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Research Article

Transposable elements *P* and *gypsy* in natural populations of *Drosophila willistoni*

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Abstract

The presence and integrity of the *P* transposon and the *gypsy* retrotransposon in the genome of 18 samples of natural *Drosophila willistoni* populations collected from a large area of South America were Southern blot screened using *Drosophila melanogaster* probes. The aim of this screening was provide further knowledge-base on the geographical distribution of *D. willistoni* and to carry out an inter-population analysis of the *P* and *gypsy* elements present in the genomes of the populations analyzed. The fragment patterns obtained indicate that both the *P* and *gypsy* elements are ancient in the *D. willistoni* genome, but whereas the *gypsy* retroelement appears to be invariable and stable the *P* element varies between populations and appears to still have some capacity for mobilization.

Key words: Drosophila willistoni, transposable element, *P*, *gypsy*.

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Introduction

The fruit fly *Drosophila* willistoni is widely distributed in the Neotropics, highly polymorphic for chromosomal inversions and possesses a high level of genetic variability expressed by several genetic markers. It is capable of exploiting very different environments for feeding and breeding throughout a wide geographical range stretching from Florida and Mexico in North America to northern Argentine in South America (Spassky *et al*., 1971). Because of this, *D. willistoni* and its sibling species has been the subject of many evolutionary studies and is considered an organism of choice for this type of research.

The transposable elements (TEs) *P* and *gypsy* are some of the best-known mobile sequences in *Drosophila melanogaster* and are widely represented in the repetitive portion of the *Drosophila* genome, appearing to be resident members of the genome of several species (Bayev *et al*., 1984; Daniels and Strausbaugh, 1986; Stacey *et al*., 1986; Daniels *et al*., 1990; Loreto *et al*., 1998a, b). These elements are members of two large groups of mobile sequences (Finnegan, 1989) represented by Class I retrotransposons such as *gypsy* and *412* which use an intermediary RNA molecule and a reverse transcriptase to transpose and Class II DNA transposons such as the *P* and *hobo* transposons which use a transposase to mobilize.

The *gypsy* element is a long 7.3 kb retrotransposon containing 0.5 kb of well-conserved long terminal repeats (LTRs) and is widely distributed in *Drosophila* and the subgenus *Sophophora* (Stacey *et al*., 1986; Loreto *et al*., 1998b), the retroviral properties of *gypsy* probably explaining this wide distribution (Bayev *et al*., 1984; Terzian *et al*., 2000; Vázquez-Manrique *et al*., 2000; Mejlumian *et al*., 2002; Pélisson *et al*., 2002; Heredia *et al*., 2004). The strong pattern similarity between *gypsy* strains found by Bayev *et al*. (1984) using Southern blotting suggests that this element invaded the *D. melanogaster* genome early in the evolutionary history of this species, the same appearing to be true for *Drosophila simulans* (Loreto *et al*., 1998b). However, little information is available about how *gypsy* element sequences evolved in the genomes of different *Drosophila* populations around the world.

The complete *P* element is 2.9 kb long and codifies two differentially spliced polypeptides (66 kDa and 89 kDa) in *D. melanogaster* (O'Hare and Rubin, 1983), differing in only one nucleotide over the entire element from the sequence in *D. willistoni* (Daniels *et al*., 1990). In addition to autonomous elements, there are smaller, non-

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autonomous *P* elements which are heterogeneous in size (ranging from about 500 to 2500 pb) and which are derived from autonomous elements by deletion of some internal sequences. The *P* element has been extensively studied because of its ability to promote a phenomenon known as hybrid dysgenesis (a syndrome of related traits including male recombination, gonad atrophy, mutations and chromosome aberrations) in the offspring of crosses in which males carrying *P* elements (termed P strains) are mated to females that lack autonomously mobile *P* elements (termed M strains) (Kidwell *et al*., 1977). We recently detected a similar phenomenon in *D. willistoni* characterized by gonad atrophy in the F_1 generation of crosses between certain *D. willistoni* strains (Regner *et al*., 1999), but the involvement of *P* elements as a causal agent was considered unlikely because both *D. willistoni* strains have *P* elements in their genomes (Regner *et al*., 1996). In fact, no *D. willistoni* strains have been recorded as being free of *P* elements (Lansman *et al*., 1985; Regner *et al*., 1998).

