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Genetic composition of seven microsatellite DNA loci of females and males from a natural population of *Drosophila mediopunctata* collected in a highland Araucaria forest fragment of Brazil.

dos Santos, Camila Heloise, Rogério P. Mateus, and Luciana P.B. Machado. Laboratório de Genética e Evolução, Departamento de Ciências Biológicas, Campus CEDETEG, UNICENTRO – Universidade Estadual do Centro-Oeste, R. Simeão Camargo Varela de Sá, 03, 85040-080, Guarapuava-PR, Brazil. e-mail address: rogeriopmateus@gmail.com

Introduction

As evolutionary processes primarily act shaping the genetic variability of a population, studies on genetic variability of natural populations are extremely important to understand the evolutionary history of a species. *Drosophila mediopunctata* belongs to the *tripunctata* group (Frota-Pessoa, 1954), which is endemic of the Neotropical region and includes 64 species that inhabit forest fragments (Vilela and Bächli, 2000). It is the second largest group of *Drosophila* in this region, and the largest of Neotropical forests (Klaczko, 2006). *Drosophila mediopunctata* is a strictly forest dwelling species that is not associated to human habitats, it has a wide geographic distribution and can be found in Brazil and El Salvador (Val *et al.*, 1981). It is very abundant in some areas, particularly in the south of its distribution or in high altitudes during the coldest months of the year (Saavedra *et al.*, 1995).

Laborda *et al.* (2009) described 134 microsatellite DNA loci for *D. mediopunctata*, and Cavasini *et al.* (2015) establish the chromosomal location of seventeen of these loci, one from each of the five major linkage groups previously published (Laborda *et al.*, 2012), and twelve new loci. So, a very important and well-described genetic marker is available for this species. Thus, the objective of this work was to perform a preliminary analysis of the genetic composition of seven microsatellite DNA loci of naturally collected females of *D. mediopunctata* and their offspring (from which we were able to infer the parental male composition), obtained from a highland Araucaria forest fragment in the South of Brazil.

Material and Methods

This work was performed in a fragment of highland Araucaria Forest phytophysiognomy (Mixed Ombrophylous Forest) of the Atlantic Forest biome, named Parque Municipal das Araucárias (25°23'36” S, 51°27'19” W), where *Araucaria angustifolia* (Coniferae: Araucariaceae) is the predominant vegetal species. This fragment is located in the third plateau of the State of Parana, Brazil, in the subregion named as Plateau of Guarapuava by Maack (1981). According to the climatic classification of Köeppen, this subregion has humid and superhumid mesothermic climate, without dry seasons and with fresh summers. The mean temperatures in hottest months are below 22ºC. Frosts are severe and frequent, and the average temperature in the coldest month is 12.9ºC.

The collection was performed according to dos Santos *et al.* (2010), using fermented bananas and oranges on plates on the ground. The collected females of *D. mediopunctata* were put individually into vials containing standard *Drosophila* culture medium (banana/agar) and the offspring (when produced) were transferred to a new vial, thus obtaining isofemale lines. From each isofemale line, the parental female and 15 F1 females were separated and stored at -20°C in 1.5 mL microtubes containing 70% ethanol. DNA extractions were performed using Promega Kit, EDTA (pH = 8.0), Proteinase K (20 mg/mL), and isopropanol.

Seven microsatellite loci (SSR034, SSR057, SSR087, SSR095, SSR096, SSR099, and SSR118) were amplified through PCR using a touchdown program, varying the annealing temperature from 65°C to 48°C, lowering 1°C each cycle, as described by Laborda *et al.* (2009). The PCR products were resolved in 6% PAGE (Machado *et al.*, 2003) and stained using silver nitrate (Sanguinetti *et al.*, 1994). All gels were photographed using the L-Pix (Loccus) image system, and the alleles were numbered sequentially from the smallest to the largest. From our sample we were able to identify the genetic composition of the collected parental female and to identify the parental male composition through the F1 female. The presence of null alleles was detected as described in Machado *et al.* (2010).

