The copia retrotransposon and horizontal transfer in Drosophila willistoni

P. M. Rubin1, E. L. S. Loreto*, C. M. A. Carareto3 and V. L. S. Valente4
1 Programa de Pós-Graduação em Biodiversidade Animal, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
2 Departamento de Biologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
3 Departamento de Biologia, Universidade Estadual Paulista (UNESP), São José do Rio Preto, São Paulo, Brazil
4 Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Rio Grande do Sul, Porto Alegre, Brazil

(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 (Biemont & 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). 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 polypeptide of 1409 amino acids. This polypeptide 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 Biemont & 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...
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.
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 x reaction buffer, 200 µM of NTPs, 20 pmol of each primer and 2-5 mM of MgCl₂. 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. 2b).

For the Southern blot analyses, approximately 6 µg of genomic DNA was digested with EcoRI. 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 hybridization 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. 2a).

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

The Southern blot analyses revealed a strong hybridization signal of copia in D. melanogaster and the absence of signal in Drosophila immigrans and Drosophila paramediodriata, 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. 2a).

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
showed the expected fragment in the high stringency Southern blot was performed and showed specific amplifications for each species. Also, region of 18S and 28S rDNA and exclude the possibility of PCR contamination (ITS by Jordan population, and two of the strains previously studied many South American populations, one Mexican populations. We have enlarged these analyses to showing a polymorphic pattern among geographic regions did not harbour the element, They showed, also, some populations from these D. willistoni populations of Florida and Nicaragua. We found that all these populations are D. willistoni with the highest observed similarity being 89.5% (see 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 original 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 Zapriónus indians (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 Goni 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.

References