The study described in this paper was designed to contribute to knowledge of the dynamics of mobile sequences in different populations of a widely distributed species within a broad territory. Our study used Neotropical *D. willistoni* because the wide distribution of this drosophilid over very diverse environments means that different selective forces have been operating on the *D. willistoni* genome, our specific interest being to understanding the role of transposable elements as an intrinsic source of variability subject to natural selection. We choose the Class I *gypsy* retrotransposable and Class II *P* transposable elements because of their different mobilization mechanism. We also used *D. willistoni* as a model to ascertain the copy number of these mobile sequences in a complex host genome characterized by a wide repertoire of genetic variability (Ehrman and Powell, 1982). The objective of our work was to characterize the presence or absence and the fragment patterns of *P* and *gypsy* insertions generated by restriction enzymes in the genome of natural populations of *D. willistoni* using Southern blot assays.

Materials and Methods

Strains of *Drosophila willistoni*

We studied 18 *Drosophila willistoni* populations (Table 1) collected at various times from several areas (Figure 1) of its geographical distribution as defined by Spassky *et al*., (1971). The flies were maintained in the laboratory by mass crosses and reared on corn flour culture medium (Marques *et al*., 1966) at 17 ± 1 °C and 60% relative humidity.

DNA extraction, probes and Southern blotting

For each population, approximately 100 adult *D. willistoni* were macerated in liquid nitrogen in a 1.5 mL microcentrifuge tube and 750 µL of lysis buffer (Tris-HCl 0.1 M; EDTA 0.1 M; SDS 1% and NaCl 0.06 M) added to the homogenized mass which was then incubated for 30 min at 65 °C. After incubation, proteins and lipids were solvent-extracted by adding an equal volume of phenol (pH 8.0) and slowly agitating the mixture for 10 min before centrifugation at 9,447x g for 10 min, the aqueous layer being transferred to a new tube and extracted with an equal volume of chloroform:isoamyl alcohol (24:1 v/v), centrifuged as above and the supernatant extracted with chloroform. The DNA was precipitated from the aqueous phase by the addition of 20% (v/v) of 1M NaCl and two volumes of 100% ethanol followed by centrifugation at 9,447x g for 5 min, the aqueous phase being discarded and the pellet rinsed three times with 70% (v/v) aqueous ethanol, dried and resuspended in 30 µL of Tris-EDTA (TE). After extraction, the DNA was digested with RNAse and tested with a suitable restriction endonucleases to assess its quality before use.

Approximately 10 µg of DNA per sample were digested with the *Xho*I, *Ava*II and *Bam*HI restriction enzymes (Invitrogen). These enzymes were chosen because the fragment pattern they generate provides information on the presence of complete or deleted elements, probable copy

Table 1 - Geographical origin of the Drosophila *willistoni* populations investigated in the present study.

Population	Origin*	Year collected
Flo	Florida, USA[#]	-
Mex	Apazapan, Mexico	1997
Ecu	Jaton Sacha, Ecuador	1997
Man	Manaus, Amazonas state, Brazil	1986
Tra	Traquateva, Pará state, Brazil	1990
Par	Belém, Pará state, Brazil	1997
Wip	Ipitanga, Bahia state, Brazil	1965
Cip_1	Cipó Hill, Minas Gerais state, MG, Brazil	1995
Cip_2	Cipó Hill , Minas Gerais state, MG, Brazil	1996
Rib	Ribeirão Preto, São Paulo state, Brazil	1995
Mel	Island of Mel, Paraná state, Brazil	1994
Tab	Tabuleiro Hill, Santa Catarina state, Brazil	1997
Isc	Island of Santa Catarina, Santa Catarina state, Brazil	1997
Tur	Turvo Park, Rio Grande do Sul state, Brazil	1994
Dla	Dois Lajeados, Rio Grande do Sul state, Brazil	1995
Msa	Morro Santana, Rio Grande do Sul state, Brazil	1995
Pir	Piriápolis, Uruguay	1995
Cor	Coronilla, Uruguay	1995

*Most northerly location first.
[#]Bowling Green Center strain, collection date unknown.

