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Inbreeding effects in *Solanum lycocarpum* A. St.-Hil populations, an endangered species of the Brazilian Cerrado

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ABSTRACT. The inbreeding effective population size is an estimate of inbreeding and genetic drift in populations. It is an important tool for conservation genetics because it represents the number of individuals that are effectively contributing alleles to the subsequent generations. Several studies have been published in the last decades on the genetic structure of natural plant populations of the Cerrado, the Central-Brazilian savannahs, but most of them do not present effective size estimates. The objective of this study was to show such estimates for *Solanum lycocarpum*, a Cerrado species that is in danger of genetic erosion. We utilized microsatellites, isozymes, and 2 natural populations for each marker to estimate the population inbreeding effective size of a group of populations ($N_{s(v)}^*$) and the minimum number of populations that should be conserved ($S_{(ref)}^*$) in order to retain an effective number of 500. For the 2 markers that were utilized, only approximately 12%

of the individuals are effective in the populations. The value obtained for $S_{(r,g)}^s$ was approximately 80.

Key words: Effective size; Conservation; Genetic structure; Lobeira; Population genetics

INTRODUCTION

Population effective size is an estimate of genetic representativeness and a critical parameter in species conservation because it predicts the number of individuals that effectively contribute with alleles to the next generation (Vencovsky and Crossa, 2003; Crossa and Vencovsky, 2011). The effective size is the size of an ideal population that would have accumulated the same loss of heterozygosity due to genetic drift as that of the actual population, and it is influenced by the reproductive system and the crossing rates of the populations (Crossa and Vencovsky, 2011).

On the basis of mathematical modeling, Vencovsky and Crossa (1999, 2003) proposed that, for long-term genetic conservation, the ideal effective size that is retained in a population should be at least 500, and they showed how to estimate the minimal number of populations that should be conserved to retain such effective size. Nevertheless, few studies on population genetic structure for conservation purposes present that estimate. Although both the current ecosystem devastation and the danger of genetic erosion in plants are frequently denounced, little has been done to provide practical information for conservation, such as the minimal number of populations to be protected.

Solanum lycocarpum A. St.-Hil is a typical species of Cerrado vegetation and has been considered as a model to study the conservation genetics of plant species (Martins, 2005). Largely distributed across Cerrado's areas (Stehmann et al., 2013), this species presents flowers and fruits throughout the year (Moura et al., 2010). It is a cross-pollinated species, and the pollinator and dispersing agents can carry the genes over long distances (Oliveira-Filho and Oliveira, 1988; Courternay, 1994). In addition, the population genetic structure of this species is relatively well-studied (Martins et al., 2006, 2011; Moura et al., 2009, 2011a,b, 2012), where it was reported that this species is in danger of genetic erosion (Moura et al., 2011a, 2012). However, the estimate of the minimal number of populations for conservation is still lacking. Therefore, the objective of this study is to provide both a genetic effective population size estimate and an estimate of the minimal number of populations for conservation purposes, which can be used as guidelines to establish protection areas.

MATERIAL AND METHODS

The estimation of the genetic population parameters was done with 2 kinds of molecular marker. Two natural populations with 60 individuals each were genotyped with nuclear microsatellite loci (SSR), and 2 populations (1 with 45 individuals and the other with 60 individuals) were genotyped with isozyme loci. For each marker, a disturbed area without native vegetation (cattle's pasture) and a protected area where the native vegetation is maintained and with less anthropogenic disturbance were analyzed. A description of the populations, the sampling method, the markers' characterization, and the genetic

structure is given in Moura et al. (2009, 2011a,b).

The inbreeding effective population size ($N_{e(v)}$) was estimated according to Vencovsky and Crossa (1999). Because it is not possible to calculate the total number of natural populations, an estimate was obtained with Equation 1, where S is the number of populations studied and F_{st} is an index of the inbreeding that is produced in the consolidated group of populations by its subdivision into separate populations.

$$N_{e(v)} = \frac{S}{2F_{st}} \quad (\text{Equation 1})$$

The number of populations ($S_{(ref)}$) that should be conserved to retain an effective size of 500 ($N_{e(ref)}$) was estimated with Equation 2 according to Vencovsky and Crossa (1999, 2003), in which θ_p is the genetic divergence between populations.

$$N_{e(ref)} = \frac{S_{(ref)}}{2x\theta_p} \quad (\text{Equation 2})$$

RESULTS

The effective size of the consolidated group of populations [$N_{e(v)}$] was similar for both markers that were used (12.5 and 12.3). The minimal number of populations to be conserved in order to retain an effective size of 500 was also similar (80 and 81) for SSR and isozymes markers (Table 1).

Table 1. Population estimates for *in situ* conservation of *Solanum lycocarpum*.

Marker	N	θ_p	$N_{e(v)}$	$S_{(ref)}$
SSR	120	0.080*	12.5	80
Isozymes	101	0.081**	12.3	81

N = number of individuals sampled; θ_p = estimate of genetic divergence among populations; $N_{e(v)}$ = effective size for the group of populations; $S_{(ref)}$ = minimal number of populations to be conserved to keep an effective size of 500. *Moura et al., 2009, 2011a; **Moura et al., 2011b.

DISCUSSION

The genetic representativeness for the group of populations [$N_{e(v)}$] was similar for both markers. $N_{e(v)}$ indicates the number of individuals that are effective in the population, i.e., how many of them are able to leave descendants to the following generations. Here, we observed that only approximately 12% of the individuals that were genotyped for both markers were effective in the population.

The value that was obtained for $S_{(ref)}$ means that approximately 80 populations of *S. lycocarpum* should be maintained in order to retain an effective size of 500. Taking into account the present scenario of devastation in the Cerrado vegetation and the reduced number of protected areas [only 1-3% (Ratter et al., 1997; Felfili et al., 2001; Aguiar et al., 2004)], these numbers ought to be considered with caution because the Cerrado remnants might not be ef-

fectively playing their role in genetic conservation in the long run. The non-protected areas are especially vulnerable to anthropic action and can easily yield monocultures.

Martins (2005) determined that the maximum seed migration distance in *S. lycocarpum* is about 40 km. In agreement with those results, Moura et al. (2011a,b) observed that populations that are 43 and 45 km apart showed a significant level of genetic divergence. Therefore, we suggest that at least 80 populations, each separated from the nearest by less than 40 km, should be conserved.

The protected areas in the Cerrado are frequently surrounded by extensive monocultures, within which few, if any, native vegetation remnants can be found. These areas are thus analogous to islands in the middle of an ocean of soybeans or sugarcane, and many of them may become genetically isolated in the short run. Consequently, studies aiming at an effective strategy of genetic conservation need to include contemporary gene flow, minimum viable area, minimum number of populations to be conserved, population size, and maximum among-population distance. The minimum amount of information for the long-run conservation of most species is still unknown, while the agricultural frontier expands into the Cerrado and makes its species more vulnerable to genetic erosion.

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