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Genetic variability analysis of two natural populations of *Drosophila antonietae* (Diptera; Drosophilidae).

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Abstract

Drosophila antonietae belongs to the *D. buzzatti* cluster (*D. repleta* group), and its populations are always associated with the cactus *Cereus hildmaniannus*. It differs from the other *D. buzzatii* cluster species by showing a higher genetic homogeneity among its populations for different markers despite the wide geographic distribution of this species. Gene flow restriction by distance, along with a relatively recent fragmentation maintenance of ancient polymorphism, has been used as the most likely explanations for this pattern. All previous analyses were, however, realized with populations located in the high and low portions of the Parana-Uruguay rivers basin. No population located in the middle portion was analyzed so far. Thus, this work aimed to analyze two *D. antonietae* populations located in the middle portion of Parana-Uruguay rivers basin, Rio do Poço-Guarapuava/PR and Cantagalo/PR, not yet sampled, using 10 allozymic loci, to try to correlate our findings with previously published data for this species. Our results showed that the allozymic genetic variabilities of both *D. antonietae* populations are in agreement with that expected for the species, and the most likely factors responsible for the high within population diversity (Cantagalo – Ho = 0.2337; Rio do Poço – Ho = 0.3425) and low among population differentiation (D = 0.0307; I = 0.9698; Fst = 0.0081) must be gene flow restricted by distance with recent fragmentation and maintenance of ancient polymorphism.

Introduction

Studies using different species of the *Drosophila buzzatii* cluster (*D. repleta* group) showed that they constitute an interesting biological system for evolutionary research as they present polymorphisms, polytypism, ecological specificities and different levels of among population

differentiation (Vilela and Sene, 1977; Kuhn *et al*., 1996; Madi-Ravazzi *et al.*, 1997; Lapenta *et al*., 1998; Machado *et al*., 2002, 2006; Manfrin and Sene, 2006; Soto *et al*., 2010). Such species are cactophilic and this ecological specificity probably made their populations to follow the xerophitic vegetations expansions and retractions that have occurred during the paleoclimate changes (Bigarella *et al*., 1975; Ab'Saber, 2000; Manfrin *et al*., 2001).

Drosophila antonietae belongs to the *D. buzzatii* cluster and has specificity to *Cereus hildmaniannus* cactus, which is distributed over the Parana-Uruguay rivers basin, in the South and Southeast regions of Brazil and to the north of the eastern border of the Argentinean Chaco (Tidon-Sklorz and Sene, 2001). It differs from the other *D. buzzatii* cluster species by showing a higher homogeneity among its populations for several markers, despite its wider geographic distribution (Baimai *et al*., 1983; Ruiz *et al*., 2000; Manfrin *et al*., 2001; de Brito *et al*., 2002; Mateus and Sene, 2003, 2007; Machado *et al*., 2010; Mateus *et al*., 2010; Monteiro *et al*., 2010). Most of these studies, however, analyzed populations from the higher and lower portions of the Parana-Uruguay river basin. Populations of the middle portion were not surveyed yet.

The present work analyzed the genetic variability of *Drosophila antonietae* populations from the middle portion of the Parana-Uruguay rivers basin, not yet sampled, using allozymic loci, aiming to correlate our findings with previously published allozyme population data from the higher and lower portions of the Parana-Uruguay rivers basin (Mateus and Sene, 2007; Mateus *et al.*, 2010). Thus, we will be able to contribute to clarifying the historical and ecological events that influenced the evolutionary history of this species.

Material and Methods

Collections

Drosophila antonietae specimens were collected in November 2008 in two areas of *Cereus hildmaniannus* occurrence in the Cavernoso river, an Iguassu river tributary, located in the middle portion of the Parana-Uruguay rivers basin, named as Cantagalo-PR (25º 25' 00.0" S, 52º 04' 14.9" W) and Rio do Poço/Guarapuava-PR (25º 17' 29.8" S, 51º 53' 08.8" W), both in the Parana State, Brazil. The collections were performed as described by Sene *et al*. (1981), using a mixture of banana, orange, and yeast as bait.

All *D. antonietae* males were identified through the aedeagus morphology according to Vilela (1983). Following the protocol proposed by Mateus *et al.* (2005), thorax and abdomen were individually dry stored at -20ºC for the allozymic analyses. The respective heads were stored in 70% ethanol at -20ºC for further analyses using DNA markers.

Allozyme Analyses

The genetic variability analysis of the two *D. antonietae* populations were performed using 57 specimens from Cantagalo and 23 from Rio do Poço, according to the method described by Mateus and Sene (2003). The following allozymic systems were applied: Alcohol dehydrogenase (ADH), Esterase (EST), Alpha-glycerol-phosphate dehydrogenase (1-GPDH), Hexokinase (HK), Isocitrate dehydrogenase (IDH), Malic enzyme (ME), Malate dehydrogenase (MDH), and Phosphoglucomutase (PGM). All stained gels were photographed using a 10.2 MP Sony câmera coupled to the L-Pix (Loccus) image capture system. The images were used in the genotyping, and the different loci and alleles were named according to Mateus and Sene (2003).

Populational Analyses

The population genetic analyses were performed using the TFPGA software (Miller, 1997). As *Adh-2*, *Hk-3* and *Pgm* loci were detected in only one population, they were excluded before calculating genetic distances and identities, and also F statistics. The heterozygote deficiency (Fis) and genetic differentiation (Fst) were measured using the qualitative guideline proposed by Wright (1978): 0 - 0.05 = low; 0.05 - 0.15 = moderate; 0.15 - 0.25 = high; above 0.25 = very high.

