Genet. Res., Camb. (2011), **93**, *pp.* 175–180. doi:10.1017/S0016672310000625

SHORT PAPER
The *copia* retrotransposon and horizontal transfer in *Drosophila willistoni*

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(*Received 17 June 2010 and in revised form 18 November 2010; first published online 31 March 2011*)

Summary

The copia element is a retrotransposon that is hypothesized to have been horizontally transferred from *Drosophila melanogaster* to some populations of *Drosophila willistoni* in Florida. Here we have used PCR and Southern blots to screen for sequences similar to *copia* element in South American populations of *D. willistoni*, as well as in strains previously shown to be carriers of the element. We have not found the canonical copia element in any of these populations. Unlike the *P* element, which invaded the *D. melanogaster* genome from *D. willistoni* and quickly spread worldwide, the canonical *copia* element appears to have transferred in the opposite direction and has not spread. This may be explained by differences in the requirements for transposition and in the host control of transposition.

1. Introduction

Transposable elements (TEs) are a significant component of almost all genomes studied thus far. They are greatly variable, having different transposition mechanisms, and a large sequence diversity. They are a source of genetic diversity for their hosts because TE mobilization is known to promote a repertory of different mutations. For example, disruption of coding sequences or gene regulatory elements can generate new coding sequences or even establish new regulatory gene networks (Biémont & Vieira, 2006; Feschotte, 2008). They are also associated with chromosome rearrangements and genome restructuring, thus, TEs are known to play an important role in genome evolution (Pritham, 2009).

The *copia* retrotransposon belongs to the *copia* superfamily, which is characterized by the order of the open reading frames (ORFs) of the enzymes integrase, reverse transcriptase and RNase H in a polyprotein domain (Wicker *et al.*, 2007). This element was first identified in *Drosophila melanogaster* and inserted into the *white* locus, promoting a *white-apricot* mutation. *Copia* is 5·4 kb long, has long terminal repeats (LTRs) of 276 bp and a single ORF of 4227 nucleotides, which codify a polyprotein of 1409 amino acids. This polyprotein is similar to the products of the *gag* and *pol* genes of retroviruses (Mount & Rubin, 1985). Sequences showing similarity to the *copia* retrotransposon were identified by Southern blot analyses of 52 species in the *Drosophila* genus. Twenty-two of these species belong to the *melanogaster* group, seven to the *willistoni* group, seven to the *obscura* group, six to the *saltans* group, two to the *immigrans* group and one to the *mesophragmatica* and *pinicula* groups (Martin *et al.*, 1983; Stacey *et al.*, 1986). Complete or partial sequences of *copia* were described in 13 species (reviewed in Biémont & Cizeron, 1999 and Almeida & Carareto, 2006; and FlyBase section on natural transposons, see Supplementary Table 1 available at http://journals.cambridge.org/GRH).

In a phylogenetic analysis of the *copia* element in the genus *Drosophila*, Jordan & McDonald (1998) demonstrated that this TE diverged into two different families, and that three subfamilies can be found within the species more related to *D. melanogaster*, a taxon formed by nine species called the *melanogaster* subgroup. In a subsequent study, Jordan *et al.* (1999) showed that *D. melanogaster* and *D. willistoni*, which

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Table 1. *List of analysed species and strains, with their respective collection sites and the results of Southern blot and PCR analyses in relation to the presence (+), absence (−) or weak signal (?) for 5′LTR-URL sequence of the copia retrotransposon*