Figure 1 - Map of the Americas indicating the original collection sites of the *Drosophila willistoni* populations (see also Table 1).

Figure 2 - Structure of the *P* canonic transposable element (A) and the *gypsy* retroelement (B) of *Drosophila melanogaster*. The numbers above each element refer to the sites recognized by the restriction enzymes used in this study.

number and the occurrence of restriction sites in the genomes of the flies based on the expected the restriction pattern the *D. melanogaster* elements. The probes used were the complete 2.9 kb sequence of the *D. melanogaster P* canonic element, contained in the pπ 25.1 plasmid (O'Hare and Rubin, 1983) and a 6.9 kb fragment liberated from the pGGHS plasmid by the *D. melanogaster gypsy* retroelement restriction enzyme *Xho*I (Dorsett *et al.*, 1989). For each sample, the DNA fragments were separated on 1% (w/v) agarose gel and transferred to a Hybond N^+ membrane (GE Healthcare) and hybridized to the random prime-labeled probes at 60 °C in a mixture containing 0.1% (w/v) SDS, 5% dextran sulfate and a 20-fold dilution of liquid block (Gene Image kit, GE Healthcare) in 5X SSC. After hybridization, the filters were washed at 60 °C by agitating for 15 min with 1X SSC followed by 0.1% SDS and then with 0.5X SSC followed by 1% SDS. Hybridized fragments were detection using the Gene Image CPD-Star kit (GE Healthcare) according to the manufacturer's instructions.

Results and Discussion

Digestion of the *D. melanogaster P* element with the *Ava*II restriction enzyme resulted in three fragments (1.8, 0.54 and 0.48 kb) (Figure 2A), the occurrence of these fragments being diagnostic for the presence of complete *P* element in the genome. The internal sequence recognized by the *Xho*I restriction enzyme is present only once in the canonical *P* element (Figure 2A), because of which the *P* element copy number can be estimated as half the number of Southern blot bands produced when the *P* element is cleaved with *Xho*I.

In the case of the *D. melanogaster gypsy* element (Figure 2B), the sequence recognized by the *Xho*I restriction enzyme only occurs in LTRs and a 6.9 kb fragment is generated when the *gypsy* element is cleaved by *Xho*I. Since the sequence recognized by *Bam*HI does not occur in the *D. melanogaster gypsy* element the number of Southern blot bands produced when *D. melanogaster* DNA is treated with *Bam*HI can be used to estimate the gypsy copy number. This reasoning can also be applied to studies of *D. willistoni*, assuming, of course, that the corresponding *D. willistoni* elements have the same restriction sites as those in *D. melanogaster*.

All of the *D. willistoni* populations studied produced three restriction sites after treatment with *Ava*II, showing that these populations contained complete *P* element sequences as shown in the banding patterns of the five populations given in Figure 3A. However, in two populations (Cor and Dla) the 0.48 kb fragment was very weak and was only visible in blots with higher exposure times (data not shown) and there were cases where we also observed several other bands that probably were either deleted copies or fragments of other *P* elements with divergent *Ava*II cleavage sites (Figure 3A). Other authors have found comparable results for *P* elements in other *D. willistoni* populations (Daniels *et al*., 1990; Regner *et al*., 1998) and no *D. willistoni* population yet investigated, including those in our study, have proved to be free of *P* elements, the population with the lowest *P* element copy number (one complete copy) as detected by *Ava*II digestion being from the southern Brazilian island *Ilha das Cobras* (Regner *et al.* 1998).

