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Short communication

Gene flow affects the genetic diversity of *Copaifera langsdorffii* Desf. in Cerrado

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ABSTRACT

Gene flow allows connectivity of geographically isolated populations and which genetic diversity is reduced. Understanding how gene flow occurs is essential to conservation and selection of priority populations for intervention. In this study, the rates of migration and selfing of *Copaifera langsdorffii* Desf. sampled in Cerrado fragments in the State of São Paulo, Brazil, were estimated through computer simulations. Different selfing and migration rates were tested and the combination that showed values of observed and expected heterozygosity closest to those previously obtained with microsatellite markers was selected. According to the simulations, selfing and migration were low. Due to the high geographical distances among the fragments, gene flow was limited and may have led to low genetic diversity in the fragments. It is recommend enriching the area with high genetic diversity germplasm and reducing disturbance to promote the occurrence of pollinators, in order to increase the genetic diversity.

Keywords: fragmentation, reproductive isolation, genetic diversity, EASYPOP, microsatellites

Introduction

Ecosystems vulnerability is an issue of great concern worldwide. Human disturbances, such as land clearing, negatively affect the environmental sustainability of forest ecosystems that lead to habitat fragmentation (Schlaepfer *et al*. 2018).

Among the consequences of habitat fragmentation, it propitiates the occurrence of inbreeding and genetic drift, especially when there is a lack of connectivity among fragments through gene flow, thus, reducing the genetic diversity, which is important for environmental sustainability (Duminil *et al*. 2016). For this reason, many conservation programs are focused on reducing gene flow barriers between isolated populations (Birnbaum *et al*. 2003). Understanding the gene flow pattern between geographically isolated populations enables conservationists to plan conservation strategies, including the selection of priority populations for intervention (Ellstrand 1992).

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Moreover, genetic diversity is affected by the reproductive system of a species. Selfing limits pollen and seed dispersion and the potential for gene recombination, in contrast to crossing. For this reason, allogamous species are expected to preserve higher genetic diversity than autogamous species (Dias *et al*. 2004).

In this study, the rates of gene flow and selfing of *Copaifera langsdorffii* trees located in Cerrado fragments were estimated through computer simulations.

Easypop version 2.0.1 (Balloux 2001) was used to simulate different rates of selfing (0.1, 0.3 or 0.5) and migration (from 0.1 to 0.9, with steps of 0.1) of four *Copaifera langsdorffii* fragments. All fragments (Assis, Itirapina, Pedregulho and Brotas) are located in the Cerrado biome in the State of São Paulo, Brazil. Criteria used for selection were the values of observed and expected heterozygosity ($H_O = 0.68$; $H_E = 0.87$) reported by Antiqueira *et al*. (2014).

For the simulations, we considered a diploid hermaphrodite species, with non-random mating and no clonal reproduction. A spatial migration model was considered, with coordinates based on the geographic location (latitude and longitude) of the genotyped populations. Regarding the mutation settings, there were assumed eight loci evolving according to the single-step mutation model (SSM), with a proportion of 0.1 *K*-allele model (KAM) events, under 103 possible allelic states (Antiqueira *et al*. 2014). We considered a mutation rate of 0.0001 mutations per locus per generation and assumed 100 generations. Each combination of selfing and migration rates was replicated 100 times. The observed and expected heterozygosities from Antiqueira *et al*. (2014) were used to select the model settings that presented values closest to the field observations. The two independent sample *t*-tests at a 5% level of probability were used to compare the observed and expected heterozygosities of the selected combination of selfing and migration rates with other combinations.

According to the simulations, there is an increase in heterozygosity (observed and expected) as selfing reduces and migration rate increases. The closest values of H_O and H_E to those obtained using microsatellite markers ($H_O = 0.68$; $H_E = 0.87$) were obtained under the selfing of 0.3 and the migration rate of 0.1 and these values were significantly different from the other rates, based on two independent sample t-tests at a 5% level of probability of error (Figure 1).