Species	Strain and/or origin (year)	Southern blot	PCR
D. immigrans (negative control)	Zarate, Argentina (2006)	−	−
D. paramediostriata (negative control)	Morro Santana, Porto Alegre, RS, Brazil (old laboratory strain)	−	−
D. melanogaster (positive control)	Harwick	+	+
D. nebulosa	Santa Maria, RS, Brazil (2006)	? (weak signal)	−
D. willistoni	WIP4, Ipitanga, BA, Brazil (1960)	? (weak signal)	−
D. willistoni	St Kitts-Tucson Stock Center (2005)	? (weak signal)	−
D. willistoni	Morro Santana, Porto Alegre, RS, Brazil (1995)	−	−
D. willistoni	17A2, Eldorado do Sul, RS, Brazil (1992)	? (weak signal)	−
D. willistoni	EM1.00 *white* – Montevideo, Uruguay	? (weak signal)	−
D. willistoni	Q14.F11 *white*[a] – Montevideo, Uruguay	? (weak signal)	−
D. willistoni	Ey10.00 *white*[cf] – Montevideo, Uruguay	? (weak signal)	−
D. willistoni	EM1.00 *yellow* – Montevideo, Uruguay	−	−
D. willistoni	Q14.F1 *yellow* – Montevideo, Uruguay	−	−
D. willistoni	TB46.02 yellow – Montevideo, Uruguay	−	−
D. willistoni	14030-0811.00 – Santa Maria de Ostuna, Nicaragua (1954)	−	−
D. willistoni	14030-0811.02 – Royal Palm Park, Florida	−	−
D. willistoni	Montevideo, Uruguay	−	−
D. willistoni	Serra Talhada, Pernambuco, Brazil.	−	−
D. paulistorum	Porto Alegre, RS, Brazil (old laboratory strain)	? (weak signal)	−
D. paulistorum	Orinocana semi-species (old laboratory strain)	? (weak signal)	−
D. paulistorum	Andino-brasilian semi-species (old laboratory strain)	? (weak signal)	−
D. insularis	St. Kitts (old laboratory strain)	? (weak signal)	−
D. equinoxialis	Mexico (old laboratory strain)	? (weak signal)	−

[a] White apricot mutant.
[cf] White-coffee mutant.

belong to different species groups, share *copia* LTRs with 99% sequence identity, whereas the sibling species *D. melanogaster* and *Drosophila simulans* share only 90% sequence identity. Since the sibling species possess a far more recent common ancestor, estimated at 2–3 million years ago (MYA) (Lachaise & Silvain, 2004) and *D. melanogaster* and *D. willistoni* diverged about 62 MYA (Tamura *et al.*, 2004), the authors suggested horizontal transposon transfer (HTT) as a possible explanation for the incongruence observed in the sequence similarity between *copia* elements in these species.

Only two occurrences of HTT between *D. willistoni* and *D. melanogaster* have been reported (Loreto *et al.*, 2008), namely that of the *P* element, which was the first well-documented observation of HTT in eukaryotes (Daniels *et al.*, 1990; Quesneville & Anxolabéhère, 1998) and of the *copia* element. In the first case, it was shown that the *P* element invaded the *D. melanogaster* genome and quickly spread worldwide. According to Jordan *et al.* (1999), the HTT of *copia* element occurred in the opposite direction, namely, from *D. melanogaster* to *D. willistoni*. Another important difference between both HTT events is that the *P* element is a class II TE, whereas *copia* is a class I TE (Wicker *et al.*, 2007); it was suggested that the elements of different classes behave differently during HTT (Loreto *et al.*, 2008; Schaack *et al.*, 2010). As pointed out by Schaack *et al.* (2010), this difference can be attributed to the fact that the DNA HTT was reported first and was more closely investigated. In contrast, no study has been conducted to understand the dynamics of the *copia* element invasion in the *D. willistoni* species in other locations in its wide geographical distribution. To address this point, we performed a population analysis of the *copia* element distribution in South American samples of *D. willistoni*, as well as two strains originally used by Jordan *et al.* (1999). Other species of the *D. willistoni* group were also screened.

2. Materials and methods

Nucleotide sequences with similarity to the 5′LTR-URL region of the *copia* retroelement were screened by PCR and Southern blot analyses in species and strains of the *willistoni* group (Table 1). The identification of the cryptic species of the *willistoni* group was confirmed by isozyme patterns of acid phosphatase (*Acph1*) (Garcia *et al.*, 2006). Table 1 also shows the species used as positive and negative controls for *copia* presence in the molecular assays.