The *D. willistoni P* element *Xho*I banding pattern showed variation in the expected *D. melanogaster* 1.8 and 0.48 kb fragment pattern, as shown by the 10 populations given in Figure 3B. This variation may have been caused by the following: i) variation in the position of the *Xho*I restriction site within the element due to loss of the expected site and/or gain of new sites; ii) different copies of the *P* element may be located at different genomic sites in these populations, implying activation of the *P* element at some

moment during the diversification of these populations; iii) alterations in the host genomic DNA surrounding the *P* element sequence, leading to an alteration in the length of the fragments. Although we detected variation, we also found that some bands were shared by more than one population (Figure 3B). As outlined above, the *P* element copy number was estimated by considering two bands to equal one copy because since *Xho*I recognizes one internal site in the canonical *P* element sequence of *D. melanogaster* and genomic sites downstream and upstream the element. By analyzing blots with different exposure times (data not shown) we estimated that in our populations the *P* element copy number ranged from three to seven. This low copy number was less than that seen in *D. melanogaster*, and confirms the findings of various authors (Lansman *et al*., 1985; Daniels *et al*., 1986; Daniels *et al.*, 1990; Regner *et al*., 1998). Regner *et al*. (1998) screened 11 natural *D.* willistoni populations in an attempt to find strains without *P* elements, but none of them contained the M strains reported in *D. melanogaster*. The findings of Clark and Kidwell (1997) reporting the existence of different *P* element families in the *D. willistoni* genome support the idea that this element is an old resident of the genome of this fly, and that different *P* sequences are the result of its evolutionary diversification over time. Silva and Kidwell (2000) have determined that the canonical *P* subfamily invaded the species of the *saltans* and *willistoni* groups in several independent horizontal transfer events within the last three million years. However, the very high degree of sequence similarity among *D. willistoni P* elements, which contrasts sharply with the recent results from *D. sturtevanti*, suggests that the sampled *D. willistoni* canonical elements last shared a common ancestor much more recently than the time of diversification of the two species (Silva and Kidwell, 2004). These authors also emphasize that if a given sample is representative of all canonical *P* elements in *D. willistoni*, then this species might have been one of the last fruit fly species within the two New World Sophophora groups to be invaded by canonical *P* elements. Castro and Carareto (2004a,b) observed the presence of a highly homologous sequence in both *Drosophila prosaltans* and *Drosophila saltans*, suggesting that *P* elements might have been be present in the common ancestor of the *saltans* subgroup.

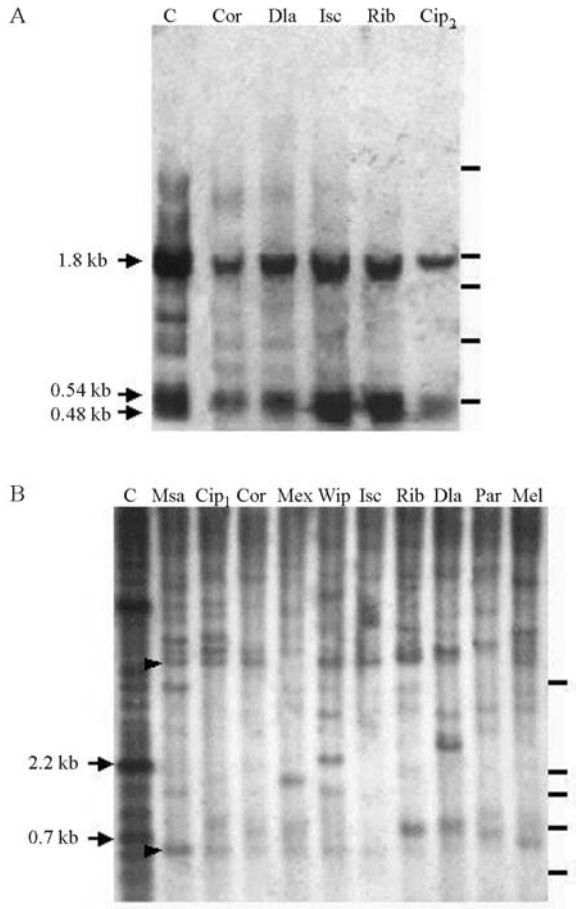

Figure 3 - Southern blot hybridization of genomic DNA from *Drosophila willistoni* strains probed with the complete sequence of *Drosophila melanogaster P* canonic element contained in the pπ 25.1 plasmid, lane C is *D. melanogaster* (Harwich strain). The Genomic DNA from the different *D. willistoni* populations was digested with either *Ava*II (Figure 3A) or *Xho*I (Figure 3B). Arrows indicate the fragments expected for *D. melanogaster*. Bars on the right represent the 1 kb Plus DNA Ladder (Invitrogen) fragments (5, 2, 1.65, 1 and 0.5 kb). Arrowheads indicate bands shared by more than one population.

