Genetics and Molecular Biology, 26, 2, 175-179 (2003)

www.sbg.org.br

Research Article

Geographic polymorphism of *P* element in populations of *Drosophila sturtevanti*

Luciane M. de Almeida[1], Francisco Langeani[2] and Claudia M.A. Carareto[1]

[1]*Departamento de Biologia, Universidade Estadual Paulista (UNESP), São José do Rio Preto, SP, Brazil.*
[2]*Departamento de Zoologia e Botânica, Universidade Estadual Paulista (UNESP), São José do Rio Preto, SP, Brazil.*

Abstract

The aim of this report was to detect full-sized *P* element sequences in eight strains of *Drosophila sturtevanti* populations from distant geographic regions and to assess the structural geographic variation among *P* element sequences. PCR analysis confirmed the presence of a putative complete *P* element in all strains. Southern blot analysis indicated bands shared by all strains, and bands restricted to geographically related strains. Parsimony analysis corroborated the hybridization pattern that reflected the geographic relationships.

Key Words: canonical *P* elements, *Drosophila, P* element polymorphism*, saltans* group, transposable elements.

Received: August 9, 2002; accepted: December 4, 2003.

Introduction

The *P* element, one of the best studied transposable elements in eukaryotes, was first discovered in *Drosophila melanogaster* (Kidwell *et al.*, 1977; Bingham *et al.*, 1982) in which multiple copies per genome are typically present but only a few are autonomous. The complete canonical *P* element is 2.9 kb long and has 31 bp inverted terminal repeats, 11 bp inverted subterminal repeats and four ORFs that encode the transposase (O'Hare and Rubin, 1983). The *P* element structure, distribution of insertions, and transposition and regulatory mechanisms have been extensively studied in *D. melanogaster*, but only a few reports have addressed these characteristics in the *saltans* species group (Daniels and Strausbaugh, 1986; Daniels *et al.*, 1990; Clark *et al.*, 1995; Clark and Kidwell, 1997; Clark *et al.,* 1998; Silva and Kidwell, 2000). These studies have shown that *P* elements from the *saltans* and *willistoni* species groups form four subfamilies. Of these, the most prevalent is the canonical *P* element, which form a compact subfamily with a maximum sequence divergence of 10% (Clark and Kidwell, 1997). The *P* element subfamilies in the *saltans* and *willistoni* species groups have been described based mainly on partial sequences (Clark *et al.*, 1995; Clark and Kidwell, 1997; Haring *et al.*, 2000; Silva and Kidwell, 2000). There has been no assessment of whether the sequences from the different subfamilies are complete or defective, or whether strains of the same species are polymorphic in their *P* element structure.

Since *D. sturtevanti* (*sturtevanti* subgroup) is the most widespread species of the *saltans* group, it is ideal for investigating the existence of full-sized sequences and structural geographic variation in the *P* elements of this group. In this work, we analyzed eight strains of *D. sturtevanti* from different geographic regions (from Mexico to the extreme south of Brazil) in order to assess the geographic polymorphism in the transposable element of this species.

Send correspondence to Cláudia Márcia A. Carareto. Departamento de Biologia, Rua Cristovão Colombo, 2265, Jardim Nazareth, 15054-000, São José do Rio Preto, SP, Brazil. Phone: +55-17-2212382. E-mail: carareto@bio.ibilce.unesp.br.

Materials and Methods

Fly stocks

The *D. sturtevanti* strains used in this study were from (1) Apazapan, Veracruz (APA; 19°11' N 96°10' W), and Matlapa, San Luis Potosi, Veracruz (MAT; 22°10' N 101° W) in Mexico; both strains were collected in 1998 by J.C. Silva, University of Arizona, Tucson, USA; (2) Villavicencio (COL; 4°09' N 73°38' W) in Colombia, (H 193.3, The Genetics Foundation, University of Texas, Austin, Texas, USA); (3) Santana do Riacho, MG (I_{27}, 19° S 44° W), Mirassol, SP (BRA; 20°47' S 49°28' W), São José do Rio Preto, SP (RP_1 and RP_2; 20°50' S 49°20' W and 20°60' S 49°18' W, respectively) and Novo Horizonte, SP (NHO; 21°29' S 49°18' W), in Brazil. The BRA strain was established with flies collected in 1971 by W.J. Tadei

