Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 35 (2005) 583–594
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Multiple events of horizontal transfer of the *Minos* transposable element between *Drosophila* species

Luciane M. de Almeida, Claudia M.A. Carareto*

Departamento de Biologia, UNESP – Universidade Estadual Paulista, 15054-000 São José do Rio Preto, SP, Brazil

Received 15 July 2004; revised 17 November 2004
Available online 1 February 2005

Abstract

In this study the *Minos* element was analyzed in 26 species of the *repleta* group and seven species of the *saltans* group of the genus *Drosophila*. The PCR and Southern blot analysis showed a wide occurrence of the *Minos* transposable element among species of the *repleta* and the *saltans* groups and also a low number of insertions in both genomes. Three different analyses, nucleotide divergence, historical associations, and comparisons between substitution rates (d_N and d_S) of *Minos* and *Adh* host gene sequences, suggest the occurrence of horizontal transfer between *repleta* and *saltans* species. These data reinforce and extend the Arcà and Savakis [Genetica 108 (2000) 263] results and suggest five events of horizontal transfer to explain the present *Minos* distribution: between *D. saltans* and the ancestor of the *mulleri* and the *mojavensis* clusters; between *D. hydei* and the ancestor of the *mulleri* and the *mojavensis* clusters; between *D. mojavensis* and *D. aldrichi*; between *D. buzzatii* and *D. serido*; and between *D. spenceri* and *D. emarginata*. An alternative explanation would be that repeated events of horizontal transfer involving *D. hydei*, which is a cosmopolitan species that diverged from the others *repleta* species as long as 14 Mya, could have spread *Minos* within the *repleta* group and to *D. saltans*. The data presented in this article support a model in which distribution of *Minos* transposon among *Drosophila* species is determined by horizontal transmission balanced by vertical inactivation and extinction.

Keywords: *Minos* element; *Minos* insertion number; Horizontal transfer; *Drosophila repleta* group; *Drosophila saltans* group

1. Introduction

Minos is a mobile element that belongs to the widespread *Tc1/mariner* superfamily. It was first identified as a dispersed repetitive sequence inserted into the rDNA locus of *Drosophila hydei* (Franz and Savakis, 1991). *Minos* is relatively small (1.6–1.8 kb) and is characterized by 255 bp perfect inverted terminal repeats (nucleotides 1–255 and 1521–1775) and the presence of two long non-overlapping open reading frames (ORFs) on the same strand that are separated by a putative 60-nucleotide intron (Franz et al., 1994). It has been shown to be active in several dipteran (Franz and Savakis, 1991; Kapetanaki et al., 2002; O'Brochta et al., 2003) and lepidopteran (Shimizu et al., 2000) species. In addition to insects, *Minos* is also active in other invertebrates (Sasakura et al., 2003), mouse tissues in vivo (Drabek et al., 2003; Zagoraiou et al., 2001) and human cells in culture (Klinakis et al., 2000), where it has been used for transposon-mediated mutagenesis.

Despite its biological importance as a tool for genetic manipulation, relatively little is known about *Minos* evolution in the *Drosophila* genus. Several types of events can occur between transposable elements and their hosts during their evolutionary relationships, which can be denominated as historical associations. For example, transposable elements may diverge with their host (co-divergence), diverge independently of their hosts, become extinct or move from one species to

* Corresponding author. Fax: +55 17 2212390.
E-mail address: carareto@ibilce.unesp.br (C.M.A. Carareto).

1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2004.11.026

another (horizontal transfer). Knowledge about *Minos* distribution among species, its number of insertions within genomes and structural variation among sequences is necessary to understand the evolutionary history of this transposable element (TE).

There are two manners in which transposable elements can be transferred between species. The first is strictly mating dependent (vertical transmission), and the second occurs between species that are reproductively isolated (horizontal transmission). Vertical transmission predicts that all descendants of an ancestral species that harbors that element should also harbor homologous variants of the element. Additionally, the sequence conservation of an element should be congruent with the phylogeny of the host species. These assumptions can be violated in the case of ancestral polymorphism, stochastic losses, and selection. On the other hand, the occurrence of horizontal transmission predicts that the distribution of the element may be discontinuous and that the sequence conservation of the element is not necessarily related to the phylogeny of the species in which it resides.