A few other instances of horizontal transfer involving *P* elements have already been documented (Clark and Kidwell, 1997; Loreto *et al*., 1998a; Castro and Carareto, 2004a), suggesting that this phenomenon is present in other taxa carrying *P* elements.

Castro and Carareto (2004a) analyzed eight species of the *saltans* group and detected transposase mRNA in germline tissues of *D. prosaltans* and *D. saltans* and repressor mRNA in the somatic tissues of *D. saltans* and *Drosophila sturtevanti*. Sequencing analysis suggested that these transcripts might belong to the canonical subfamily and that they can be transpositionally active only in *D. saltans*. The fact that *D. willistoni* samples in our study presented some different patterns of fragments produced by *Xho*I digestion of their genomic DNA suggests a recent activation of the *P* element. It still seems, however, that a better understanding of *P* element distribution is needed and its putative activity needs proving.

Sequences sharing some homology to the *gypsy* retroelement probe were also detected in all the *D. willistoni* populations screened. The cleavage of *gypsy* with *Xho*I (Figure 4A shows the pattern of 10 of the 18 populations studied), *Ava*II (Figure 4B shows the pattern of five of the 18 populations studied) and *Bam*HI (Figure 4C shows the pattern of 10 of the 18 populations studied), produced a certain conserved pattern of fragments among the different populations studied. Although the hybridization signals indicated only weak homology between the *D. melanogaster*