(UNESP, São José do Rio Preto, SP), I_{27} was established in 1995 by C.R. Vilela (USP, São Paulo, SP), RP_1 in 1997 by L.M. Almeida and RP_2 and NHO in 1998 by F.R. Torres (UNESP, São José do Rio Preto, SP). The Harwich-w strain of *D. melanogaster*, a strong P strain isolated by M.G. Kidwell (University of Arizona, Tucson, AZ, USA), was used as a positive control.

PCR amplification

Genomic DNA from each strain was amplified by PCR using the primer M-IR which anneals to the terminal repeats (nucleotides 14 to 31 and 2894 to 2877) of full-sized, and internally deleted copies of *P* elements (Haring *et al.*, 1995). The reaction mixture consisted of 200 ng of genomic DNA, 2 mM $MgCl_2$, 0.16 mM of each dNTP, 0.25 pmol of pM-IR/µL, 0.1 unit of Taq DNA polymerase and 1x buffer. Temperature cycling involved heating the solutions to 94 °C for 7 min, followed by 30 cycles of 94 °C for 45 s, 57 °C for 45 s, 72 °C for 1.5 min, and a final extension at 72 °C for 5 min.

Southern blot analysis

The *P* element insertion sites and the variation in the restriction fragment sizes were investigated using Southern blot analysis (Sambrook *et al.*, 1989). For both methods, a chemioluminescent hybridization system (ECL™ direct nucleic acid labelling and detection systems, Amersham Life Science) was used according to the manufacturer's instructions.

Total genomic DNA was isolated from pools of about 50 individuals, as described by Jowett (1986). Approximately 10 µg of DNA from each strain were digested with the appropriate restriction endonucleases (Figure 1). The DNA fragments were then electrophoresed, transferred to a nylon membrane, and fixed. The blot was hybridized with ECL hybridization buffer and the appropriate probe. The membrane was washed for 40 min in primary buffer (6 M urea, 0.4% SDS and 0.5 M xSSC) at 42 °C, and for 10 min in secondary buffer (2xSSC) at room temperature.

Restriction fragment lenght polymorphism (RFLP) and probes

To assess *P* element restriction fragment polymorphism, genomic DNA was digested with the endonucleases *Ava* II and *Acc* I and probed with the 896 bp *Pvu* II fragment of the *P* element extracted from pπ 25.1 by digesting the *D. melanogaster P* element sequence with *Pvu* II. Digestion of the canonical *P* element by *Ava* II generated three internal fragments of 478 bp, 544 bp and 1838 bp in length; whereas *Acc* I generated a single 2360 kb internal fragment that embraced 81% of the complete sequence. Figure 1 depicts the restriction sites for the enzymes used and the positions where the probes hybridized.

Evolutionary analysis

Relationships among the *P* element sequences of each strain based on the absence (0) or presence (1) of restriction fragments were inferred using the maximum parsimony method, as implemented in PAUP v.4.0b10 (Swofford, 1997). Maximum parsimony searches were done using the branch-and-bound algorithm. Bootstrap analyses were done using parsimony and consisted of 500 replicates with the branch-and-bound algorithm.

