populations, provided no excision occurred. Using the same rationale, strains that possess higher distance values must possess several copies inserted at different places within the genome, which might mean that these sequences remained transpositionally active, in at least one of the populations studied, after the divergence of the populations. The localization of the insertions in the nine strains of *D. sturtevanti* shows 16.7% polymorphism for the O-type and 24.8% polymorphism for the canonical *P* element ($t = 2.65; P < 0.0001$). On the other hand, the mean divergence (table 4) for the O-type insertions (20.0%) in *D. saltans* is not significantly different from that for the canonical elements (18.3%) ($t = 3.14; P = 0.9554$).

According to the second argument, if we compare the divergence of each element between species, and the means are not significantly different, we can assume that the element might have been submitted to equivalent evolutionary dynamics in both species; therefore, they might have been inactive since their last common ancestor. On the contrary, if the average distances are significantly different, those elements occupy more differentiated genomic sites; therefore, they must have remained active after the diversification of the species. The mean divergence (table 4) of the canonical *P* elements was significantly more polymorphic ($t = 4.88, P < 0.001$) between strains of *D. sturtevanti* (24.8%) than between those of *D. saltans* (18.3%). However, the difference between the pairwise distances of the O-type *P* elements of *D. sturtevanti* (16.7%) and *D. saltans* (20.0%) was not significant ($t = 1.27; P = 0.209$).

Discussion

The *P* element family presents a relatively low copy number in the *saltans* group of *Drosophila* compared to the number of canonical *P* elements in *D. melanogaster*. The number of genomic insertions, regardless of the subfamilies (canonical and noncanonical), evaluated by Southern blotting, within the genomes of 10 species of the *saltans* group varied from one copy each in *D. neocordata* and *D. emarginata* to 15 copies in *D. prosaltans* (Castro and Carareto 2004a). On the other hand, analyses by *in situ* hybridization in polytene chromosomes revealed the occurrence of only one, or at most two, euchromatic copies of O-type elements in *D. austrosaltans, D. lusaltans* and *D. prosaltans* (Haring *et al*. 2000).

Total genomic hybridization pattern by Southern blotting has been widely used to analyse the number of genomic insertions of TE, despite the fact that this method produces underestimates of the abundance of TE. This underestimation is mainly due to the low resolution of agarose gels which can result in overlapping of restriction fragments of similar sizes that contain different insertions, making it difficult to detect heterochromatic copies (Maside *et al*. 2001), and impossible to distinguish them in tandem insertions when the TE do not have restriction sites for the endonucleases used. Together, these factors lead to a pronounced underestimate of the TE

copy number in the host genome. Another technique—*in situ* hybridization of polytene chromosomes—is very efficient for evaluating the number of *Drosophila* TE inserted into euchromatin, but not very effective for evaluating the number and the location of TE within heterochromatin. This is an important limitation, since heterochromatin is one of the genomic regions with the greatest abundance of TE in *Drosophila* (Charlesworth *et al*. 1994; Bartolomé *et al*. 2002; Rizzon *et al*. 2002).

Considering these limitations, we used the TD method in an attempt to generate a fingerprint that would allow a more reliable estimation of the copy number of the two *P*-element subfamilies. The values obtained were significantly higher than those estimated by hybridization in previous evaluations done in our laboratory (Castro and Carareto 2004a). Using the same strains of *D. saltans* (Sal_Mexico1, Sal_Mexico2, Sal_Mexico3 and Sal_Guatemala), the average number of the canonical *P* element was 11 when estimated by hybridization (Castro and Carareto 2004a) and 16 by TD. Even larger was the difference observed in strains of *D. sturtevanti* (Stu_Mexico and Stu_Brazil MG), in which the average copy number of the canonical *P* element was also 11 when estimated by hybridization (Castro and Carareto 2004a) and as high as 24 when estimated by TD. Adding the canonical and the O-type *P* elements together, the average number of *P* elements is much greater than that observed in both species by hybridization, 35 in *D. sturtevanti* and 29 in *D. saltans*.

The variability among the populations studied here in copy number of *P* elements can be due to several factors, among which one of the most important is the transpositional dynamics of these elements during their active periods. Stochastic losses, originating from genetic drift in the original populations, or even more recently, given the fact that the analysed strains are maintained as small laboratory populations, might also have affected the variability. However, if this was the case, one would expect that in the longer-established strains (*D. saltans*: Sal_Guatemala, Sal_El Salvador, Sal_Mexico3; *D. sturtevanti*: Stu_Colombia, Stu_Costa Rica; see table 1) a reduced copy number would be observed. This was not the case, compared to those of the more recent strains (*D. sturtevanti*: Stu_Paraguay, Stu_Brazil SP2, Stu_Brazil RJ; see table 1). Another factor, extrinsic to the evolutionary dynamics of the sequences, which could have some influence on such variation, is related to the nature of the analysis itself. Although presenting the advantage of allowing the amplification of sequences of each *P* element subfamily by the use of specific primers, TD has a limitation too. If the restriction sites for the endonuclease used are not as close as about 2–3 kb from the TE insertion, that insertion cannot be detected, owing to the inability of *Taq* DNA polymerase to amplify longer fragments (Guimond *et al*. 2003). In an attempt to minimize this limitation, two endonucleases were tested, of which *Eco*RI produced the best fingerprint and was, therefore, the selected enzyme for further studies. Despite that limitation, the larger number of genomic insertions detected compared to our previous data suggests that TD is more sensitive in estimating the number of TE genomic insertions, as well as in highlighting the intrinsic polymorphism among populations.

