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Micropia transposable element occurrence in *Drosophila* species of the *saltans* group.

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Introduction

Transposable elements origin and distribution in different species and whether they are recent or old components of the host genome are still subjects of research. The presence of a transposable element in all organisms of a species group suggests that they are old components of these genomes. Members of the retrotransposon family *micropia* were discovered as constituents of wild-type Y chromosomal fertility genes from *Drosophila hydei* (Hennig *et al.*, 1983; Huijser *et al*., 1988), but they also occur in autosomes and X chromosomes. The presence of the *micropia* retrotransposon in different *Drosophila* species was determined by Southern analysis using the *micropia* element of *D. hydei* as a probe. It has been found in several species of the all subgroups of the *repleta* group, but with patchy distribution (Lankenau *et al*., 1994); in the *immigrans* group, in *D. immigrans*, in the *funebris* group, in *D. funebris*, and in the *mellanica* group, in *D. mellanica* (Lankenau, 1993); and in three of the four species groups of the subgenus Sophophora: *melanogaster* group (*D. melanogaster*, *D. simulans*, *D. birchii*, *D. yakuba*, *D. ananassae*); *willistoni* group (*D. willistoni*); and the *saltans* group (*D. saltans*) (Lankenau and Hennig, 1990; Lankenau *et al*., 1988; Lankenau *et al*., 1990; Lankenau *et al*., 1994). *Micropia* has been studied in details only in species of the *repleta* group, *D. hydei* in special, and in *D. melanogaster*. It has a typical retrotransposon structure with approximately 5.5 kb length and a 4 kb open reading frame encoding putative products that show homology to the nucleocapsid, protease, reverse transcriptase, RNase H and integrase products of vertebrate retroviruses (Lankenau *et al*., 1988; Lankenau *et al*., 1989). The general structure of this retrotransposon is well conserved in both species, but the LTRs are completely different. The overall sequence homology ranges between 70% and 90% on the amino acid level. *Micropia* encodes a 5.0 kb transcript that is expressed in both testes and somatic tissues of males and females. Although the great similarity, *micropia* in *D. hydei* produces an antisense RNA overlapping the RNaseH and parts of the transcriptase reverse that is not expressed in *D. melanogaster*. Since only *D. saltans* was screened for the presence of *micropia* in its genome, this study aimed to contribute to the knowledge about *micropia* distribution in the *saltans* species group.

Material and Methods

Species: The species of *D. saltans* group studied are listed in Table 1.

PCR reactions: PCR reactions were performed in 25µl volumes using approximately 200ng of template DNA, 100 µM of each dNTP, 12 pmol of each primer, 1.5 mM of $MgCl_2$ and 1 unit of Taq DNA Polymerase (GIBCO-BRL) in 1× Polymerase Buffer. After an initial denaturation for 3 min at

95°C, 40 cycles consisting of a 1-min denaturation at 95 °C, a 1-min annealing at 52°C and a 2-min extension at 72°C steps were followed. An additional extension step of 10 min at 72°C was performed after the last cycle. The amplified fragments were separated by electrophoresis in a 1% agarose gel. The primers used were #2813 (5'- TTAACTCCTAGAGTTCATCGCTGG- 3') and #2814 (5'- CATGTACCTGGTTAACTACTGACC - 3') which amplify a 386 bp fragment from a highly conserved sequence of *micropia* (from nucleotide 2813 to 3198).

Dot blot hybridization: Denaturated DNA (5 mg) was applied directly to the nylon membrane and hybridized with a 3.1 kb *micropia* fragment excised with *Eco*RI from dhMiF$_2$ plasmid (Huijser *et al*., 1988). Hybridization and detection were performed with ECL™ direct nucleic acid labeling and detection systems according to manufacturer's instructions.