Results and Discussion

Identification of putative full-sized *P* elements

We analyzed strains of *D. sturtevanti* collected in Mexico (19°11' N 96°10' W), Colombia (4°09' N 73°38' W) and southeastern Brazil (19° S 44° W to 21°29' S

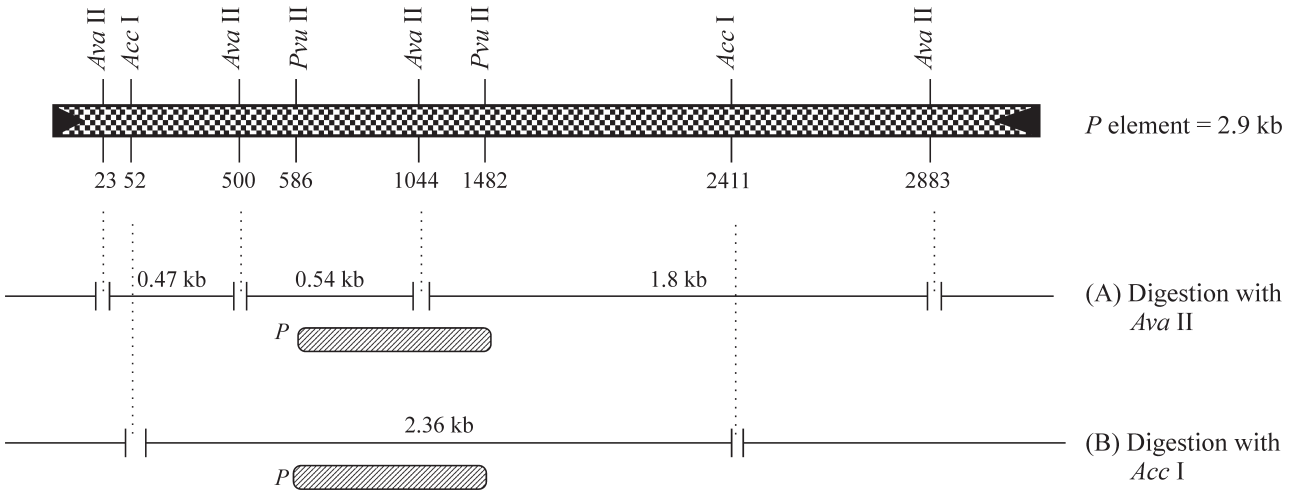

Figure 1 - Partial restriction site of the canonical *P* element with schematic representations of the restriction sites *Ava*II (A) and *Acc*I (B). The probe (*Pvu*II fragment of *P* element contained in pπ 25.1) used in RFLP analysis is also presented (P).

49°18' W) in order to assess the existence of full-sized *P* element sequences and to determine whether these sequences showed geographical variation based on their endonuclease restriction sites. The occurrence of an approximately 2.9 kb fragment amplified in all strains (apparently the same as amplified in the *P* element of the *D. melanogaster* positive control) revealed the presence of at least one potentially full-length element (Figure 2) and several smaller defective sequences. This complete element may belong to the canonical subfamily but could also be a more divergent sequence since our amplification conditions were not stringent. In addition to the 2.9 kb fragment, a slightly smaller fragment was also observed only in *D. sturtevanti*. This second sequence could belong to a *P* element subfamily different from the canonical one since pM-IR can amplify the complete *P* element sequence belonging to subfamily M or others such as subfamily T in species of the *obscura* group (Hagemann *et al*., 1996).

P element RFLP analysis

Two RFLP maps were used to evaluate the geographic variation of *P* element. The restriction enzyme *Ava* II cleaves a full-sized *P* element at four sites (positions 23, 500, 1044 and 2883) to generate internal fragments of 478 bp, 544 bp and 1838 bp. The absence of one of these fragments, or variation in their sizes, indicates internal deletions or the absence of restriction sites as a result of point mutations. Figure 3A shows the *Ava* II digests hybridized with the 0.9 kb *Pvu* II fragment carried by pπ 25.1. Since this probe hybridized with the 0.54 kb and 1.8 kb *Ava* II fragments, we expected to find these bands, or smaller ones, as a result of deletions. Both fragments were present in all *D. sturtevanti* strains and also in *D. melanogaster*-Harwich. All of the strains had fragments smaller than 1.8 kb, indicating internal deletions. Several of these bands were shared by all of the *D. sturtevanti* strains (black arrows), but some of them were restricted to Colombian and Mexican strains (dotted arrows), and another was restricted to Brazilian strains (hatched arrows). The bands larger than 1.8 kb represented polymorphisms caused by mutations in the first two *Ava* II restriction sites. Southern blotting also showed that all of the *D. sturtevanti* strains, but not *D. melanogaster*, had bands with a molecular weight > 1.8 kb.

