Microsatellite heterologous amplification in individual samples of *Drosophila griseolineata.*

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Abstract

In this work we tested the transference of 20 microsatellite loci described for *Drosophila mediopunctata* (*tripunctata* group) in individual samples of *D. griseolineata* (*guaramunu* group). The samples were collected in Caieira da Barra do Sul (27°48'S; 48°33'W), a secondary Atlantic Forest fragment, located south of Florianópolis city, Santa Catarina state, Brazil. According to the literature, among the 20 loci, 18 showed good quality amplification in a pool of individuals of a *D. griseolineata* strain. However, our results showed that only seven (35%) of the 20 analyzed loci presented positive amplification in individual samples from the natural population. This amplification rate was even lower to that obtained in a previous work with *D. maculifrons*, species of the same *guaramunu* group of *D. griseolineata*, and slightly higher to that for *D. ornatifrons* (of the *guarani* group, closely related to *guaramunu* and *tripunctata* groups). These results indicated that the transferability of the microsatellite loci described for *D. mediopunctata* to species of closely related groups is very low in individual samples, which are suitable for populational analyses. Thus, we present here the seven loci, with their respective primer optimal annealing temperatures, which showed to be appropriate for use in future natural population structure analyses of *Drosophila griseolineata*.

Introduction

The microsatellite molecular marker is a suitable tool to qualify and quantify the genetic variability of natural populations, as well as to determine the possible causes of how this variation is distributed. Microsatellite loci are frequently used in population analyses of *Drosophila* because, among other reasons, most of the times the described primers for one species can be applied in other related species (Machado *et al.*, 2003; Moraes and Sene, 2007; Laborda *et al.*, 2009a; Tractz *et al.*, 2012). The transferability is possible when the flanking regions of the microsatellite loci are conserved among taxa (Peakall *et al.*, 1998). However, in heterologous amplifications a higher frequency of null alleles is expected as a result of mutations in the flanking regions, which prevent primer annealing (Callen *et al.*, 1993; Estoup and Cornuet, 1999; Dakin and Avise, 2004).

Laborda *et al.* (2009b) described more than one hundred microsatellite loci for *Drosophila mediopunctata* (*tripunctata* group) and tested their cross-amplification in species of different groups of *Drosophila* (Laborda *et al*., 2009a). Tractz *et al.* (2012) analyzed the applicability of 18 microsatellite loci in individual samples from natural populations of *D. maculifrons* and *D. ornatifrons* (*guaramunu* and *guarani* groups, respectively, according to Robe *et al.*, 2010). They observed a transferability rate of only 28% in *D. ornatifrons* and of 50% in *D. maculifrons*, despite of Laborda *et al.* (2009a) have indicated that these loci presented good quality amplification in a pool of individuals from isofemale lines of these species. Silva *et al.* (2015) applied with success the loci suggested by Tractz *et al.* (2012) in estimates of effective population size and gene flow rate in two natural populations of *D. maculifrons* collected in conservation areas of Araucaria forest (Mixed Ombrophilous Forest).

Therefore, due to the conflicted results about the success of transferability using individual DNA samples (Tractz *et al.*, 2012) and pool of individuals from isofemale lines of *Drosophila* (Laborda *et al.*, 2009a), the main goal of this work was to evaluate the applicability in *D. griseolineata* of the microsatellite loci described for *D. mediopunctata*. To those that showed positive amplification in individual samples of *D.*

griseolineata, their optimal annealing temperatures were determined in order to achieve a better quality amplification and, consequently, a higher reliability in the population analyses to which these loci could be applied.

Material and Methods

The *Drosophila griseolineata* samples were supplied by Prof. Daniela C. De Toni, from the Departamento de Biologia Celular, Embriologia e Genética of the Universidade Federal de Santa Catarina – UFSC. Specimens were collected in a secondary Atlantic forest fragment that depicts high stage of forest regeneration, located in Caieira da Barra do Sul (27°48'S; 48°33'W), south of the Florianópolis city, Santa Catarina state, Brazil.

Table 1. Quality, rate of amplification and optimal annealing temperature of microsatellite loci of *Drosophila mediopunctata* in *Drosophila griseolineata* collected in Caieira da Barra do Sul, Florianópolis-SC, Brazil. + = positive and good quality amplification; +/- = positive, but weak amplification; - = absence of amplification.