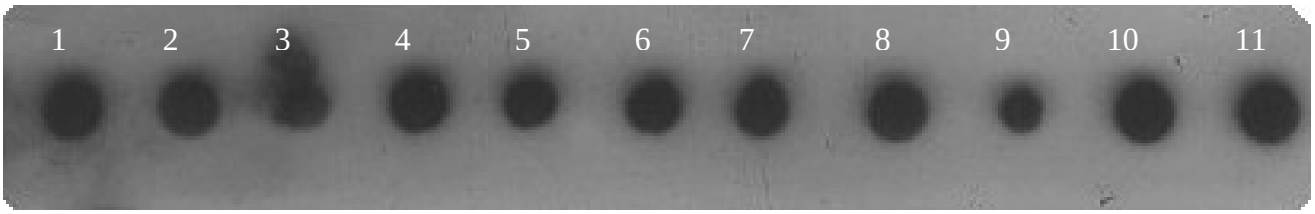

Figure 1. Dot blot of *D. saltans* species group probed with a 3.1 kb *micropia* fragment. The genomic DNAs were blotted as follow: 1. *D. neocordata*; 2. *D. emarginata*; 3. *D. parasaltans*; 4. *D. subsaltans*; 5. *D. milleri*; 6. *D. dacunhai*; 7. *D. sturtevanti*; 8. *D. austrosaltans*; 9. *D. saltans*; 10. *D. prosaltans*; 11. *micropia* (dhMiF$_2$ plasmid).

Results and Discussion

M*icropia* retrotransposon occurs in *D. saltans* as reported by Lankenau *et al*. (1988), Lankenau and Hennig (1990), Lankenau *et al*., (1990), Lankenau *et al*. (1994), and Biémont and Cizeron (1999), in their revision about the distribution of transposable elements in *Drosophila* species. As the occurrence of *micropia* has not been reported for other species of the *saltans* group, we have searched for its occurrence in 10 species, one at least belonging to each *saltans* species subgroups (*cordata*, *elliptica*, *parasaltans, sturtevanti* and *saltans*). All the species possess this retrotransposon in their genomes

Table 1. Species of *saltans* group of *Drosophila* used in this study.

Subgroup	Species	Geographic Origin	Collection
neocordata	D. neocordata	Minas Gerais-Brazil	Stock Center n. 14041-0831.0
elliptica	*D. emarginata*	Vera Cruz- Mexico	Stock Center n. 14042-0841.6
parasaltans	*D. parasaltans*	Tapuruquara- Bazil	Bicudo, H.E.M.C.-UNESP
	D. subsaltans	Belém-Brazil	Stock Center n. 14044-0872.0
sturtevanti	*D. milleri*	Puerto Rico	Stock Center n. 1403-0861.0
	D. dacunhai	Haiti	Stock Center n. 1403-0854.0
	D. sturtevanti	Matlapa- Mexico	Silva, J - University of Arizona
saltans	*D. austrosaltans*	Pirassununga-Brazil	Bicudo, H.E.M.C.- UNESP
	D. saltans	Chilpancingo-Mexico	Bicudo, H.E.M.C.- UNESP
	D. prosaltans	Trinidad Tobago	Bicudo, H.E.M.C.- UNESP

(Figures 1 and 2). These results reinforce the hypothesis of *micropia*'s wide distribution in *Drosophila* genera (Lankenau *et al*., 1994), suggesting that this element may be an old genome component possibly introduced in *Drosophila* genera before its diversification. Although it has a wide distribution, it appears that there are two groups of *micropia* elements in *Drosophila* species. One group, represented by *micropia* from *D. melanogaster*, does not possess the testis-specific antisense promoter, whereas the other group, represented by *D. hydei* elements, expresses such RNA in primary spermatocytes. So far, from six species tested that contain copies of *micropia* five also express the 1.0 kb antisense RNA; all these five species belong to the of *repleta* group (Lankenau *et al*., 1994).