Figure 3B shows the *Acc* I digest hybridized with the *Pvu* II fragment. *Acc* I cleaves the canonical *P* element close to its ends (positions 53 and 2412) to generate a single 2360 kb internal fragment that embraces 81% of the complete sequence. This blot allowed us to include in the analysis of polymorphism the initial sequence the of *P* element that did not hybridized in the experiments with *Ava* II (53 bp up to 500 bp). The *D. melanogaster*-Harwich positive control showed the expected 2.4 kb band (column 9) and smaller ones derived from defective elements. The Brazil-

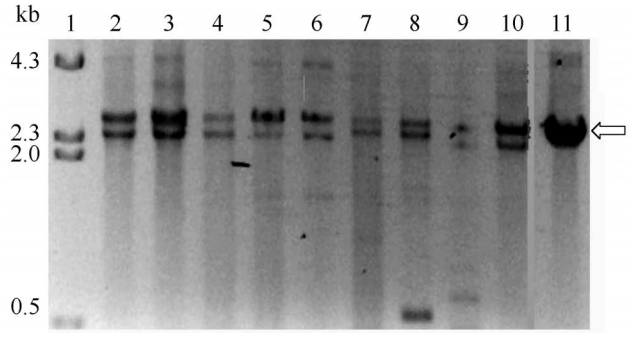

Figure 2 - PCR amplification of the 2.9 kb full-size sequence of the *P* element in *D. sturtevanti*. 1. λ *Hind* III DNA marker, 2. COL, 3. APA, 4. MAT, 5. I_{27}, 6. BRA, 7. RP_1, 8. NHO, 9. RP_2 and 10. pπ 25.1 (positive control), (⇦) 2.9 kb band.

