Migration rate and genetic diversity of two *Drosophila maculifrons* (Duda, 1927) populations from Highland Araucaria Forest Fragments in Southern Brazil

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Flies from the Drosophilidae family have been suggested as appropriated for the assessment of the effects of habitat fragmentation (e.g., Mata et al., 2010). Cavasini et al. (2014) evaluated the Drosophilidae assemblages of Araucaria Forest fragments in Guarapuava/PR (southern of Brazil), including one studied here. They concluded that the areas are in intermediate state of conservation, and the size of the preserved area and/or connection with other fragments are important and should be considered for the establishment of conservation units. In this context, we analyzed the genetic variability of *D. maculifrons* (Duda, 1927), a forest dwelling species that belongs to the *guaramunu* group, collected in two conservation units of highland Araucaria Forest fragments in Guarapuava/PR, in order to establish parameters of genetic diversity and levels of gene flow. Thus, we expect to obtain insights about the connectivity level and its importance to conservation.

The two areas (Parque Municipal das Araucárias – PMA: 25° 23' 36" S, 51° 27' 19" W, with 43 ha; Parque Municipal São Francisco da Esperança – SSF: 25° 03' 52" S, 51° 17' 37" W, with 84,7 ha) are located 36 kilometers apart from each other and at about 1,100 m above the sea level. The genetic variability was analyzed using nine allozymatic (*Est*, *Gpdh*, *Idh*, *Me*, *Pgm*, *Hk*, *Mdh-1*, *Mdh-2* and *Mdh-3* – Mateus and Sene, 2003) and nine microsatellite loci (034, 053, 057, 087, 095, 096, 099, 102 and 118 – Laborda et al., 2009). For allozyme data, the parameter Θ and migration rates M were inferred through the MIGRATE-n v3.6.4 software (Beerli, 2012). Assuming an average mutation rate of $1.28x10^{-6}$ per locus per generation (Voelker et al., 1980), average Θ estimates were translated to estimates of average effective population sizes (i.e. $Ne = \Theta/4\mu$) for each population.

The allozymatic genetic diversities (Ho) for both populations (Table 1) were lower than those found by Saavedra et al. (1995) for *D. maculifrons* (0.2831) in the Rio Grande do Sul state, Brazil. They were also lower than those found by Machado et al. (2012) for *D. ornatifrons* (Duda, 1927), a closely related species of *D. maculifrons*, collected in the same areas (PMA – 0.3609; SSF – 0.4060). It is noteworthy that, for allozymes, *D. maculifrons* and *D. ornatifrons* of SSF showed higher genetic diversity. For microsatellites, the Ho values obtained (Table 1) were lower than those found by Heinz (2012) for *D. mediopunctata* (Dobzhansky and Pavan, 1943), a closely related species of the *tripunctata* group, in two areas of Guarapuava/PR, PMA (0.5385) and Fazenda Brandalise (0.5062).

For allozymes, the genetic distance indexes resulted in a low to moderate differentiation between PMA and SSF (Nei's $D = 0.0248$; Fst = 0.0556). Low genetic differentiation for *D. maculifrons* was also recently detected for COI and COII mitochondrial genes (Cenzi de Ré et al., 2014). On the other hand, for microsatellites, low genetic differentiation was not observed ($D = 0.4174$; Fst = 0.0901), probably because of its high variability. Therefore, despite the two populations are somewhat genetically similar (see Table 1), they depict some degree of differentiation. This is corroborated by PMA showing one locus less than SSF for both markers, and both populations presented several exclusive alleles (PMA: allozymes - 5; microsatellites - 13; SSF: allozymes - 3; microsatellites - 33).

The migration rates and population size estimations showed that SSF supplies much more migrants to PMA than otherwise ($M_{SSF \rightarrow PMA} = 2{,}160$; $M_{PMA \rightarrow SSF} = 12.416$), contributing to a higher average effective population size to PMA ($Ne_{PMA} = 4.629 \times 10^{16}$; $Ne_{SSF} = 8.496 \times 10^{3}$). Theses results indicated that size (of the fragment) matters regarding migration. SSF is a conservation unit twice larger than PMA (not taking in account that SSF is surrounded by several other fragments of Araucaria forest in private properties, which must double or even triple the total size of the area, and PMA is surrounded by crop plantations and Guarapuava city limits) and this is probably driving the high rates of migrants between these areas (e.g., Schiffer et al., 2007). Moreover, the amount of migration detected is probably the main cause of the low genetic differentiation found for allozymes here, but other evolutionary forces, such as genetic drift and mutation rates, for example, must be in action to generate and maintain the differentiation detected for microsatellites.

Table 1. Allozymes and microsatellites genetic variability parameters for two *Drosophila maculifrons* natural populations from Guarapuava/PR (Brazil).

Markers	Populations[1]	N[2]	N.L.[3]	N.A.[4]	% (0.95)[5]	H.W.[6]	Ho[7]	He[8]
Allozymes	PMA	51	8	3.00	37.5	66.7	0.1943	0.2933
	SSF	50	9	2.44	55.6	60.0	0.2538	0.2651
Microsatellites	PMA	32	8	6.25	100	0	0.3994	0.7470
	SSF	41	9	7.78	100	11.1	0.3838	0.7444

[1]PMA = Parque Municipal das Araucárias, SSF = Parque Municipal São Francisco da Esperança; [2]Sample size; [3]Number of loci; [4]Average number of alleles per loci; [5]Proportion of polymorphic loci (more frequent allele not more than 95%); [6]Proportion of loci out of Hardy-Weinberg expectations among the polymorphic; [7]Mean observed heterozygosity; [8]Mean expected heterozygosity.

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