included in this poorly resolved "western" clade of *D. aldrichi* based on *cox1* data (D. Oliveira, unpubl. data). Therefore, the identity of our recently discovered "*D. wheeleri*" from Sonora is still tentative, and resolution will require further collections and additional sequence data to resolve the phylogenetic relationships within this species cluster.

Cultures of these species are available from the author, and Sonoran "*D. wheeleri*" has been deposited at the *Drosophila* species stock center.

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Isoenzymatic analysis of South American species of the *Drosophila tripunctata* group (Diptera, Drosophilidae).

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Abstract

The *Drosophila tripunctata* species group is endemic of the Neotropical region, is the second largest Drosophilidae group in this region, and the largest forest group of the genus. The South-Center region of the Parana State, Brazil, is characterized by a wet, cool season, and represents the most preserved redoubt of Araucaria rain forest remnants. In this work we tested four allozyme

systems in 11 neotropical species of the *D. tripunctata* group that occur in this region to analyze their variability and potential use as genetic markers in studies of conservation and forest fragmentation effects over genetic variability of forest dwelling *Drosophila* species. A total of 5 loci and 20 alleles (6 in *Idh*, 5 in *Mdh-1*, 3 in *Mdh-2*, 3 in *Me*, and 3 in *Gpdh*) were detected. All loci showed more than one allele. *Idh* and *Mdh-1* presented the highest number of alleles (6 and 5, respectively) when compared to the others, which showed 3 different alleles each. All species were polymorphic in at least two loci. Overall, *D. pruinifacies* showed to be more divergent to the other species regarding its allelic composition of the loci analyzed. These data revealed a good potential to be used in following works about populational, conservational, and evolutionary aspects related to the effect of fragmentation on the *Drosophila* communities in the subtropical rain forests of South America.

Introduction

The *Drosophila tripunctata* species group is endemic of the Neotropical region, with exception of *D. tripunctata* which occurs in the Neartic (Vilela, 1992). It is the second largest Drosophilidae group in the neotropic, enclosing 64 nominal species, and the largest forest group of the genus (Vilela and Bächli, 2000; Klaczko, 2006). According to Sene *et al.* (1980), it is abundant in forests, occurs in low frequencies in the Cerrado biome and dunes, and is absent in the Caatinga. Pavan (1959) suggests that this group is more frequent near rivers and lakes during the coldest months of the year.

The South-Center region of the Parana State, Brazil, is characterized by a wet, cool season, with hoar frosts being common and severe, and the average temperature is below 22^{o}C during the warmest months. This region represents the most preserved redoubt of Araucaria rain forest remnants. The nature of land-use change in recent decades has not only resulted in a dramatic decrease in total forest cover, but also in an increasingly skewed size distribution of forest remnants. Forest fragmentation is an important process contributing to the present-day concern over the loss of biodiversity and rates of species extinction.

Very few works have been conducted regarding allozyme variation in natural populations of *D. tripunctata* species group, most of them using *D. tripunctata* (Lacy, 1982, 1983). Therefore, the aim of this work was to test four allozyme systems in 11 neotropical species of the *D. tripunctata* group that occur in the south-center region of Paraná, to analyze their variability and potential use as genetic markers in studies of conservation and forest fragmentation effects over natural populations genetic variability of forest dwelling *Drosophila* species.

Material and Methods

Surveys were conducted during the year 2006 in two fragments of mixed rain forest (Araucaria rain forest) located in the municipality of Guarapuava, Paraná state, Brazil: "Parque Municipal das Araucárias" (25^{o}23'36"S, 51^{o}27'19"W) and "Fazenda Brandalise" (25^{o}18'21"S, 51^{o}25'12"W). The following species of the *Drosophila tripunctata* group were obtained: *Drosophila angustibucca*, *D. bandeirantorum*, *D. bifilum*, *D. medioimpressa*, *D. mediopicta*, *D. mediopunctata*, *D. mediosignata*, *D. mediostriata*, *D. platitarsus*, *D. prosimilis*, and *D. pruinifacies*.

The specimens were analyzed in 14% starch gel (Penetrose 30™) stained for four isoenzymatic systems, according to Mateus and Sene (2003): Isocitrate dehydrogenase (IDH), Malate dehydrogenase (MDH), Malic enzyme (ME), and Alpha-glycerol-phosphate dehydrogenase (1-GPDH).

Results and Discussion

The electrophoretic analyses resulted in a total of 5 loci and 20 alleles (6 in *Idh*, 5 in *Mdh-1*, 3 in *Mdh-2*, 3 in *Me* and 3 in *Gpdh*) for the four isoenzymatic systems tested. Figure 1 shows part of the results obtained. Lacy (1982) also had detected 5 loci for the same 4 isoenzymatic systems tested here, with MDH showing two loci (*Mdh-1* and *Mdh-2*). For the *Idh* locus, the more cathodic enzyme was detected only in *D. mediopicta*. On the other hand, for the *Mdh-1* and *Gpdh* loci, the slower enzymes were found only in *D. pruinifacies* specimens. All loci showed more than one allele. *Idh* and *Mdh-1* presented the highest number of alleles (6 and 5, respectively) when compared to the others, which showed 3 different alleles each.

