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Screening for transposable elements in South America invasive species *Zaprionus indianus* and *Drosophila malerkotliana*.

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Transposable elements (TEs) have usually been studied focusing on distribution, structure, activity and evolution. Such analyses primarily aim at understanding the impact of TEs in the genetic dynamics and evolution of the host genomes. A kind of impact analysis that has not been enough explored is to look at genomic dynamics of TEs under stressful conditions, like environment invasion and colonization. According to the "wake-up" hypothesis (Vieira *et al.*, 1999), there is a tendency of mobilization intensification in colonizing populations resulting in an increasing of copy number. An example is given by the comparison between *D. melanogaster* and *D. simulans*, two cosmopolitan sibling species. *D. melanogaster* has dispersed around the world some centuries before *D. simulans* (Capy and Gilbert, 2004) and among 34 transposable elements analyzed, *D. melanogaster* appears to have higher insertion site numbers than *D. simulans* for 29 TEs when populations of America, Asia, Europe, Australia and Africa, the last one being the native place of both species, are compared (Vieira *et al.*, 1999).

Besides *D. melanogaster* and *D. simulans*, numerous other Drosophilidae were able to invade new habitats and had recently colonized the Brazilian territory mainly by antropic activity; among them, *Zaprionus indianus* (from Africa) and *D. malerkotliana* (from Asia). *Zaprionus indianus* was first collected in 1998, in a fig culture at Valinhos city, Sao Paulo (Vilela, 1999), and now it can be collected in several regions of Brazil (Vilela, 1999; Castro and Valente, 2001; Kato *et al.*, 2004), Uruguay (Gõni *et al.*, 2002), and southern North America (van der Linde *et al.*, 2006). *D. malerkotliana* has invaded South America in the 1970's decade (Val and Sene, 1980) and nowadays it is frequently collected in southern Brazil. The recent invasion of a continent by a species provides a useful tool for studying the dynamics of TEs during colonizing stress, because we can monitor populations since the first steps of introduction. To do that, it is necessary to accumulate information about distribution, history, and dynamics of its transposable elements. The goal of this study was then to make an initial search for transposable elements in *Z. indianus* and *D. malerkotliana* genomes to guide further studies on these invasive species.

Seven retrotransposons were searched (*copia*, *mdg-1*, *412*, *gypsy*, *297*, *micropia,* and *roo/B104*), two non-LTR retrotransposons (*jockey* and *doc*), and one transposon (*bari-1*) in a Brazilian population of *Z. indianus* (Mirassol, SP) and *D. malerkotliana* (Onda Verde, SP) by the Dot blotting method. The probes were prepared by two different methods: (1) PCR reactions were performed in 25 μl using approximately 50 ng of each TE plasmid, 0.4 μM of each specific primer

(Table 1), 200 mM of dNTPs, 1.5 mM of $MgCl_2$ and 1U of Taq Platinum Polymerase (Invitrogen) in 1× PCR buffer; for amplification we used an initial denaturation step of 3 min at 94°C, 30 cycles consisting of 30 sec at 94°C, 30 sec at 56°C and 1 min at 72°C, finally a additional extension step of 10 min at 72°C were performed; (2) 5 μg of each plasmid was digested with appropriate restriction enzymes (Table 1). The fragments were isolated from agarose 1% gels, purified using GFX PCR DNA and Gel Band Purification (GE Healthcare), and probed using Gene Images Random Prime Labelling Module (GE Healthcare) according to the manufacturer's instructions. For screening the TEs, 8 mg of genomic DNA from each strain was dropped in the Hybond N+ nylon membranes (GE Healthcare). For hybridization and detection we used the chemioluminescent hybridization system Gene Images (GE Healthcare) at high stringency (60°C) according to the manufacturer's instructions. Ultra pure water and 1 mg of each plasmid were used as negative and positive controls, respectively.

Table 1. Plasmids, primers and restriction enzymes used to prepare probes for detection of transposable elements in *Z. indianus* and *D. malerkotliana*.