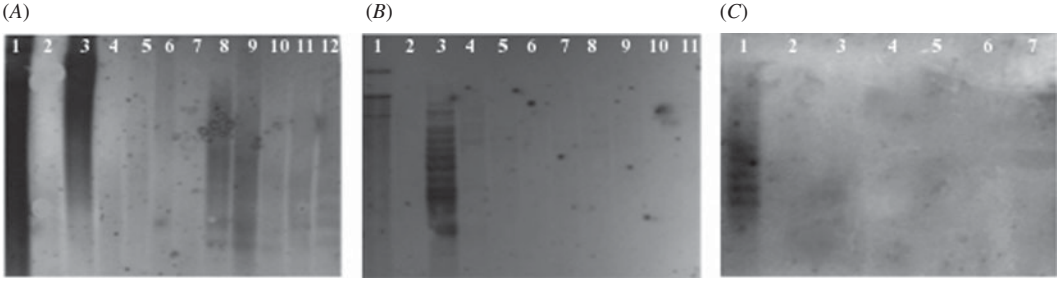

Fig. 1. Southern blot using a probe of 440 bp 5′LTR-URL region of *copia* retrotransposon from *D. melanogaster*. (*A*) (1) PTZ18 plasmid, (2) *D. paramediostriata*, (3) *D. melanogaster*, (4) *D. willistoni* Wip4, (5) *D. willistoni* 17A2, (6) *D. willistoni* Tucson Stock Center, (7) *D. willistoni* Morro Santana, (8) *D. paulistorum* POA, (9) *D. paulistorum* Andino-brasileira, (10) *D. paulistorum* Orinocana, (11) *D. insularis* and (12) *D. equinoxialis*. (*B*) (1) PTZ18 plasmid, (2) *D. immigrans*, (3) *D. melanogaster*, (4) *D. nebulosa*, (5) *D. willistoni* Wip4, (6) *D. willistoni* EM1.00, (7) *D. willistoni* Q14.F11, (8) *D. willistoni* Ey10.00, (9) *D. willistoni* EM1.00, (10) *D. willistoni* Q14.F1 and (11) *D. willistoni* TB46.02. (*C*) (1) *D. melanogaster*, (2) *D. willistoni* Royal Palm Park, Florida, (3) *D. willistoni* Santa Maria de Ostuna, Nicaragua, (4) *D. willistoni* Serra Talhada, (5) *D. willistoni* Montevideo, (6) *D. willistoni* 17A2 and (7) *D. willistoni* Wip4.

Genomic DNA was prepared from adult flies as previously described (Oliveira *et al*., 2009). PCR analyses were performed in 50 μl reactions using 50 ng of genomic DNA, 1 U of Taq DNA Polymerase (Invitrogen), 1 × reaction buffer, 200 μM of NTPs, 20 pmol of each primer and 2·5 mM of $MgCl_2$. The primers used are specific to the 5′LTR-URL region of *copia* retrotransposon from *D. melanogaster*, amplifying a 440 bp fragment (Jordan & McDonald, 1998). The amplification conditions were 94 °C for 5 min, 30 cycles at 94 °C for 45 s, 52 °C for 60 s and 72 °C for 60 s, and the final step at 72 °C for 5 min. To exclude possible PCR contamination, a 786 bp fragment of gene *COII* was sequenced using primers TL2J3037 and TKN3785 (Simon *et al*., 1994). PCRs with these primers were also used as controls for DNA quality (Fig. 2*b*).

For the Southern blot analyses, approximately 6 μg of genomic DNA was digested with *Eco*RI. The DNA fragments were fractioned by agarose gel electrophoresis (0·8 %) and transferred to a nylon membrane (Hybond N+, GE Healthcare). The 440 bp fragment corresponding to the *copia* 5′LTR-URL region of the PTZ18 plasmid produced by PCR was used for the hybridization probe, as previously described (Almeida & Carareto, 2006). The membranes were hybridized at 60 °C. In order to label and detect the *copia* sequences, the AlkPhos® kit and the CPD Star Detection kit (GE Healthcare) were used according to the manufacturer's instructions.

The membranes were rehybridized with a second probe corresponding to a single copy gene, the *white* gene. In this case, the primers used in probe amplification were forward: GCGCCACGAAAACATTT ACT and reverse: ACATCGAGCCTGCATCTCTT. These rehybridizations have been used as a control for *copia* hybridization.