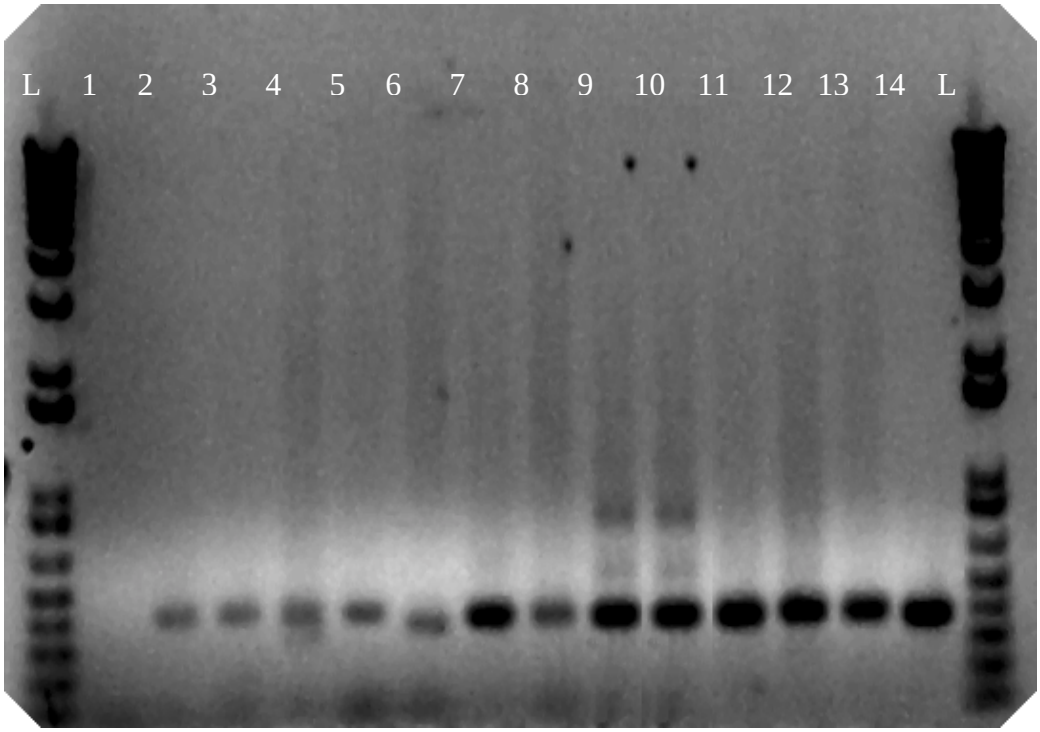

Figure 2. PCR amplification of *micropia* internal sequence from *D. saltans* species group and control DNAs. 1. negative control; 2. *D. neocordata*; 3. *D. emarginata*; 4. *D. parasaltans*; 5. *D. subsaltans*; 6. *D. milleri*; 7. *D. dacunhai*; 8. *D. sturtevanti*; 9. *D. austrosaltans*; 10. *D. saltans*; 11. *D. prosaltans*; 12. *D. simulans*; 13. *D. melanogaster*; 14. *micropia* ($dhMiF_2$ plasmid). L = 1 kb plus DNA ladder.

We here present a contribution to the knowledge of the distribution of *micropia* retrotransposon in the *saltans* group species. According to literature, this is the first description of *micropia* occurrence in species of the subgroups *cordata* (*D. neocordata*), *elliptica* (*D. emarginata), sturtevanti* (*D. milleri*, *D. dacunhai*, *D. sturtevanti*), and *parasaltans* (*D. parasaltans* and *D. subsaltans,*) and in other species of the *saltans* subgroup than *D. saltans* (*D. austrosaltans* and *D. prosaltans*). Since the *saltans* group belongs to the Sophophora subgenus, additional analyses are in progress in our laboratory aiming to determine whether the antisense RNA has still been transcribed in these species, or not, as in *D. melanogater*, a species closer to *saltans* than to *repleta* species group.

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