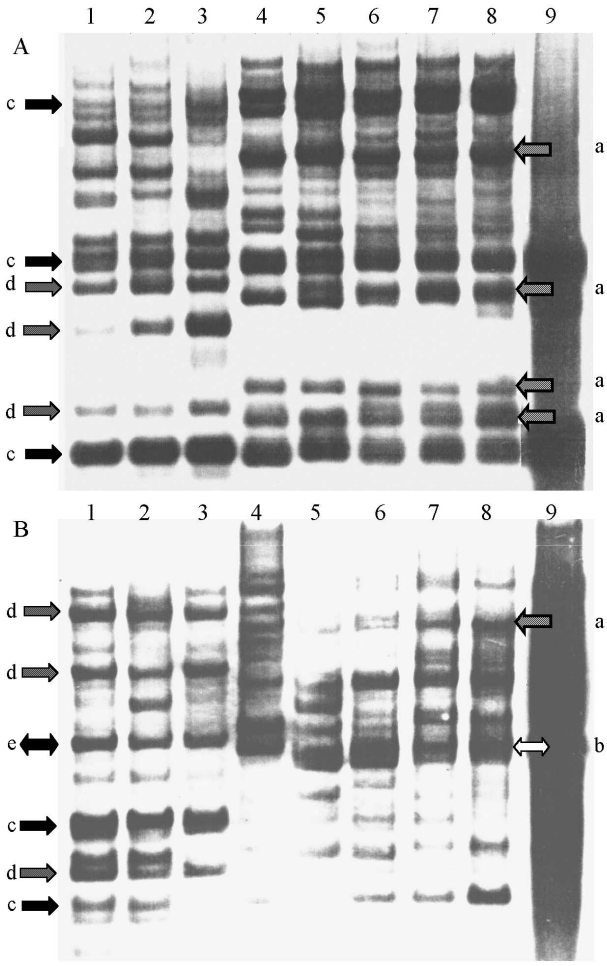

Figure 3 - Southern blot of *D. sturtevanti* genomic DNA digested with restriction endonucleases: **A**. Digested with *Ava*II and probed with the *Pvu*II fragment of the *P* element from pπ 25.1. **B**. Digested with *Acc*I and probed with the *Pvu*II fragment of the *P* element from pπ 25.1 (samples: 1- COL, 2- APA, 3- MAT, 4- I_{27}, 5- BRA, 6- RP_1, 7- NHO, 8- RP_2 and 9- *D. melanogaster* Harwich). Bands shared by all strains are marked by black arrows (c); those shared by Colombian and Mexican strains by dotted arrows (d) and those restricted to Brazilian strains by hatched arrows (a). The 2.4 kb band (b) shared by Brazilian strains was slightly larger than the 2.4 kb band (e) shared by Mexican and Colombian strains.

ian strains of *D. sturtevanti* showed bands corresponding to 2.4 kb (white double arrow); whereas, the Mexican and Colombian strains had a slightly larger band (black double arrow). In addition to the similarity between the Mexican-Colombian strains on the one hand and the Brazilian strains on the other, there were other fragments common to these groups as shown by the dotted arrows for the Mexican-Colombian group and the hatched arrows for the Brazilian group. As in the *Ava* II analysis, bands larger than 2.4 kb were present in all *D. sturtevanti* strains but not in *D. melanogaster*.

The PCR analysis and the *Ava* II and *Puv* II blots together indicate the presence of apparently full-sized *P* elements in *D. sturtevanti,* although divergent *P* element sequences were seen when the Brazilian strains were compared with the other three. RFLP analysis revealed a group of sequences in *D. sturtevanti* that were common to most of the strains and another group restricted to the most geographically related strains. The fragments common to all strains (black arrows in Figure 3A and B) may represent sequences that have been inactive since the dispersion of *D. sturtevanti* in the Americas and have not undergone mutation at the restriction sites for the enzymes used in this study. The sequences common to the Colombian and Mexican strains (dotted arrows) and to the Brazilian (hatched arrows) were different and probably originated more recently in the ancestral populations of each group. The existence of different groups of sequences coexisting in the same genome reflects the presence of multiples *P* element subfamilies in the *saltans* species group and may have resulted from different horizontal transfer events (Silva and Kidwell, 2000).

Figure 4 summarizes the results of the parsimony analysis used to determine the relationships among the *P* element restriction fragments of each strain. Of 73 characters, 9 were constant, 14 were uninformative, and 50 were parsimony informative. All of the six equally parsimonious trees corroborated the hybridization pattern that reflected geographic relationships; *i.e.*, the presence of a group of sequences clearly associated with the Brazilian strains (100% of 500 bootstrap replicates). Although the tree shows different relationships among the Brazilian strains, bootstrap analysis indicated that the branches would collapse to create a polytomie if the maximum branch length were zero.

The lack of significant variation noted above was confirmed by the similar banding patterns of strains BRA, RP_1 and RP_2. Together with the bi-directionality of the low hybrid dysgenesis indices previously reported for this species (Almeida, 2000; Almeida and Carareto, 2002), this statistical homogeneity allows us to reinforce the suggestion of *P* element inactivity in *D. sturtevanti*. These findings also contribute to our knowledge of the structural differentiation and geographical variation in *P* elements of *D. sturtevanti*.