The low selfing rate can be justified by the reproductive mode of this species. According to Carvalho (2003), *C. langsdorffii* is predominantly allogamous. Outcrossing in hermaphrodite plants, like *C. langsdorffii* is usually enforced by self-incompatibility, an important mechanism in flowering plants that prevents selfing and consequently generating and mantaining the genetic diversity (Takayama & Isogai, 2005). Therefore, although most flowering plants are hermaphrodites, outcross prevails in the plant kingdom. In a study on the reproductive system of *Copaifera langsdorffii* from a preserved area in Minas Gerais, Brazil, Oliveira *et al*. (2002) found high rates of multilocus and unilocus outcrossing (0.917 and 0.877, respectively).

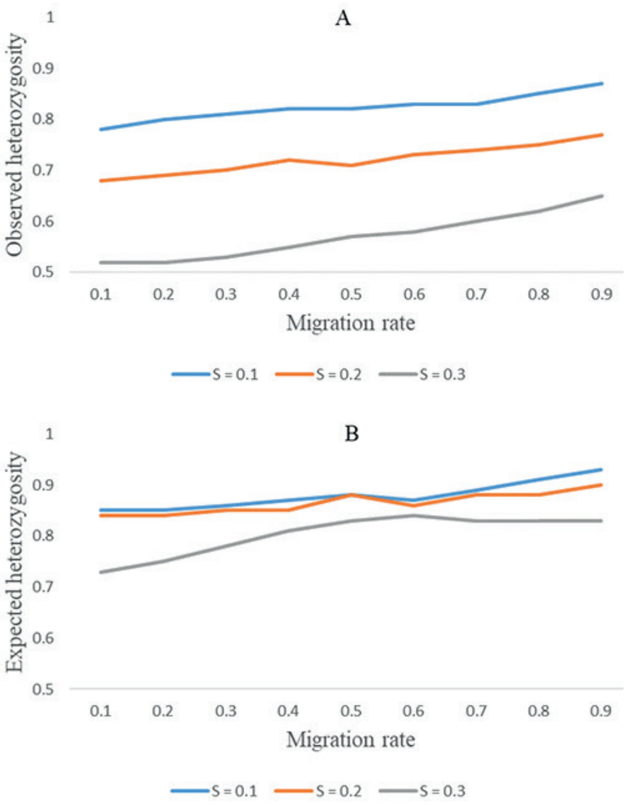

Figure 1. Estimates of observed (**A**) and expected (**B**) heterozygosities generated by simulations, for different rates of selfing (S) and migration.

In regard to migration, there is low gene flow among the studied fragments. Gene flow via pollen in *Copaifera langsdorffii* is performed by bees and seed dispersal is zoochoric. The main animal seed dispersers are monkeys and birds, which both swallow arils and eventually regurgitate the seeds close to the mother plant, limiting gene flow (Martins *et al*. 2008; Sebbenn *et al*. 2011). Assis and Pedregulho are the most distant fragments (460 km away), followed by Assis – Itirapina (350 km), Pedregulho – Itirapina (280 km), Pedregulho – Brotas (280 km), Assis – Brotas (275 km) and Itirapina – Brotas (24 km) (Antiqueira *et al*. 2014). Thus, the long distances between the fragments and the dispersal syndrome in this species can justify the low gene flow obtained in our simulations.

According to Ellstrand (1992), gene flow in plant populations is an evolutionary force that promotes the homogenization of gene frequencies between populations in contrast to genetic drift that promotes genetic differentiation between populations. For this reason, various conservation programs focus on reducing barriers (physical or distance barriers) to gene flow in fragmented populations (Birnbaum *et al*. 2003). In a study by Sebbenn *et al*. (2011), there was restricted gene flow in a fragmented population of *Copaifera langsdorffii* from the Brazilian Atlantic forest.

This study reinforces the importance of tree species genetic diversity in order to ensure its conservation. The studied populations of *C. langsdorffii* result from a long fragmentation process by anthropic activities. On the other hand, the long distances that separate these populations limit the connectivity through gene flow. Therefore, local strategies must be adopted to contain genetic erosion, such as enriching the area with high genetic diversity germplasm and reducing disturbance to promote the occurrence of pollinators in these fragments.

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