Table 1. Genetic composition of seven microsatellite DNA loci of females and males of *Drosophila mediopunctata* obtained from the natural population of Parque Municipal das Araucárias, Guarapuava/PR. X = presence; - = absence

		Alleles									
Loci	Sex	null	1	2	3	4	5	6	7	8	9
SSR034	F	X	X	X	-	-	X	X	X	X	X
	M	-	X	X	X	X	-	X	X	X	X
SSR057	F	-	X	X	X	-	-	-	-	-	-
	M	-	X	X	X	X	-	-	-	-	-
SSR087	F	-	X	X	X	-	-	-	-	-	-
	M	-	X	X	X	-	-	-	-	-	-
SSR095	F	-	X	X	X	X	X	-	-	-	-
	M	-	X	X	X	X	X	X	-	-	-
SSR096	F	X	X	X	X	X	-	-	-	-	-
	M	-	X	X	X	X	-	-	-	-	-
SSR099	F	-	X	X	X	X	-	-	-	-	-
	M	-	X	X	X	-	-	-	-	-	-
SSR118	F	X	X	-	X	X	X	-	-	-	-
	M	-	X	X	X	X	X	X	-	-	-

Results and Discussion

The collections and establishment of isofemale lines from Parque Municipal das Araucarias resulted in 8 strains of *Drosophila mediopunctata*. From each of these 8 strains, the analysis of the parental female and their 15 F1 females generated the genetic composition of females and males presented in Table 1. The SSR034 was the locus with the highest number found of alleles (8 in both sexes), and SSR087 had the lowest number (3 in both sexes). Null alleles were found in three loci, SSR034, SSR096, and SSR118, out of seven analyzed. Laborda *et al.* (2009) studied the genetic variability of thirteen strains of *D. mediopunctata* and in general they detected higher number of alleles than our results. The number of alleles observed in their work was 9 for SSR034, 6 for SSR057, 5 for SSR087, 6 for SSR095, 9 for SSR096, 3 for SSR099, and 5 for SSR118. These differences in the number of alleles detected in these two samples should be due to the sample size of each work. We analyzed 8 females and their F1 females, which resulted in the inferred genetic composition of the parental males. Laborda *et al.* (2009) performed their study using 30 flies from each strain. Therefore, it is expected that if we analyze more samples our number of alleles must increase.

This work was a preliminary study that will be important to generate data to evaluate the number of parental males that contributed to the formation of each isofemale line, which will be critical to understand genetic parameters of sperm competition in *Drosophila mediopunctata*. Sperm competition is one of several aspects that are relevant to understand reproductive characters in this species.

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The invasive *Drosophila suzukii* (Diptera: Drosophilidae) uses native plant species of the Brazilian savanna as hosts.

Ramos, D.L.[1]*, P.D.F.F. Ramos[1], and L.G. Carvalheiro[1,2]. [1]Instituto de Ciências Biológicas, Universidade de Brasília, Brasília-DF, 70910-900; [2]Centre for Ecology, Evolution and Environmental Changes (CE3C), Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal. Corresponding author: davilramos91@gmail.com

Introduction

Drosophila suzukii (Matsumura), also known as spotted wing drosophila, is a native species from western Asia, characterized by damaging soft-skinned and unwounded fruits to perform oviposition (Acheampong, 2010). For this reason, this species is considered an important pest, especially in agricultural systems, bringing economic loss to diverse crops (*e.g*., strawberry, Wollmann *et al.*, 2016). In recent decades, surveys of the occurrence of this species have shown its rapid expansion around the world (*i.e*., Asia, Europe, South and North America). This wide geographic distribution of *D. suzukii* suggests high potential of colonization of different environments, as well as high thermal tolerance (Cini *et al.*, 2012). Furthermore, its short life cycle and high fecundity capacity (average production of 600 eggs throughout its life cycle) allow an explosive population growth in this species when under favorable conditions (Cini *et al.*, 2012).

In Brazil, records of this species are recent and occurred in the south and center-west of the country. Paula *et al.* (2014) were the first to find individuals of this species in the Cerrado region in 2013, a biome known for its high biodiversity, and threatened by the constant agricultural expansion (Lahsen *et al.*, 2016). However, little is known about the influence of this species on natural environments and its effect as an invasive species and fruit pest of native species. In this study, we document and discuss about *D. suzukii* as a fruit predator of three native species of Cerrado biome.

Materials and Methods

This study was conducted at IBGE Ecological Reserve (15° 56'S, 47° 53'W), a protected area located in the south of Federal District, Brazil, with more than 10,000 ha (Paula *et al.*, 2014). This site is embedded in