Results and Discussion

The Cantagalo population showed 70% and Rio do Poço showed 80% of polymorphic loci. The mean observed heterozygosity (Ho) was 0.2337 for Cantagalo and 0.3425 for Rio do Poço, and the mean expected heterozygosities were 0.4260 and 0.5340, respectively. For both populations, only three loci (*Est-1*, *Est-2*, and *Idh*) showed significant departure from the Hardy-Weinberg equilibrium (Table 1).

Table 1. Allele frequency for the 10 allozymatic loci analyzed for two *Drosophila antonietae* populations, Cantagalo and Rio do Poço. Numbers in bold indicate loci with departure from the Hardy-Weinberg Equilibrium; n = sample size; % = proportion of polymorphic loci; Ho = mean observed heterozygosity; He = mean expected heterozygosity.

Alleles	Cantagalo	Rio do Poço	Alleles	Cantagalo	Rio do Poço
Adh-2	(n = 02)	(n = 00)	*Hk-1*	(n = 08)	(n = 06)
1.00	1.00	-	1.00	1.00	1.00
Est-1	(n = 27)	(n = 19)	*Mdh*	(n = 36)	(n = 14)
0.95	**0.3704**	**0.1842**	1.00	1.00	1.00
1.00	**0.2037**	**0.4474**	*Idh*	(n = 39)	(n = 17)
1.05	**0.1111**	**0.0263**	0.95	**0.0897**	0.1176
1.10	**0.1296**	**0.1053**	1.00	**0.7692**	0.5882
1.15	**0.1852**	**0.2368**	1.02	**0.1410**	0.2941
Est-2	(n = 25)	(n = 20)	*Me*	(n = 06)	(n = 10)
0.95	**0.2200**	**0.2500**	0.95	0.1667	0.3000
1.00	**0.4000**	**0.4250**	1.00	0.8333	0.7000
1.05	**0.2200**	**0.2250**	*Pgm*	(n = 07)	(n = 00)
1.10	**0.1600**	**0.1000**	0.90	0.2143	-
1-Gpdh	(n = 37)	(n = 08)	0.95	0.2143	-
1.00	0.7432	0.5625	1.00	0.2857	-
1.05	0.2568	0.4375	1.05	0.2857	-
Hk-3	(n = 00)	(n = 05)	%	70%	80%
0.95	-	0.4000	Ho	0.2337	0.3425
1.00	-	0.6000	He	0.4260	0.5340

The mean observed heterozygosity of both *D. antonietae* populations (Cantagalo – Ho = 0.2337; Rio do Poço – Ho = 0.3425) were higher than those obtained by Mateus *et al*. (2010) for one *D. antonietae* populations (0.179) and for one *D. gouveai* population (0.063), another species of the *D. buzzatii* cluster. But when compared to the Mateus and Sene (2007) results, who analyzed 11 different *D. antonietae* populations, only the Ho from Rio do Poço was higher than their average (0.319). This demonstrates that the values obtained here were similar to those previously obtained for the same species using the same genetic marker. Moreover, the Ho obtained for the two *D.*

antonietae populations analyzed in this work were higher than the Ho for cactophilic (0.087) and non-cactophilic (0.160) *Drosophila* species (allozyme data from Zouros, 1973; Johnson, 1974; Barker and Mulley, 1976; and Moraes and Sene, 2002).

This higher diversity of *Drosophila antonietae* over other cactophilic species was also found using other markers. Monteiro *et al*. (2010), analyzing aedeagus morphometry, suggest that the maintenance of within population diversity could be the result of gene flow restricted by distance, which can be favored by the corridor formation of the host cacti in the Parana-Uruguay rivers basin, facilitating the genetic exchange among neighboring populations. Mateus and Sene (2007) and Machado *et al*. (2010), using allozyme and microsatellite DNA polymorphisms, respectively, detected moderate genetic differentiation among *D. antonietae* populations. Mateus and Sene (2007) proposed historical gene flow and natural selection over allozyme loci as the main factors to explain the pattern observed, not discarding the maintenance of ancient polymorphism hypothesis, as also suggested by Machado *et al*. (2010).

Table 2. Wright´s F statistics for the polymorphic allozymic loci of *Drosophila antonietae* populations from Cantagalo and Rio do Poço. C.I. = confidence interval.

Locus	Fis	Fit	Fst
Est-1	0.1619	0.1967	0.0416
Est-2	0.4841	0.4682	-0.0308
1-Gpdh	-0.3085	-0.2417	0.0511
Idh	0.1106	0.1457	0.0394
Me	0.6952	0.6724	-0.0749
All loci	0.2494	0.2555	0.0081
C.I. 95% - minimum	-0.0564	-0.0328	-0.0405
- maximum	0.5303	0.5114	0.0450

Nei (1972) genetic distance (0.0307) and identity (0.9698) are in agreement with Avise and Smith (1977) and Thorpe (1983) for the case of same species populations, which tend to show similarity above 0.9 and distance below 0.1. When these values where compared to the data of Mateus and Sene (2007), they were lower than the majority of their pair-wise comparisons, demonstrating that these two populations are genetically closer to each other than to many others of the same species. These results are in agreement with the Wright´s F statistics analysis results (Table 2), which showed that both populations have no heterozygote deficiency and are not differentiated from each other as the Fis and Fst, respectively, were not significantly different from zero.

Thus, we can conclude that our results obtained for two *Drosophila antonietae* populations, Cantagalo and Rio do Poço, are in agreement with the expected for the species and that the most likely factors responsible for the high within population diversity and low among population differentiation must be gene flow restricted by distance with recent fragmentation and maintenance of ancient polymorphism.

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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