L.P.B., L. Madi-Ravazzi, and W.J. Tadei 2006, Braz. J. Biol. 66: 279-293; Machado, L.P.B., R.P. Mateus, F.M. Sene, and M.H. Manfrin 2010, Biol. J. of Linn. Soc. 100: 573-584; Madi-Ravazzi, L., H.E.M.C. Bicudo, and J.A. Manzato 1997, Cytobios 89: 21-30; Manfrin, M.H., and F.M. Sene 2006, Genetica 126: 57–75; Manfrin, M.H, R.O.A. de Brito, and F.M Sene 2001, Ann. Entomol. Soc. Am. 94: 333-346; Mateus, R.P. and F.M. Sene 2003, Biochem. Genet. 41: 219-233; Mateus, R.P. and F.M. Sene 2007, J. Zool. Syst. Evol. Res. 45: 136-143; Mateus, R.P., L.P.B. Machado, and F.M. Sene 2005, Dros. Inf. Serv. 88: 46-48; Mateus, R.P., L.P.B. Machado, E.M. Moraes, and F.M. Sene 2010, Biochem. Syst. Ecol. 38: 410-415; Miller, M.P., 1997, Software can be downloaded from http://www.marksgeneticsoftware.net/tfpga.htm; Monteiro, S.G., R.P. Mateus, M.O. Moura, and F.M. Sene 2010, submitted, Contrasting patterns of within-species morphometric variation in two cactophilic species of Drosophila (Diptera: Drosophilidae). Organ. Div. Evol.; Moraes, E.M., and F.M. Sene 2002, J. Zool. Syst. Evol. Res. 40: 123–128; Nei, M., 1972, Am. Nat. 106: 283-292; Ruiz, A., A.M. Cansian, G.C.S. Kuhn, M.A.R. Alves, and F.M. Sene 2000, Genetica 108: 217–227; Sene, F.M, M.A.Q.R. Pereira, C.R. Vilela, and N.M.V. Bizzo 1981, Dros. Inf. Serv. 56: 118–121; Soto, I.M, E.M. Soto, C. Corio, V.P. Carreira, M.H. Manfrin, and E. Hasson 2010, Environ. Entomol. 39: 865-873; Thorpe, J.P., 1983, Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Protein Polymorphism: Adaptive and Taxonomic Significance. (Oxford, G.S., and D. Rollinson, eds.). Academic Press, New York, pp. 131-152; Tidon-Sklorz, R., and F.M. Sene 2001, Ihering. Sér. Zool. 90: 141-146; Vilela, C.R., 1983, Rev. Bras. Ent. 27: 1-114; Vilela, C.R., and F.M. Sene 1977, Pap. Avul. Zool. 30: 295-299; Wright, S., 1978, *Evolution and the Genetics of Population: Variability Within and Among Natural Populations*, Vol. 4. University Chicago Press, Chicago; Zouros, E., 1973, Evolution 27: 601–621.

Allozymatic activity in samples prepared for morphometric and molecular analyses in two species of the *Drosophila guarani* group (Diptera: Drosophilidae).

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Abstract

The degree of genetic variation within populations is important and has received some attention in the literature as it can be an indication of the overall species vitality and of its population´s potential to adapt to new environments. Such variation has been assessed through several morphological and molecular markers, which in combination give better results about the evolutionary history and population genetic structure of a species. The aim of this work was to evaluate the allozymic activity in specimens of two species of the *Drosophila guarani* group, *D. ornatifrons* and *D. maculifrons*, using a sample separation methodology that maximizes obtaining morphological and molecular data. Our results showed that there is a higher activity of the allozymic loci analyzed in the thorax and abdomen, and also that the wing and aedeagus previous separation for morphometric analyses did not change the allozyme loci visualization in the gel. No loci analyzed presented specific pattern of expression in the head, indicating that this body part could be used to perform DNA extraction for further molecular markers analyses. Thus, the same specimen can be

analyzed for different markers, increasing the power of investigation of the evolutionary history and genetic population structure of the species of interest. Moreover, this methodology diminishes the need to collect many individuals and/or to perform several collection trips to the same area, therefore, maximizing the sample utilization obtaining results for several different markers (morphological and molecular) from the same sample.

Introduction

The population genetic variation has been accessed through several morphological and molecular markers, which, in combination, give better results about the evolutionary history and population genetic structure of a species (see Ruiz *et al*., 2000; Moraes and Sene, 2007; Gamper e*t al*., 2010; Jenner, 2010, as examples). Allozyme electrophoresis has been used in populational and evolutionary research of several organisms, including *Drosophila*, because it analyses the genetic variability of a population through the direct product of a gene (Lapenta *et al.*, 1998; Mateus and Sene, 2003, 2007; Moraes and Sene, 2002; Zawadzki *et al*., 2005, 2008). Thus, the aim of this work was to evaluate the allozymic activity in two species of the *Drosophila guarani* group, *D. ornatifrons* and *D. maculifrons*, using a sample separation methodology that maximizes obtaining morphological and molecular data.