Sequence searches for *copia* element were carried out for the *D. willistoni* genome in the FlyBase BLAST database (http://flybase.bio.indiana.edu/blast/) (Altschul *et al*., 1990) using the complete *copia* sequence for query (Accession number X02599; Mount & Rubin, 1985). Hits with scores above 100 were selected for further analysis. The sequenced strain of *D. willistoni* (Gd-H4-1 strain, Stock Center number: 14030-0811.94, of Drosophila 12 Genomes Consortium, 2007) comes from the Guadalupe Island (west Coast of Mexico's Baja California Peninsula).

3. Results and discussion

The Southern blot analyses revealed a strong hybridization signal of *copia* in *D. melanogaster* and the absence of signal in *Drosophila immigrans* and *Drosophila paramediostriata*, as expected. In the *willistoni* group, a few weak signals were obtained in *Drosophila nebulosa*, *Drosophila paulistorum*, *Drosophila insularis*, *Drosophila equinoxialis* and in some strains of *D. willistoni*. Strains previously shown by Jordan *et al*. (1999) as possessing *copia* did not present a hybridization signal in our studies (Table 1, Fig. 1). These faint signals were weaker than those obtained by hybridization with the *D. willistoni white* gene probe, which was used as a hybridization control (data not shown). These results indicate that the similarity between the canonical *copia* sequence, used as a probe, and the *copia*-related sequences occurring in the genomes of the investigated species was very low.

In agreement with the Southern blot analysis, only *D. melanogaster* produced the expected 440 bp fragment in the PCR assays. No amplification was obtained in the other species, even in the *D. willistoni* strains previously studied by Jordan *et al*. (1999) (Fig. 2*a*).

The *in silico* analysis for the available *D. willistoni* genome produced 25 hits. However, all of these hits were degenerate sequences, and the longest hit was

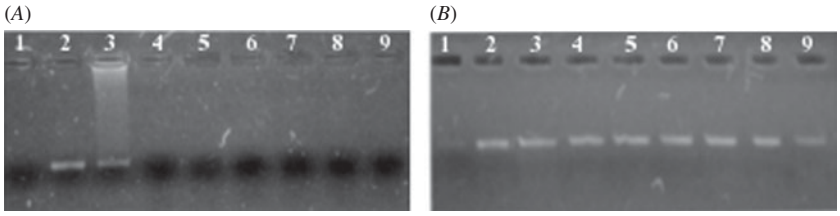

Fig. 2. (*A*) PCR using the primers copPCS and copLTR (Jordan & McDonald, 1998), specific to the 5′LTR-URL region of the *copia* retrotransposon from *D. melanogaster*, amplifying a 440 bp fragment. (1) negative control, (2) PTZ18 plasmid, (3) *D. melanogaster*, (4) *D. willistoni* Royal Palm Park, Florida, (5) *D. willistoni* Santa Maria de Ostuna, Nicaragua, (6) *D. willistoni* Montevideo, (7) *D. willistoni* Serra Talhada, (8) *D. willistoni* 17A2 and (9) *D. willistoni* Wip4. (*B*) PCR control using primers TL2J3037 and TKN3785 (Simon *et al*., 1994) for amplification of a fragment of 786 bp of gene *COII*. (1) negative control, (2) *D. melanogaster*, (3) *D. willistoni* Royal Palm Park, Florida, (4) *D. willistoni* Santa Maria de Ostuna, Nicaragua, (5) *D. willistoni* Montevideo, (6) *D. willistoni* Serra Talhada, (7) *D. willistoni* 17A2 and (8) *D. willistoni* Wip4.

1360 bp long. The similarity level was also variable, with the highest observed similarity being 89·5% (see Supplementary Figure 1 available at http://journals.cambridge.org/GRH). These results suggest that these sequences correspond to old *copia*-like sequences that have been active for a long in the *D. willistoni* genome because the obtained sequences are degenerated and do not correspond to canonical *copia* elements.

In light of these results, the presence of the *copia* element in species of the *willistoni* group, as detected through Southern blot analysis with medium stringency hybridization (Martin *et al*., 1983; Stacey *et al*., 1986), has been verified and are in agreement with the faint signals we have observed in this study. In fact, the *D. willistoni* genome showed sequences that have enough similarity to be detected by Southern blot analyses. For this reason, the sequences previously hybridized are probably from a *copia* related element, but not the canonical *copia* element.

