However, the duration of second and subsequent mounts gradually shortened. The incomplete sperm transfer in the first mount must have been the cause for second and subsequent mounts.

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Esterase loci differences, specificities, and body expression patterns in species of the *Drosophila guarani* group (Diptera; Drosophilidae).

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Abstract

The level of genetic differentiation within populations has received considerable attention as it can indicate the vitality and the potentiality of the population to respond to environmental changes. Several works have combined morphological, isoenzymes, and DNA sequencing to produce better and more complete results about the evolutionary history and genetic structure of populations and species. Thus, the aim of this work was to evaluate the esterase loci differences, specificities, and body expression patterns in two species of *Drosophila guarani* group, in order to obtain a tool for future studies using a combined analysis of isoenzymes, DNA, and morphology. Our esterase loci analysis showed that there are genetic composition differences between species and that these markers could be used in studies of natural populations' genetic variability. However, to obtain better results for *D. maculifrons*, the individual sample should not have the body cut in parts, such as head, thorax and abdomen, because there is head specific locus.

Introduction

Isoenzyme electrophoresis has been used in population and evolutionary researches since 1966 as a way to evaluate populational genetic variability through the direct product of gene expression (Mateus *et al*., 2005). Esterases in *Drosophila* form a polymorphic group of isoenzymes (Johnson, 1974) and can be related with several body functions, such as juvenile hormone levels regulation, digestive processes, reproductive behavior, and insecticide degradation. These enzymes have been detected in all life phases and in many tissues of this group of organisms, which demonstrates the importance of this class of enzymes in the insect normal development (Karotam *et al*., 1993; Gu and Zera, 1994; Feyereisen *et al*., 1995).

The *D. guarani* group belongs to the quinaria-tripuntacta section of the *Drosophila* subgenus and has 16 neotropical species (Bächli, 2009). According to Gottschalk *et al*. (2008), six of these species have been recorded in the Brazilian territory: *D. alexandrei*, *D. guaru*, *D. ornatifrons*, *D. griseolineata*, *D. guaraja*, and *D. maculifrons*. Like other Brazilian species, the *D. guarani* group has been poorly studied, and the data on ecology, systematics, genetics, and evolution its species are scarse. Therefore, much more work related to these aspects should be done.

In this context, this study evaluated for the first time the esterase loci genetic differences and body expression patterns using two species of the *D. guarani* group: *D. ornatifrons* (*D. guarani* subgroup), and *D. maculifrons* (*D. guaramunu* subgroup). For the first species, esterase pattern of the whole body was investigated, whereas for the second, body expression pattern (head, thorax and abdomen) and also esterase specificity to α- and β-naphthyl acetates were analyzed in order to provide a tool for future combined works using isozyme, DNA, and morphology data.

Material and Methods

The *Drosophila* species were collected in 2008 in a conservation area in Guarapuava, Parana, Brazil (25°23'36" N, 51°27'19" W), named Parque Municipal das Araucárias, which covers approximately 100 ha, and 42.75% of the total area is composed by Araucaria forest. Flies collected were identified, and individuals of the species *D. maculifrons* and *D. ornatifrons* were stored individually at -20°C for later electrophoretic analysis.

The analysis of esterase activity was performed through electrophoresis on 10% polyacrylamide gel, according to the method described by Ceron (1988). The identification of esterases in the gel was performed using α- and β-naphthyl acetates as substrates, and the products of the reactions were stained by Fast Blue-RR salt. For *D. ornatifrons*, the esterase pattern of the whole body was analyzed. For *D. maculifrons*, the pattern of body expression (head, thorax and abdomen) and also esterase specificity for α- and β-naphthyl acetates were investigated. The enzyme loci were numbered in ascending order of anodal mobility.