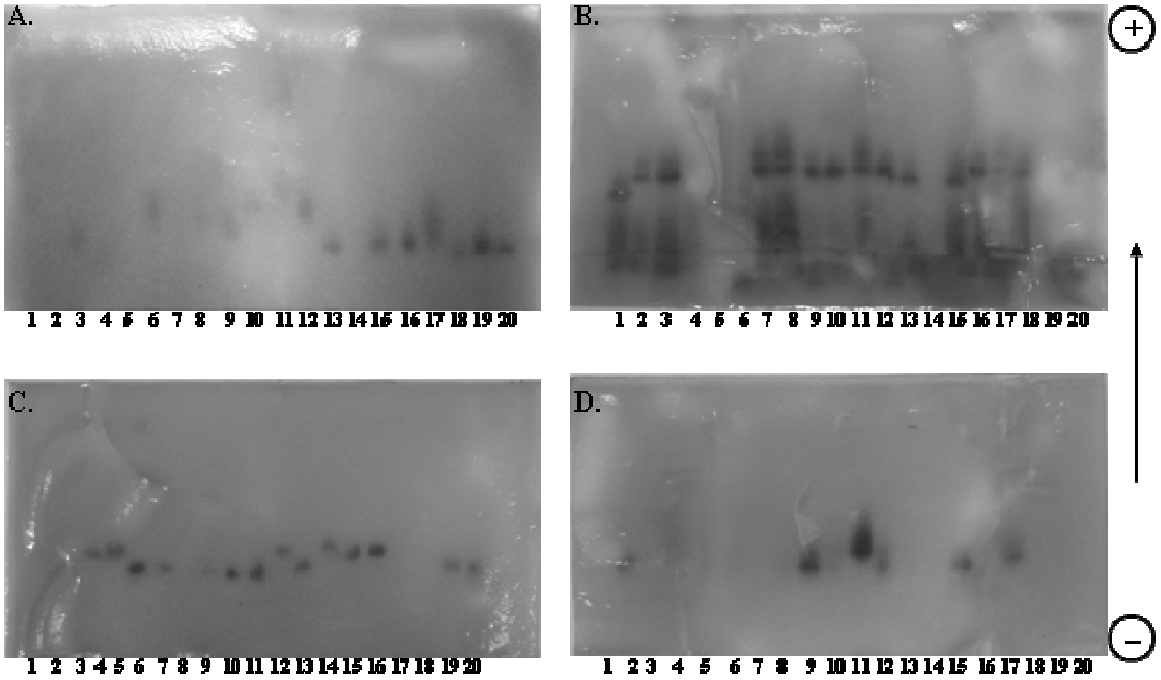

Figure 1. Starch gel (14%) isoenzymatic profile of *Drosophila tripunctata* species group. A. IDH. B. MDH. C. ME. D. 1-GPDH. Samples: 1 to 17 – "Parque Municipal das Araucárias"; 18 a 20 – "Fazenda Brandalise". 1 and 2 - *D. mediopicta*; 3 and 4 - *D. prosimilis*; 5 to 8 - *D. mediostriata*; 9 and 10 - *D. mediopunctata*; 11 to 14 - *D. bandeirantorum*; 15 to 17 - *D. medioimpressa*; 18 and 19 - *D. mediopicta*; 20 - *D. prosimilis*.

Drosophila bandeirantorum was polymorphic for all loci. *Drosophila mediosignata* and *D. mediostriata* presented more than one allele in 3 loci (*Idh*, *Mdh-1* and *Me*; *Idh*, *Mdh-2* and *Me*, respectively). *Drosophila medioimpressa*, *D. mediopicta*, *D. mediopunctata*, and *D. pruinifacies* were polymorphic for 2 loci (*Mdh-1* and *1-Gpdh*; *Idh* and *Mdh-1*; *Idh* and *Mdh-2*; *Mdh-1* and *Mdh-2*, respectively). Overall, *D. pruinifacies* showed to be more divergent to the other species regarding its allelic composition of the loci analyzed.

These data are preliminary and reveal important features of the genetic variability of the *D. tripunctata* group species surveyed, and both the genetic marker and *Drosophila* group have good potential to be used in following works about populational, consevational, and evolutionary aspects

related to the effect of fragmentation on the *Drosophila* communities in the subtropical rain forests of South America.

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Methodology tune up for the assessment of desiccation resistance in natural populations of *Drosophila buzzatii*.

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Introduction

Arid environments impose strong selective pressures on organisms. Particularly, insects and other terrestrial arthropods are susceptible to water loss due to their small size, especially in desert environments (Gibbs *et al*., 1997). Comparative interspecific studies have showed that in the genus *Drosophila*, species from arid environments exhibit physiological characteristics that allow the maintenance of water balance (Marron *et al*., 2003). Intraspecific studies have focused mainly on *D. melanogaster*. These studies do not agree on the mechanisms involved in the ability to cope with desiccation conditions. In order to investigate how hydric stress explains ecological responses and distribution patterns, it appears appropriate to assess the adjustment to dry conditions and its intraspecific variation in desert dwelling species.

Certainly, populations can differ in a trait at a geographic scale, which could also be related to ecological diversity and/or mechanistic variations at lower organization levels. In this sense, a comparative study must focus on the genetic basis of the genotype-environment interaction (Mackay and Anholt, 2007), and how it provides support for a species dealing with challenging and heterogeneous conditions. Such a study requires a prior assessment of experimental conditions in pilot studies, in order to adjust the technique to the selected model species.

The objective of this work was to tune up a method for measuring desiccation resistance in *D. buzzatti*, a cactophilic and widely distributed species in southern South America, and finally to set an experimental design that allows us to detect within and among population variation and phenotypic plasticity by means of the response of flies to different desiccation treatments. The specific questions addressed are: i) how intense should a desiccation treatment be to exert an effect on survival?; ii) how subtle should desiccation be to reveal a differential effect among populations differently adjusted to dry conditions?; iii) which is the most accurate and representative response variable to measure the effect of desiccation treatments among individuals?; iv) does variation in the response to desiccation vary between males and females?; v) is there standing genetic variation (differences among isofemale lines) in the response to desiccation?; vi) does desiccation resistance vary among populations?