Although sequences from canonical *P* elements have been shown to possess indels and stop codons that prevent their functioning (Silva and Kidwell 2004), the occurrence of complete elements and their capacity for undergoing transcription (Castro and Carareto 2004b) makes their immobilization in species of the *saltans* group a nondefinitive conclusion. In this work, we used pairwise distances to infer the relative status of mobilization of canonical and O-type elements in *D. sturtevanti* and *D. saltans*. The smaller numbers of the O-type sequences in comparison to the canonical subfamily, both in *D. sturtevanti* and in *D. saltans*, is expected for inactive elements, based on the life cycle of transposons proposed by Kaplan *et al*. (1985). According to these authors, the longer the period the element is harboured by a host genome, the smaller its copy number, due to longer periods of inactivation and degradation. Accordingly, if a given TE is represented by several fixed insertions in different populations, it can be assumed that it is inactive and has been an ancestral component of those genomes.

The distance analyses corroborate the hypothesis that the O-type subfamily is an ancestral character, and that the canonical subfamily is prone to mobilization or has undergone recent inactivation. First, the locations of the canonical *P* elements within the genome of *D. sturtevanti* are more variable (greater distances) than those of the O-type; this polymorphism can be generated by insertion or excision of a copy from a given site. Second, the significantly greater pairwise distances between the strains of *D. sturtevanti* (24.8%) than between those of *D. saltans* (18.3%) suggest that the canonical *P* elements occupy more differentiated genomic locations in the first species than in the second, and must therefore have remained transpositionally active in *D. sturtevanti* until more recently. This situation would be expected if the element had initiated its life cycle more recently in *D. sturtevanti* than in *D. saltans*. This possibility is supported by the hypothesis of horizontal transfer of canonical *P* elements from *D. saltans* to *D. sturtevanti* proposed by Castro and Carareto (2004b). On the other hand, the pairwise distances of the O-type elements between strains of *D. saltans* (20.0%) and of *D. sturtevanti* (16.7%) do not differ, suggesting that the positions of the O-type genomic insertions are equally variable in both species. This situation would be expected if the element had remained inactive since the last common ancestor and was thus subjected to equivalent evolutionary dynamics in both species. The inactivity of the O-type element in the *saltans* group is also indicated by the degradation of its sequences in *D. prosaltans* (Haring *et al*. 2000), and in *D. sturtevanti* and *D. saltans* (N. de Setta and C. M. A. Carareto, unpublished data). These results, together with the greater abundance of canonical *P* elements, suggest a possible transpositional activity, or at least a recent immo-

bilization, of the canonical subfamily in *D. sturtevanti*. The variable genomic localization of the canonical *P* elements in *D. sturtevanti* discussed here reinforces the observation of Silva and Kidwell (2004) that groups of canonical *P* elements could have developed separately in different populations of *D. sturtevanti* and that there could still exist pockets of transpositional activity in some of them.

Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Brazil) research grants nos. 03/12545-0 to C. M. A. C. and 04/02851-9 to M.A.V.S. F.R.L. was recipient of a FAPESP fellowship (03/12740-7).

References

Almeida L. M., Langeani F. and Cararetо C. M. A. 2003 Geographic polymorphism of *P* element populations of *Drosophila sturtevanti*. *Genet. Mol. Biol*. **26**, 175–179.

Bartolomé C., Maside X. and Charlesworth B. 2002 On the abundance and distribution of transposable elements in the genome of *Drosophila melanogaster*. *Mol. Biol. Evol*. **19**, 926–937.

Castro J. P. and Cararetо C. M. A. 2004a P elements in the *saltans* group of *Drosophila*: a new evaluation of their distribution and number of genomic insertion sites. *Mol. Phylogenet. Evol*. **32**, 383–387.

Castro J. P. and Cararetо C. M. A. 2004b Canonical *P* elements are transcriptionally active in the *saltans* group of *Drosophila. J. Mol. Evol*. **59**, 31–40.

Castro J. P. and Cararetо C. M. A. 2004c *Drosophila melanogaster P* transposable elements: mechanisms of transposition and regulation. *Genetica* **121**, 107–118.

Charlesworth B., Jarne P. and Assimacopoulos S. 1994 The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. III. Element abundances in heterochromatin. *Genet. Res*. **64**, 183–197.

Clark J. B. and Kidwell M. G. 1997 A phylogenetic perspective on *P* transposable element evolution in *Drosophila. Proc. Natl. Acad. Sci. USA* **94**, 11428–11433.