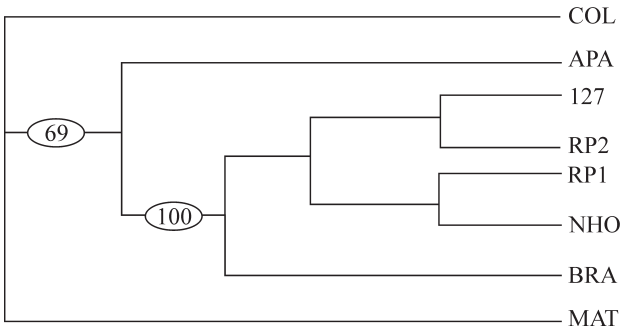

Figure 4 - Relationships estimated by parsimony analysis using the branch-and-bound method based on restriction maps of *P* elements in eight strains of *D. sturtevanti*. All characters were unordered and gaps were treated as missing data. This tree is one of the six most parsimonious reconstructions and required 99 steps. The consistency index is 0.6465 and the retention index is 0.6067. The numbers in the ovals indicate the percent of 500 bootstrap replications that contain the indicated clade. The strains are denoted by codes (APA - Apazapan and MAT - Matlapa, both from Mexico; COL - Villavicencio, Colombia, and I_{27} - Santana do Riacho, BRA- Mirassol, RP_1 and RP_2, São José do Rio Preto, and NHO - Novo Horizonte, Brazil.

Acknowledgments

The authors thank M.G. Kidwell, J.C. Silva, C.R. Vilela, W.J. Tadei and F.R. Torres for providing us with strains of *D. sturtevanti*. M.G. Kidwell, A.J. Holyoake and J.P. Castro provided helpful comments on an early version of the manuscript and S. Aranha edited the manuscript. This work was supported by FAPESP (Grant nos. 95/7192-2 and 98/08734-1 to C.M.A.C and 97/14646-5 to L.M.A) and CNPq.

References

Almeida LM (2000) Genomic distribution of *P* element in *Drosophila sturtevanti* populations. Genet Mol Biol 23:503-504.

Almeida LM and Carareto CMA (2002) Gonadal hybrid dysgenesis in D*rosophila sturtevanti* (Diptera, Drosophilidae). Iheringia, Série Zoologia 92:71-79.

Bingham PM, Kidwell MG and Rubin GM (1982) The molecular basis of the P-M hybrid dysgenesis: the role of *P* element, a *P* strain transposon family. Cell 29:995-1004.

Clark JB, Altheide TK, Schlosser MJ and Kidwell MG (1995) Molecular evolution of *P* transposable elements in genus *Drosophila* I. The *saltans* and *willistoni* species groups. Mol Biol Evol 12:902-913.

Clark JB and Kidwell MG (1997) A phylogenetic perspective on *P* transposable element evolution in *Drosophila.* Proc Natl Acad Sci USA 94:11428-11433.

Clark JB, Kim P and Kidwell MG (1998) Molecular evolution of *P* transposable elements in genus *Drosophila* III. The *melanogaster* species group. Mol Biol Evol 15:746-755.

Daniels SB and Strausbaugh LD (1986) The distribution of *P* element sequences in *Drosophila*: the *willistoni* and *saltans* species groups. J Mol Evol 23:138-148.

Daniels SB, Peterson KR, Straubaugh LD, Kidwell MG and Chovnick A (1990) Evidence for horizontal transmission of

the *P* transposable element between *Drosophila* species. Genetics 124:339-355.

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

Haring E, Hagemann S and Pinsker W (1995) Different evolutionary behaviour of *P* element subfamilies: M-type and O-type elements in *Drosophila bifasciata* and *D. imaii*. Gene 163:197-202.

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: Roberts DB (ed) *Drosophila*: a Pratical Approach. edited by D.B. Roberts, IRL Press, Oxford, pp 275-286.

Kidwell MG, Kidwell JF and Sved J (1977) Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86:813-833.

O'Hare K and Rubin GM (1983) Structure of *P* transposable element and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell 34:25-35.

Sambrook J, Fritsch EF and Maniatis T (1989) Molecular Cloning: a Laboratory Manual 2 ed, v.1, Cold Spring Harbor Laboratory Press, New York, 9.31-9.57 pp.

Silva JC and Kidwell MG (2000) Horizontal transfer and selection in the evolution of *P* elements. Mol Biol Evol 17:1542-1557.

Swofford D (1997). PAUP: Phylogenetic analysis using parsimony. Version 4.0b10. Smithsonian Institution, Washington, D.C.