Element Plasmid [1]	Probe type	Primers / Restriction enzymes	Probe extension[2]
copia - p77E4	PCR	LTR-5´CTATTCAACCTACAA AAATAACG3´ PCS-5´ATTACGTTTAGCCTTGTCCAT3´	439 bp
micropia - dhMiF$_2$	PCR	2813-5´TTAACTCCTAGAGTTCATCGCTGG3´ 2814-5´CATGTACCTGGTTAACTACTGACC3´	387 bp
gypsy - pGGHS	PCR	GM003-5´GTACTGAACATTATCAGAATC3´ GM004-5´TCTAAGGAGTCCTCTGCAAGG3´	542 bp
412 - *pBR322*	Restriction fragment	Hind III	~850 bp
297 - *pBR322*	Restriction fragment	Eco RI	~2,3 kb
mdg-1 - *pBR322*	Restriction fragment	Eco RI / Hind III	~1,2 kb
roo/ B104 - *pBR322*	Restriction fragment	Eco RI / Hind III	~2,3 kb
doc - *pBspt Kst*	Restriction fragment	Eco RI / Hind III	~800 kb
jockey - *puC19*	Restriction fragment	Eco RI / Hind III	~1,5 kb
bari-1 - *puC8*	PCR	Br1 – 5' ATTCGTCGCAGGCTAAAAGA 3' Br2 – 5' TTGTAACACCACCTTTGGCA 3'	703 bp

[1] Plasmid source: *copia* and *412* – E. Loreto (UFSM, Santa Maria, RS, Brazil); *gypsy* - D. Dorsett (Memorial Sloan-Kettering Cancer Center, USA); *micropia* – D.H Lankenau (University of Heidelberg, Heidelberg, Germany); *297*, *mdg-1*, *roo/B104* and *doc* – C. Vieira (Université Lyon I, Lyon, France); *jockey* – D.J. Begun (University of California, Davis, USA); *bari-1* – R. Caizzi (Universidade de Bari, Bari, Itália). [2] TE sequences inserted into plasmids were derived from:*D. melanogaster* (p77E4, pGGHS, *pBR322*, *pBspt Kst*, *puC19* and *puC8*) and *D. hydei* (dhMiF$_2$).

The 10 TEs investigated were identified in both *Z. indianus* and *D. malerkotliana*; however, the distribution of sequences homologous to these elements showed different hybridization signals, varying from very strong to weak signals (Figure 1).

The distribution of transposable elements of *Z. indianus* and *D. malerkotliana* is in agreement with the few data previously reported. Among the TEs here analyzed, only *gypsy* had been searched in *Z. indianus* (Heredia *et al.*, 2004), and the authors proposed that this retrotransposon was received

from *D. simulans* through an event of horizontal transfer. Likewise, our result showed a strong homology between *gypsy* probe from *D. melanogaster* and *Z. indianus* sequences, reinforcing the previous data, since *D. melanogaster* and *D. simulans* are sibling species and their *gypsy* sequences are highly similar (Heredia *et al.*, 2004). *D. malerkotliana* had just been studied for *copia* and *412* occurrences. *Copia* had been identified by weak *in situ* hybridization signal on the chromocenter (Biémont and Cizeron, 1999), and *412* had also been shown on the chromocenter both by *in situ* hybridization and Southern blotting (Cizeron *et al.*, 1998). Our analysis showed that *copia* and *412* are components of *D. malerkotliana* genome, but the hybridization signals obtained are also very weak, suggesting that both TEs of *D. malerkotliana* have low homology with those of *D. melanogaster*.