Materials and Methods

Drosophila maculifrons and *D. ornatifrons* specimens used in this study were collected in two areas: Parque Municipal das Araucárias (25°23'36" S, 51°27'19" W), municipality of Guarapuava, State of Parana, Brazil; and Estação Experimental de Zootecnia (21°10' S, 48°05' W), municipality of Sertãozinho, State of São Paulo, Brazil. The collections were performed according to Garcia *et al*. (2009), and after the species separation, the specimens of the two species of interest of the *Drosophila guarani* group were individually dry stored in 1.5 mL tubes at – 20^{o}C for further allozymic analyses.

The electrophoretic profile was compared between samples of entire individuals and samples of body parts, as follows: - head; - thorax; - thorax without wings; - abdomen; - abdomen without aedeagus; and thorax and abdomen without wings and aedeagus. Seven allozymic systems were analyzed: Alpha-glycerol-phosphate dehydrogenase (GPDH), Esterase (EST), Isocitrate dehydrogenase (IDH), Leucine amino peptidase (LAP), Malate dehydrogenase (MDH), Malic enzyme (ME), and Phosphoglucomutase (PGM), according to Mateus and Sene (2003).

Results and Discussion

Mateus *et al*. (2005) determined the body expression pattern for several allozymic systems in the cactophilic species *D. antonietae*, and concluded that the body parts could be separated in order to analyze different markers. Different *Drosophila* species, however, even phylogenetically close to each other, can differ in the allozyme loci expression pattern (Mateus *et al*., 2010), and the results of Mateus *et al*. (2005) could not be the same for other *Drosophila* species.

In previous work, Saavedra *et al.* (1995) performed an analysis of four allozymic loci polymorphisms in *Drosophila maculifrons*, a species of the *D. guarani* group, using polyacrylamide gel electrophoresis (PAGE), and Silva *et al*. (2009) evaluated the body expression pattern of esterases

in *D. maculifrons* and *D. ornatifrons*. In this work we analyzed two species of the *Drosophila guarani* group, *D. maculifrons* and *D. ornatifrons*, regarding the body expression pattern of allozymic systems other than those previously investigated and also using methodology different from Saavedra *et al*. (1995) and Silva *et al*. (2009), that is, starch gel electrophoresis. In spite of the fact that this technique has lower band definition, it maximizes the sample utilization by yielding the application of the same individual in more than one gel, which can be used to be stained for more allozymic systems.

Figure 1 shows the electrophoretic profile of some allozymatic systems analyzed in this work in *Drosophila maculifrons* and *D. ornatifrons*. The IDH, LAP, and PGM systems showed only one locus each (*Idh* – Figure 1-A, *Lap* and *Pgm*, respectively), with no difference in the activity in the body parts for both species. The GPDH systems also presented only one locus (*Gpdh*) with higher activity in the thorax in both species. For esterases (Figure 1B), it was detected one locus *D. ornatifrons* and two for *D. maculifrons*. In this species, the first locus (*Est-1*) had higher activity in the thorax and the second (*Est-2*) was poorly detected in the whole body. A second sporadic EST locus was detected specifically in the abdomen of *D. ornatifrons* (arrow in Figure 1B), but it was not considered because of its very low frequency. In the MDH system (Figure 1C), one cathode locus (*Mdh-1*) was detected that had higher activity in the thorax and abdomen. The anode locus (*Mdh-2*) had higher activity in the abdomen. In the ME system (Figure 1D), only one locus (*Me*) with higher activity in the abdomen was observed.