Loci	Amplification Quality	Annealing Temperature
Dmed^{UNICAMP}_ssr034	-	-
Dmed^{UNICAMP}_ssr039	-	-
Dmed^{UNICAMP}_ssr041	-	-
Dmed^{UNICAMP}_ssr053	-	-
Dmed^{UNICAMP}_ssr054	+	50°C
Dmed^{UNICAMP}_ssr056	-	-
Dmed^{UNICAMP}_ssr057	-	-
Dmed^{UNICAMP}_ssr065	-	-
Dmed^{UNICAMP}_ssr079	±	54°C
Dmed^{UNICAMP}_ssr087	+	56°C
Dmed^{UNICAMP}_ssr095	-	-
Dmed^{UNICAMP}_ssr096	+	54°C
Dmed^{UNICAMP}_ssr099	-	-
Dmed^{UNICAMP}_ssr102	-	-
Dmed^{UNICAMP}_ssr107	+	56°C
Dmed^{UNICAMP}_ssr115	-	-
Dmed^{UNICAMP}_ssr118	±	50°C
Dmed^{UNICAMP}_ssr121	-	-
Dmed^{UNICAMP}_ssr126	+	54°C
Dmed^{UNICAMP}_ssr133	-	-
Amplification Rate	35%	

The same 18 microsatellite loci analyzed by Tractz *et al*. (2012) in *D. maculifrons* and *D. ornatifrons* were tested in individual samples of *Drosophila griseolineata*, adding two other loci (Dmed^{UNICAMP}_ssr115 and Dmed^{UNICAMP}_ssr121), totaling 20 of the microsatellite loci originally described for *D. mediopunctata* (Laborda *et al.*, 2009b) (Table 1). According to Laborda *et al.* (2009a), 18 loci analyzed in this work showed positive and good quality amplification using DNA sample of a pool of individuals from an isofemale line of *D. griseolineata*. The Dmed^{UNICAMP}_ssr099 and Dmed^{UNICAMP}_ssr107 loci, according to those authors, did not present amplification in *D. griseolineata*.

First, all loci were tested using the same touchdown PCR conditions described by Laborda *et al*. (2009b). Those loci that showed positive amplification were submitted to tests of different temperatures during the annealing stage in order to determine the optimal temperature for each primer, using the following PCR conditions: one denaturation cycle at 94°C for 2 minutes; 25 cycles containing one minute of denaturation at 94°C, 60 seconds in the testing annealing temperature (ranging from 50°C to 60°C, increasing two degrees Celsius in each different reaction) and 72°C for two minutes. For the Dmed^{UNICAMP}_ssr079 and Dmed^{UNICAMP}_ssr118 loci, we also tested the annealing temperatures of 53°C and 55°C. The PCR products were analyzed in 6% PAGE, stained with silver nitrate (Sanguinetti *et al.*, 1994; Machado *et al.*, 2003). The optimal annealing temperature was determined verifying the fragment size according to Laborda *et al.* (2009b) that showed less unspecific amplifications that could compromise the quality of population studies.

Results and Discussion

Laborda *et al.* (2009a) tested the amplification of microsatellite loci described for *Drosophila mediopunctata* (Laborda *et al.*, 2009b), of the *tripunctata* group, in DNA samples of a pool of individuals from isofemale lines of different *Drosophila* species. In the present work, the amplification rate of several of these loci in individual DNA samples of *D. griseolineata* specimens (*guaramunu* group, according to Robe *et al.*,

2010) collected from a natural population. Eighteen out of the 20 tested loci had good amplification quality using a pool of individuals of a *D. griseolineata* strain, according to Laborda *et al.* (2009a). However, in the individual DNA samples from freshly collected specimens of this work, only seven (35%) showed amplification. Among these seven loci, two (Dmed^{UNICAMP}_ssr079 and Dmed^{UNICAMP}_ssr118) showed weaker amplification, even when annealing temperature higher (55°C) and lower (53°C) than that applied to obtain the fragment with the expected size were tested. Moreover, these loci were not amplified even using the touchdown PCR condition. The optimal annealing temperature for each primer that showed positive amplification ranged from 50°C to 56°C (Table 1).