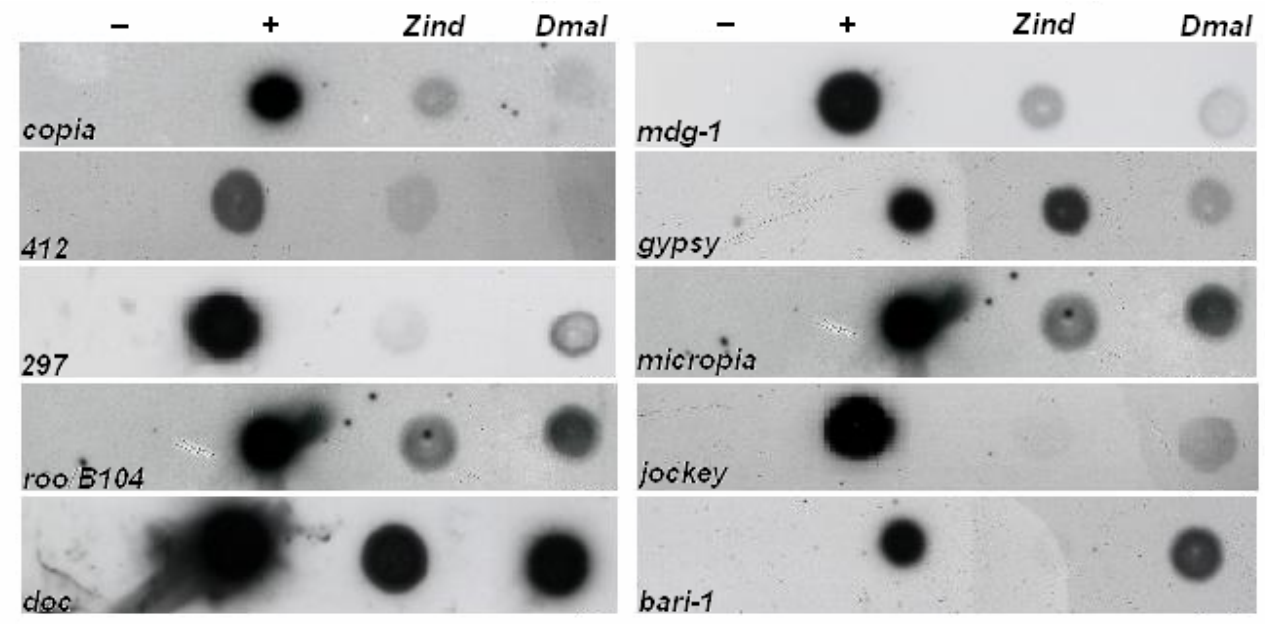

Figure 1. Dot blotting analysis of the transposable elements *copia*, *mdg-1*, *412*, *gypsy*, *297*, *micropia*, *roo/B104*, *jockey*, *doc* and *bari-1* in *Z. indianus* (*Zind*) and *D. malerkotliana* (*Dmal*). (–, negative control; +, positive control).

The results here presented show for the first time the occurrence of several transposable elements in two species scarcely studied in this respect. Except for *doc* and *gypsy* in *Z. indianus* and *doc*, *micropia* and *bari-1* in *D. malerkotliana*, all the analyses produced weak hybridization signals (Figure 1), which suggest that these TEs are divergent from those of *D. melanogaster* and *D. hydei* (for *micropia*) and they should have evolved vertically, accumulating nucleotide variation since the last common ancestor. On the other hand, *doc* and *gypsy* of *Z. indianus* and, *doc*, *micropia* and *bari-1* of *D. malerkotliana* have shown remarkably strong hybridization signals (Figure 1). Further analysis could test which is the most parsimonious hypothesis to explain that high homology between *Z. indianus* / *D. malerkotliana* and *D. melanogaster* / *D. hydei* sequences, if horizontal transfer of these elements between these species or any other alternative factor that can explain high similarity between elements of distantly related species (for example, Almeida and Carareto, 2005; Setta *et al.*, 2007). This report is the first step of a study regarding the dynamics of transposable elements in the

invasive species *Z. indianus* and *D. malerkotliana* and will be used for selecting candidate TEs for more detailed studies.

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Effects of Phloxine B and Hematoporphyrin IX on immature stages of *Drosophila melanogaster.*

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Introduction

Photosensitizers such as xanthene derivatives (*e.g*., phloxine B) and porphyrins (*e.g*., hematoporphyrin IX) are endowed with photoinsecticidal properties. Xanthene derivatives showed acute photo-toxicity against several dipterans (Ben Amor and Jori, 2000, Berni *et al*., 2002, 2003). Pujol-Lereis (2006) compared the photo-toxic effect of phloxine B (PhB) during the postembryonic development of *D. melanogaster* (*Dm*), *Haematobia irritans*, and *Ceratitis capitata*, and determined that *Dm* is affected during larval development. Low concentrations of hematoporphyrin (HP) rapidly decrease adult survival rates of *Ceratitis capitata*, *Bactrocera (Dacus) oleae*, and *Stomoxys calcitrans* (Ben Amor *et al.,* 1998, 2000).

Photosensitizer molecules react upon absorption of visible radiation with the subsequent formation of reactive oxygen species, mediating signaling cascades which either fortify antioxidant defenses of cells or switch to apoptotic death if oxidative pressure is too great (Girotti, 1998). There are two mechanisms by which the photosensitizer can react with biomolecules: type I reactions produce highly reactive oxygen species (*e.g*., the superoxide and the peroxide anions) which usually activate enzymatic antioxidant defense, and type II reactions result in the formation of singlet oxygen, leading mainly to lipid peroxidation. Studies of the effects in immature stages were carried