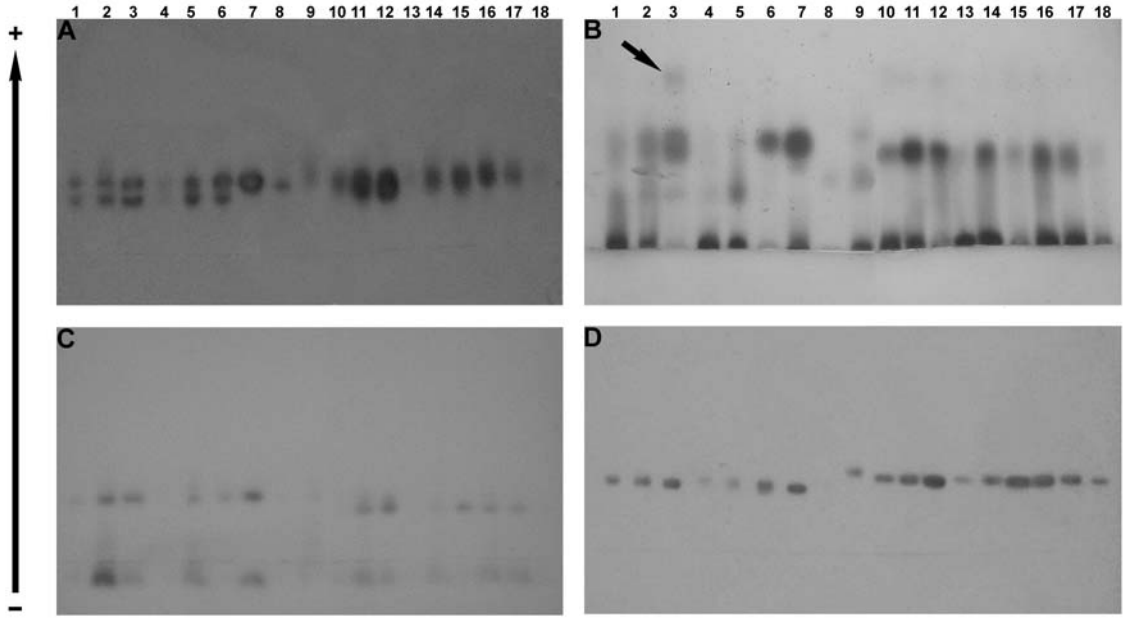

Figure 1. Electrophoretic profile for esterase in two species of the *Drosophila guarani* group. **A.** IDH; **B.** EST; **C.** MDH; **D.** ME. 1 to 9 – *D. ornatifrons*; 10 to 18 – *D. maculifrons*. 1, 4, 10 and 13 – 3 heads; 2 and 11 – 3 thorax; 3 and 12 – 3 abdomens; 5 and 14 – 3 thorax without wings; 6 and 15 – 3 abdomens without aedeagi; 7 and 16 – entire individuals; 8, 9, 17 and 18 – thorax/abdomen (without wing and aedeagus). Arrow: esterase locus specific to the abdomen in *D. ornatifrons*.

All these results demonstrated that there is higher activity for all allozyme loci analyzed in the thorax and abdomen, and that removing wings and aedeagus for morphometric analyses did not modify the detection of the allozyme loci in the gel. No locus depicted activity specific of the head, indicating that this body part could be used for DNA extraction for further nuclear and mitochondrial molecular markers studies. It was possible to observe that the allozymatic activity is much more affected by the sample quality (how many times it was defrosted? How long took the identification after collection or the manipulation before electrophoresis?) rather than the body part separation.

Thus, the specimens of *Drosophila maculifrons* and *D. ornatifrons* can have the body parts separated to be used in different analyses without interfering with the quality of the obtained data. This makes the work easier as it diminishes the need to collect many individuals and/or to perform several collection trips to the same area, therefore, maximizing the sample utilization obtaining results for several different markers (morphological and molecular) from the same sample. The possibility to investigate the same individual for different markers and, therefore, to perform a combined analysis, yields to respond to biological questions more efficiently and also contributes to wider investigations about the evolutionary history, population structure, and conservational aspects of the studied organisms.

References: Gamper, H.A., M.G. van der Heijden, and G.A. Kowalchuk 2010, New Phytol. 185: 67-82; Garcia, A.C.L., M.S. Gottschalk, M.A. Montes, V.H. Valiati, C. Rohde, and V.L.S. Valente 2009, Dros. Inf. Serv. 92: 80-87; Jenner, R.A., 2010, Arthropod Struct. Dev. 39: 143-153; Lapenta, A.S, H.E.M.C. Bicudo, C.R. Ceron, and J.A. Cordeiro 1998, Cytobios 96: 95-107; Mateus, R.P. and F. M. Sene 2003, Biochem. Genet. 41: 219-233; Mateus, R.P., and F.M. Sene 2007, J. Zool. Syst. Evol. Res. 45: 136-143; Mateus, R.P., L.P.B. Machado, and F.M. Sene 2005, Dros. Inf. Serv. 88: 46-48; Mateus, R.P., L.P.B. Machado, E.M. Moraes, and F.M. Sene 2010, Biochem. Syst. Ecol. 38: 410-415; Moraes, E.M., and F.M. Sene 2002, J. Zool. Syst. Evol. Res. 40: 123-128; Moraes, E.M., and F.M. Sene 2007, J. Zool. Syst. Evol. Res. 45: 336-344; Ruiz, A., A.M. Cansian, G.C.S. Kuhn, M.A.R. Alves, and F.M. Sene 2000, Genetica 108: 217–227; Saavedra, C.C.R., V.L.S. Valente, and M. Napp 1995, Rev. Brasil. Genet. 18: 147-164; Silva, D.C., K. dos Santos, E.C. Gustani, P.T. Rodrigues, D.P. Simão, L.P.B. Machado, and R.P. Mateus 2009, Dros. Inf. Serv. 92: 70-72; Zawadzki, C.H., E. Renesto, R.E. Reis, M.O. Moura, and R.P. Mateus 2005, Genetica 123: 271-283; Zawadzki, C.H., E. Renesto, and R.P. Mateus 2008, Biochem. Genet. 46: 755-769.

Sampling two species of the *Drosophila guarani* group in a fragment of Araucaria Forest: testing different types of baits, fermentation time, and period of the day.

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Abstract

Most of the phylogenetic relationships within the *Drosophila guarani* group still remain unclear and recent studies have been conducted with this aim. Thus, work that could indicate the distribution area and the best way to collect species of this group are very important. The aim of this work was, therefore, to test the best period of the day to collect two species of the *Drosophila*