The Dmed^{UNICAMP}_ssr107 locus, which showed no amplification for *Drosophila griseolineata* in the work of Laborda *et al.* (2009a), presented positive result in the tests performed in the present work. The proportion of *D. mediopunctata* loci that showed amplification in *D. griseolineata* (35%, Table 1) was lower than that found by Tractz *et al.* (2012) in *D. maculifrons* (50%), despite both belonging to the same group of species. The rate of amplification in *D. griseolineata* was higher when compared with *D. ornatifrons* (28%, Tractz *et al.*, 2012), of the *guarani* group. However, among the loci that showed positive amplification, only three (Dmed^{UNICAMP}_ssr087, Dmed^{UNICAMP}_ssr096, Dmed^{UNICAMP}_ssr118) coincided among *D. griseolineata* and the other two species. On the other hand, five coincident loci were obtained between *D. maculifrons* and *D. ornatifrons* (the same three above, plus Dmed^{UNICAMP}_ssr034 and Dmed^{UNICAMP}_ssr057).

These data indicated that the transferability of the loci described for *Drosophila mediopunctata* to species that belong to closely related groups is reduced in individual samples, which are more adequate for populational analyses. Moreover, despite the higher amplification rate in the *guaramunu* group to be in agreement with the close phylogenetic relationship of this group with the *tripunctata* group, closer than the relationship of the *guarani* group of *Drosophila ornatifrons* with the *tripunctata* group (Kastritsis, 1969; Kastritsis *et al.*, 1970; Hatadani *et al.*, 2009; Robe *et al.*, 2010), the higher number of in common amplified loci between *D. maculifrons* and *D. ornatifrons* than between both species of the *guaramunu* group reinforce the data of Laborda *et al.* (2009a), who postulated that there is no correlation between phylogeny and the results of interspecific amplification.

The microsatellite loci of *Drosophila mediopunctata* that showed good quality amplification in *D. griseolineata* indicated them to be adequate genetic markers to be applied in population studies using this species.

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References: Callen, D.F., A.D. Thompson, Y. Shen, H.A. Phillips, R.I. Richards, J.C. Mulley, and G.R. Sutherland 1993, Am. J. Hum. Genet. 52: 922-927; Dakin, E.E., and J.C. Avise 2004, Heredity 93: 504–509; Estoup, A., and J.M. Cornuet 1999, *In*: *Microsatellites: Evolution and Applications*. (Goldstein, D.B., and C. Schötterer, eds.). Oxford University Press, Oxford, New York, pp. 49-65; Kastritsis, C.D., 1969, J. Hered. 60: 51-57; Kastritsis, C.D., G. Pasteur, and J. Quick 1970, Canad. J. Genet. Cytol. 12: 952–959; Laborda, P.R., L.B. Klaczko, and A.P. Souza 2009a, Conserv. Genet. Resour. 1: 281-296; Laborda, P.R., G.M. Mori, and A.P. Souza 2009b, Conserv. Genet. Resour. 1: 297-307; Machado, L.P.B., M.H. Manfrin, W.A. Silva-Junior, and F.M. Sene 2003, Mol. Ecol. Notes 3: 159-161; Moraes, E.M., and F.M. Sene 2007, J. Zoolog. Syst. Evol. Res. 45: 336–344; Peakall, R., S. Gilmore, W. Keys, M. Morgante, and A. Rafalski 1998, Mol. Biol. Evol. 15: 1275–1287; Robe, L.J., V.L.S. Valente, and E.L.S. Loreto 2010, Genetica 138: 725–735; Sanguinetti, C., E. Dias Neto, and A.J.G. Simpson 1994, Biotechniques 17: 209; Silva, D.C., L.P.B. Machado, and R.P. Mateus 2015, Braz. J. Biol. 75: 254-255; Tractz, C.C., G.R. Salomon, S.V. Zorzato, L.P.B. Machado, and R.P. Mateus 2012, Dros. Inf. Serv. 95: 76-79.

Interplay between *Drosophila suzukii* and native *Drosophila* species in the Mediterranean area.

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