Clark J. B., Altheide T. K., Schlosser M. J. and Kidwell M. G. 1995 Molecular evolution of *P* transposable elements in the genus *Drosophila* I. The *saltans* and *willistoni* species groups. *Mol. Biol. Evol*. **12**, 902–913.

Daniels S. B. and Strausbaugh L. D. 1986 The distribution of *P* element sequences in *Drosophila*: the *willistoni* and *saltans* species groups. *J. Mol. Evol*. **23**, 138–148.

Daniels S. B., Peterson K. R., Strausbaugh L. D., Kidwell M. G. and Chovnick A. 1990 Evidence for horizontal transmission of the *P* element between *Drosophila* species. *Genetics* **124**, 339–355.

Ellis T. H., Poyser S. J., Knox M. R., Vershinin A. V. and Ambrose M. J. 1998 Polymorphism of insertion sites of *Ty1-copia* class retrotransposons and its use for linkage and diversity analysis in pea. *Mol. Gen. Genet*. **260**, 9–19.

Guimond N., Bideshi D. K., Pinkerton A. C., Atkinson P. W. and O'Brochta D. A. 2003 Patterns of *Hermes* transposition in *Drosophila melanogaster*. *Mol. Genet. Genomics* **268**, 779–790.

Hagemann S., Miller W. J. and Pinsker W. 1992 Identification of a complete *P* element in the genome of *Drosophila bifasciata*. *Nucleic Acids Res*. **20**, 409–413.

Hagemann S., Miller W. J. and Pinsker W. 1994 Two distinct *P* element subfamilies in the genome of *Drosophila bifasciata. Mol. Gen. Genet*. **244**, 168–175.

Hagemann S., Haring E. and Pinsker W. 1996a Repeated horizontal transfer of *P* transposons between *Scaptomyza pallida* and *Drosophila bifasciata. Genetica* **98**, 43–51.

Hagemann S., Haring E. and Pinsker W. 1996b A new *P* element subfamily from *Drosophila tristis, D. ambigua* and *D. obscura. Genome* **39**, 978–985.

Haring E., Hagemann S. and Pinsker W. 1998 Transcription and splicing patterns of *M*- and *O*-type *P* elements in *Drosophila bifasciata*, *D. helvetica*, and *Scaptomyza pallida. J. Mol. Evol*. **46**, 542–551.

Haring E., Hagemann S. and Pinsker W. 2000 Ancient and recent horizontal invasions of drosophilids by *P* elements. *J. Mol. Evol*. **51**, 577–586.

Jowett T. 1986 Preparation of nucleic acids. In *Drosophila: a practical approach* (ed. D. B. Roberts), pp. 275–286. IRL Press, Oxford.

Kaplan N., Darden T. and Langley C. H. 1985 Evolution and extinction of transposable elements in Mendelian populations. *Genetics* **109**, 459–480.

Loreto E. L., Valente V. L., Zaha A., Silva J. C. and Kidwell M. G. 2001 *Drosophila mediopunctata P* elements: a new example of horizontal transfer. *J. Hered*. **92**, 375–381.

Maside X., Bartolomé C., Assimacopoulos S. and Charlesworth B. 2001 Rates of movement and distribution of transposable elements in *Drosophila melanogaster*: *in situ* hybridization vs Southern blotting data. *Genet. Res*. **78**, 121–136.

Nouaud D., Quenesville H. and Anxolabéherè D. 2003 Recurrent exon shuffling between distant *P*-element families. *Mol. Biol. Evol*. **20**, 190–199.

Oliveira de Carvalho M., Silva J. C. and Loreto E. L. 2004 Analyses of *P*-like transposable element sequences from the genome of *Anopheles gambiae. Insect Mol. Biol*. **13**, 55–63.

Rizzon C., Marais G., Gouy M. and Biémont C. 2002 Recombination rate and the distribution of transposable elements in the *Drosophila melanogaster* genome. *Genome Res*. **12**, 400–407.

Sarkar A., Sengupta R., Krzywinski J., Wang X., Roth C. and Collins F. H. 2003 *P* elements are found in the genomes of nematoceran insects of the genus *Anopheles. Insect Biochem. Mol. Biol*. **33**, 381–387.

Silva J. C. and Kidwell M. G. 2000 Horizontal transfer and selection in the evolution of *P* elements. *Mol. Biol. Evol*. **17**, 1542–1557.

Silva J. C. and Kidwell M. G. 2004 Evolution of *P* elements in natural populations of *Drosophila willistoni* and *D. sturtevanti. Genetics* **168**, 1323–1335.

Swofford D. 2002 *Phylogenetic analysis using parsimony (* and other methods) version 4.0b10*. Smithsonian Institution, Washington, DC, and Sinauer, Sunderland.

Van den Broeck D., Maes T., Sauer M., Zethof J., De Keukeleire P., D'Hauw M. *et al*. 1998 Transposon display identifies individual transposable elements in high copy number lines. *Plant J*. **13**, 121–129.

Received 20 April 2006