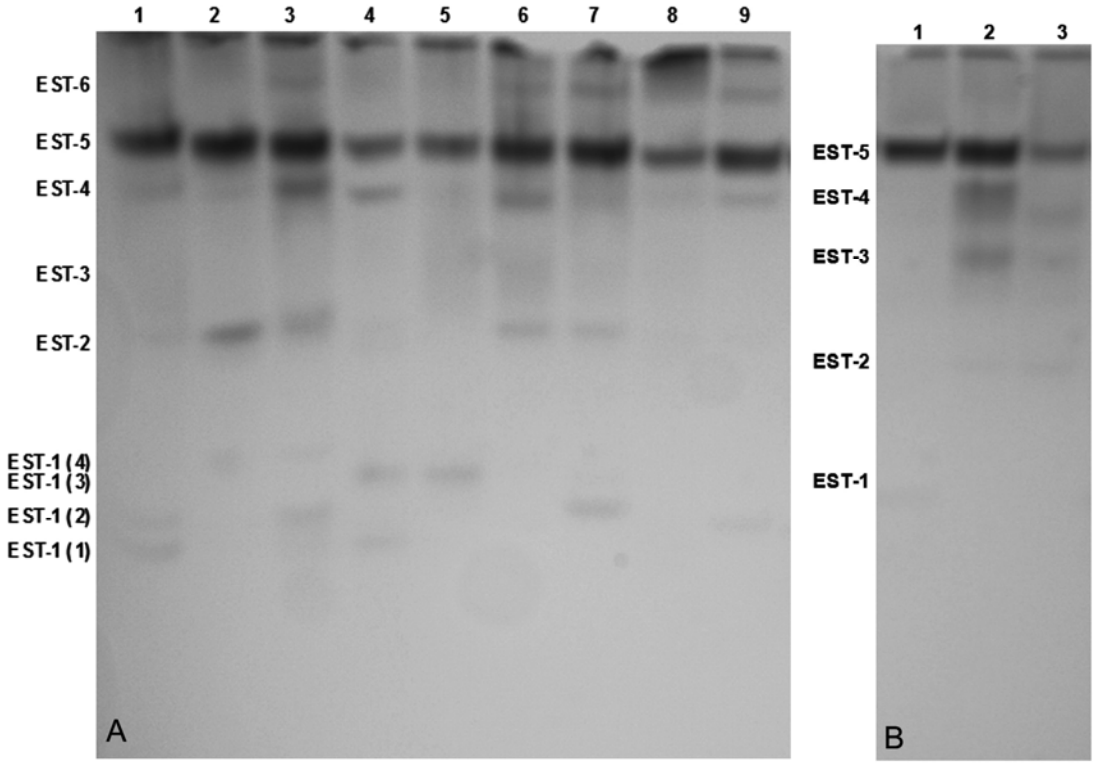

Figure 1. Esterase profile of (A) *Drosophila ornatifrons* and (B) *Drosophila maculifrons* head (1), thorax (2) and abdomen (3).

Results and Discussion

Esterase profile analysis in *D. ornatrifrons* species revealed the presence of six loci, four α-esterases (EST-1, EST-2, EST-5 and EST-6) and two β-esterases (EST-3 and EST -4). All these loci were monomorphic, except by EST-1, which presented four alleles (Figure 1-A). *Drosophila maculifrons* presented five monomorphic loci (Figure 1-B). EST-1, EST-2 and EST-5 were α-esterase exclusive as they were not stained in the presence of only β-naphthyl acetate. EST-3 and EST-4 loci were preferentially β-esterases as they were visualized when in contact with one or the other substrate independently, but in the presence of both substrates at the same time they preferentialy reacted with β-naphthyl acetate. The analysis of the esterase profile of both species revealed differences in the genetic composition, which is consistent with the patterns found for other groups of insects (Bueno *et al*., 2003; Reyes *et al*., 2004, as examples).

The esterase body expression pattern in *D. maculifrons* revealed that the EST-1 locus is exclusive of the head. EST-2, EST-3 and EST-4 were detected in the chest and abdomen. EST-5 had no specific pattern of body expression as it was observed in all body part samples. The differences in body expression pattern of esterase loci in *D. maculifrons* species suggests that individuals should not be cut in parts (head, thorax and abdomen) in studies where the aim is to access the genetic variability of these enzymes, as suggested for other *Drosophila* species (Mateus *et al*., 2005; Cavasini *et al*., 2008), because it will generate loss of information as there is head specific locus.

However, new studies about physiology, enzyme activity and gene expression should be performed in different tissues in order to investigate the functional and differential expression observed for these loci on this species. The results of such work could be related to the differences in body/tissue expression pattern during development and also under the effects of contaminants, which can affect the specificity of these enzymes.

Our results showed that there is genetic differentiation among the species studied, indicating the necessity for further studies using natural populations of *D. guarani* species group, which may contribute to clarify the phylogenetic relationships among the still controversial *D. guarani* and *D. guaramunu* subgroups (Robe *et al*., 2005). Furthermore, in the *D. maculifrons* genetic variability analysis, regarding the methodology used here, individuals should not be cut in body parts because there is a head specific locus.

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