The *copia* element has been reported to be involved in other HTT events, as observed between flies of the *melanogaster* group and between species of this group with *Zaprionus indianus* (Almeida & Carareto, 2006). Also, Jordan *et al*. (1999) described a recent HTT of the *copia* element from *D. melanogaster* to some *D. willistoni* populations of Florida and Nicaragua. They showed, also, some populations from these geographic regions did not harbour the element, showing a polymorphic pattern among *D. willistoni* populations. We have enlarged these analyses to many South American populations, one Mexican population, and two of the strains previously studied by Jordan *et al*. (1999): *D. willistoni* Royal Palm Park, Florida and *D. willistoni* Santa Maria de Ostuna, Nicaragua. We found that all these populations are void of canonical *copia* sequences.

Jordan *et al*. (1999) conducted some controls to exclude the possibility of PCR contamination (ITS region of 18S and 28S rDNA and *Adh* genes) and showed specific amplifications for each species. Also, a high stringency Southern blot was performed and showed the expected fragment in the *D. willistoni* strains, presenting a strong hybridization signal for *D. melanogaster*, a weaker signal for *D. simulans* and an even weaker one for *D. willistoni*. A possible explanation for the disagreement between the results reported here and those obtained by Jordan *et al*. (1999) is that the *D. willistoni* strains used in both studies present a large temporal separation. In fact, these strains have been maintained under laboratory conditions for a long time and could have lost their original canonical *copia* elements.

It is interesting to compare the *copia* and the *P* element HTT events between *D. melanogaster* and *D. willistoni*. In a very short period of time, which is hypothesized to be approximately 40 years, the *P* element invaded the *D. melanogaster* genome and spread worldwide (Bregliano & Kidwell, 1983). On the contrary, the *copia* element, which invaded the *D. willistoni* genome, was only found in a very restricted geographic area of the *D. willistoni* distribution. These differences can be related to the TEs themselves, for example, variations in characteristics, such as transposition rates, requirements for host specific factors, mechanisms for transposition control, or even the population's structure of the host species. Populations that are more structured are less prone to disseminate genetic material, including TEs (Vieira *et al*., 2009). *D. willistoni* has a large geographical distribution, which extends to areas from Florida to Argentina (Spassky *et al*., 1971; Dobzhansky & Powell, 1975). Strains from any part of this distribution do not show incipient reproductive isolation, except for samples from Lima, Peru, which are considered a subspecies called *D. willistoni quechua* (Ayala & Tracey, 1973; Robe *et al*., 2010). However, the existence of endemic chromosome inversions suggests some level of population restructuring in this species (Rohde *et al*., 2006).

Theoretical models predicting the events following a genome invasion by a TE highlight the fact that transposition rate is a critical factor for TE distribution, without being lost due to genetic drift (Le Rouzic & Capy, 2005). However, the transposition

rate is not the only property contributing to the geographic distribution of TEs. It is a complex trait that involves host regulatory mechanisms and perhaps environmental influences. The transposition rate of *copia* in *D. willistoni* and the relationship of this TE with the host genome are not as well characterized as those of the *P* element.

The *P* element in *D. melanogaster* is one of the best characterized TEs, and the biological mechanism for its wide-spread distribution is well understood (Engels, 1989). Our data show that even if the *copia* element was able to invade the genome of some populations of *D. willistoni* as suggested by Jordan *et al.* (1999), it was not able to spread to the *D. willistoni* populations studied here or even be maintained in the original populations under laboratory conditions. Further studies focusing on the mobilization rates and the mechanism of *copia* transposition in *D. willistoni* are required to understand the reasons why *copia* has failed to spread to the genomes of *D. willistoni* populations.

We are grateful to Ms C Cristina Parada, Dr Beatriz Goñi and Dr Yanina Panzera for strains of *Drosophila willistoni*; to Luiz F. V. Oliveira for valuable help in several aspects of this research and to Dr Lizandra Robe for suggestions. We also thank the reviewers for idea and suggestions. This study was supported by grants from CNPq, Fapergs, Proape-CAPES-PRPGP/UFSM.

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