A single previous study by Arcà and Savakis (2000) has shown the distribution of the *Minos* transposable element in 19 species of the subgenus *Sophophora* (including two species of the *saltans* group), eight of the subgenus *Drosophila* (including six species of the *repleta* group), and one species of the *Scaptodrosophila* subgenus. The analysis showed a discontinuous distribution of the *Minos* transposon in the *Sophophora* subgenus. Distance sequence analysis (Neighbor-joining) realized with only four species (*D. mojavensis*, *D. hydei*, *D. saltans*, and *D. willistoni*) showed that the *Minos* sequence of *D. mojavensis* is more similar to that of *D. saltans* than to that of its sibling species *D. hydei*. This finding led Arcà and Savakis (2000) to suggest the occurrence of horizontal transfer of *Minos* between *D. saltans* and *D. mojavensis*, two distantly related species. Aiming to broaden our knowledge about the evolutionary history of the *Minos* transposable element we extended the number of species to 33 and improved the phylogenetic analysis using the maximum-parsimony method; analysis of historical associations implemented by the TreeMap software and comparisons between substitution rates (d_N and d_S) of *Minos* and *Adh* host gene sequences.

2. Materials and methods

2.1. Fly stocks

In this study the *Minos* element was analyzed in 26 species (see Appendix A, Table 1) of three subgroups (*hydei*, *mercatorum*, and *mulleri*) of the *repleta* group and seven species of five subgroups (*cordata*, *elliptica*, *parasaltans*, *sturtevanti*, and *saltans*) of the *saltans* group of the genus *Drosophila*.

2.2. Molecular methods

About 200 ng of genomic DNA was amplified with M5 and M3 primers (for details, see Appendix B). The fragments amplified are 1068 bp long and were separated by electrophoresis in 1% agarose gel stained with ethidium bromide. The PCR products were cloned into a TA cloning vector (Invitrogen) and sequenced. For each amplified fragment two individual clones were chosen at random for sequencing. Southern analysis was also used to estimate the overall amount and the presence of putative full-sized *Minos* elements in each species (see Appendix B).

2.3. Sequence analyses

2.3.1. Evolutionary analysis by direct optimization and sensitive analysis

POY (Wheeler and Gladstein, 2000) is a new program for phylogenetic analysis of DNA sequence based on the principle of parsimony. This program implements the method of DNA analysis "direct optimization" (Wheeler, 1996) and yielding results are equivalent to those obtained with Hennig (Farris, 1988), NONA (Goloboff, 1998) or PAUP (Swofford, 2000). According to Wheeler (2000) and Schulmeister et al. (2002), the direct optimization shows advantages over conventional methods (in which the alignments are separated from the analysis). In conventional analyses a multiple alignment is generated by aligning sequences pairwise, in an order that is outlined in a guide tree or alignment topology that was determined by the sequence data themselves. This way, the order in which this pairwise alignment is performed has an influence on the positional homology statements, which consequently has an influence on the outcome of the phylogenetic analysis. Direct optimization solves this problem because POY includes the alignment procedure into the simultaneous analysis framework where a unique scheme of positional homologies (a kind of multiple alignment) is produced for each examined topology during the tree search. The optimization alignment method has been recently used by several investigator (Chavarría and Carpenter, 1994; Carpenter and Wheeler, 1999a,b; Edgecombe et al., 1999; Giribet and Ribera, 2000; Giribet et al., 2000; Janies and Mooi, 1999; Schulmeister et al., 2002; Sorenson et al., 1999; Wheeler and Hayashi, 1998), principally for its efficiency in generating phylogenetic hypotheses that entirely avoid sequence alignment.

Before derive the *Minos* element phylogeny by POY, the sequences were aligned with Clustal W (Thompson et al., 1994) and then split into eight fragments with different sizes. This procedure has the only aim of