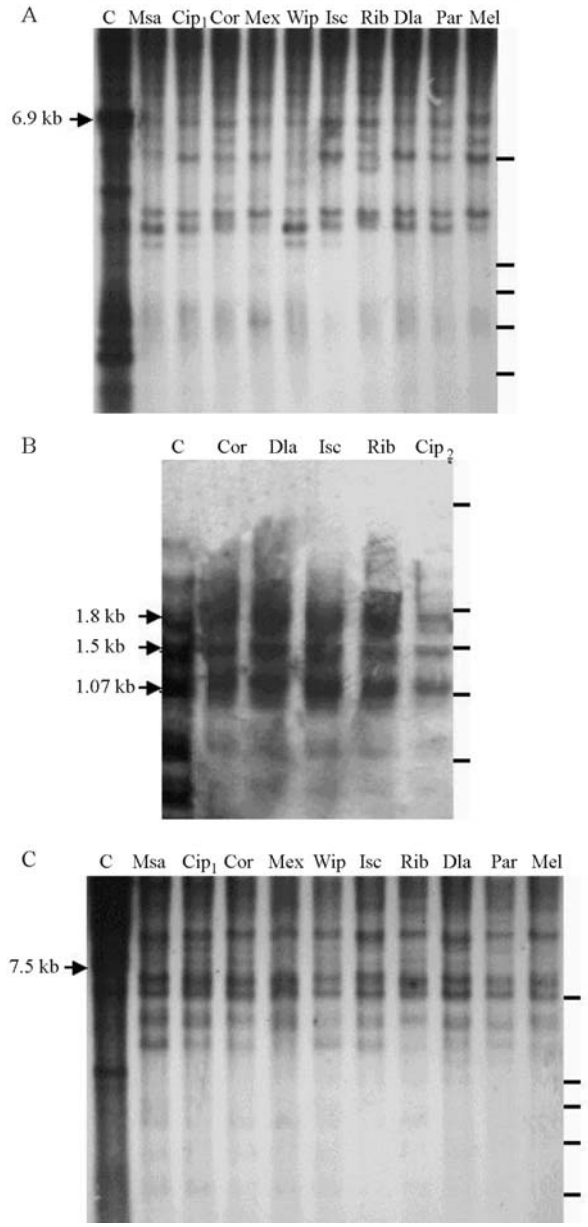

Figure 4 - Southern blot hybridization of genomic DNA from *Drosophila willistoni* strains probed with a 6.9 kb fragment liberated by the *Xho*I restriction enzyme from the *gypsy* retroelement of the *Drosophila melanogaster* pGGHS plasmid, lane C is *D. melanogaster* (Harwich strain). The Genomic DNA from the different *D. willistoni* populations was digested with *Xho*I (A), *Ava*II (B) or *Bam*HI (C). Arrows indicate the fragments expected for *D. melanogaster*. Bars on the right represent the 1 kb Plus DNA Ladder (Invitrogen) fragments (5, 2, 1.65, 1 and 0.5 kb).

gypsy probe used and the sequences present in the *D. willistoni* DNA, we observed the 6.9 kb fragment expected from *Xho*I cleavage (Figure 4A), suggesting conservation of restriction sites and the existence of complete copies of the *gypsy* retroelement in the genomes of the *D. willistoni* populations analyzed. In all the populations studied we also observed the shared lower molecular weigh bands, one of about 5.5 kb and two other bands of around 3 kb (Figure 4A) which may correspond to either deleted *gypsy* sequences and/or new internal *Xho*I restriction sites. Other authors also have also found deleted fragments of around 5.2 and 3.7 kb in *Drosophila subobscura* (Alberola and De Frutos, 1993).

The results of the *Ava*II cleavage (Figure 4B) showed a well-conserved pattern of *gypsy* fragments among the *D. willistoni* populations observed. One of the expected bands (about 1.07 kb) seemed to be present in the genome of all populations studied. The remaining fragments observed in *D. melanogaster* by *Ava*II cleavage were not detected in all our *D. willistoni* samples, suggesting divergence between the probe and the *gypsy* sequences present in the *D. willistoni* genome. However, there were other fragments which were conserved in all the *D. willistoni* populations. Since *Ava*II cuts DNA at many *gypsy* element sites only a few modifications are needed to explain this pattern, although this pattern could also be due to the putative coexistence of two types of *gypsy* element in *D. willistoni*. These alterations probably appeared before the diversification of populations.

We estimated the copy number of the *gypsy* retroelement by DNA cleavage with *Bam*HI, using several blots with different exposure times (Figure 4C) to calculate that there were between nine and sixteen sequences homologous to the probe used in the *D. willistoni* samples. However, only 10 to 30% of these bands were of a size corresponding to complete copies of the *D. melanogaster gypsy* retroelement. The great majority of the bands obtained could correspond to incomplete sequences and/or to new restriction site sequences.

It is also important to mention the similarity of the pattern of *gypsy Bam*HI fragments among the *D. willistoni* populations (Figure 4C), such conservation suggesting little or no mobilization of the *gypsy* retroelement during the time in which the geographical dispersion and the diversification of *D. willistoni* populations occurred. The results above are compatible with the data of Loreto *et al*. (1998) and Heredia *et al*. (2004), who studied other *Drosophila* species. When the *gypsy* sequences of different species are comparatively analyzed, however, the occurrence of some horizontal transfer between species is suggested (Stacey *et al*., 1986; Alberola *et al*., 1997; Terzian *et al*., 2000; Heredia *et al*., 2004).

According to Capy *et al*. (1998), such considerations are plausible in the light of the available models that try to explain the dynamics of transposable elements in the genomes of host species, these authors stating that each transposable element has a distinct evolutionary histories caused by factors intrinsic to the transposable element itself and by the regulatory strategies of the host.

By comparison with the fragment patterns revealed by the Southern blot assays performed with the probes of mobile Class I and Class II elements, our results point to the

notion that both the *P* and *gypsy* elements are ancient in *D. willistoni*, but whereas the *P* element still seems to be undergoing diversification and is susceptible to mobilization the *gypsy* retroelement appears to be stable.

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