Estimating the Selfing and Migration of *Luehea divaricata* Populations Based on Genetic Structure Data, Using the EASYPOP Program

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Abstract: Genetic structure data of five populations of the *Luehea divaricata* Mart. & Zucc., forest tree species under development in the Atlantic Forest biome, obtained by microsatellite DNA markers, were used in simulations to study their reproductive and ecological pattern. Different selfing and migration rates were tested, using the observed and expected heterozygosity of 0.55 and 0.67, respectively, obtained through the use of microsatellite markers. Closest values were obtained with the use of selfing rates of 0.3 and migration of 0.2. These results suggest the presence of some self-incompatibility system between these species, which reduces, but does not prevent the self-fertilization. The migration rate contributes to a low genetic differentiation between the populations, making the reproductive mode, responsible for the inbreeding observed in the same populations. Authors suggest continuous monitoring of the genetic variability as a guarantee for the persistence of these populations. The study focus on the importance of using computer simulations to investigate ecologic, reproductive and genetic patterns for forestry populations, thus enabling the application of suitable measures for conservation.

Key words: Computational simulations, conservation biology, inbreeding, heterozygosity.

1. Introduction

The exponential growth of the human population, which has been observed over the last decades, has been accompanied by the destruction of the planet's diverse ecosystems, including forestry. The overexploitation of forest resources and the devastation of forest areas to the expansion of agricultural activities are among the main human activities that have resulted in the destruction and fragmentation of habitats and hence the extinction of forest species [1].

Habitat fragmentation is a phenomenon that consists of the subdivision of an area, geographically isolating members of a certain population into two or more fragments. Depending on the distance between fragments, the reproductive system and distance of dispersion of pollen and seeds, the geographic isolation can result in the reproductive isolation, and consequently restriction in the crossings inside the fragments. In a long term, speciation may occur as members of separate groups can develop different evolutionary mechanisms to the point of no longer being able to cross, even if the gene flow is restored. However, the extinction of the population is most likely, due to the size of the isolates, since in small populations there is a greater performance of genetic drift and a greater occurrence of inbreeding. These events reduce genetic variability and make the population susceptible to the effects of cyclical environmental changes such as the occurrence of pests or diseases, occurrence of frosts or floods [1, 2].

The importance of genetic variability is extensive to the evolutionary success of species, since the

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evolution acts on an existing variability in the population, allowing the natural selection to preserve the individuals better adapted to a wide range of environmental variability [3].

The management of a fragmented population goes through the conservation of its genetic variability that can be accomplished by expanding the areas of its fragments with germplasm that adds genetic variability or by allowing the genetic flow between the fragments. In order to define a priority population for conservation and the conservation method to be adopted, the parameters of the genetic structure must be determined through the use of molecular markers, from which the rate of crosses and the level of genetic flow among its fragments can be estimated [4].

Computational simulations offer more accurate alternatives for the estimation of population genetic parameters, including crossover and gene flow rates. The EASYPOP program [5] was used in the present study to simulate selfing and migration rates from

microsatellite markers data obtained from five populations of *Luehea divaricata* Mart. & Zucc. (Malvaceae) forest tree species, under development in a Brazilian area of the Atlantic Forest biome, in order to provide credible information for planning their conservation.

2. Material and Methods

2.1 Research Area

Authors studied five *Luehea divaricata* Mart. & Zucc. (Malvaceae) forest populations located in the Brazilin States of Santa Catarina, Paraná, São Paulo and Minas Gerais (Fig. 1).

2.2 Model Settings

Authors used different selfing (0.1, 0.3 and 0.5) and migration rates (from 0.1 to 0.9, with steps of 0.1) in simulations with the computer program EASYPOP version 2.0.1 [5] to determine those that would result
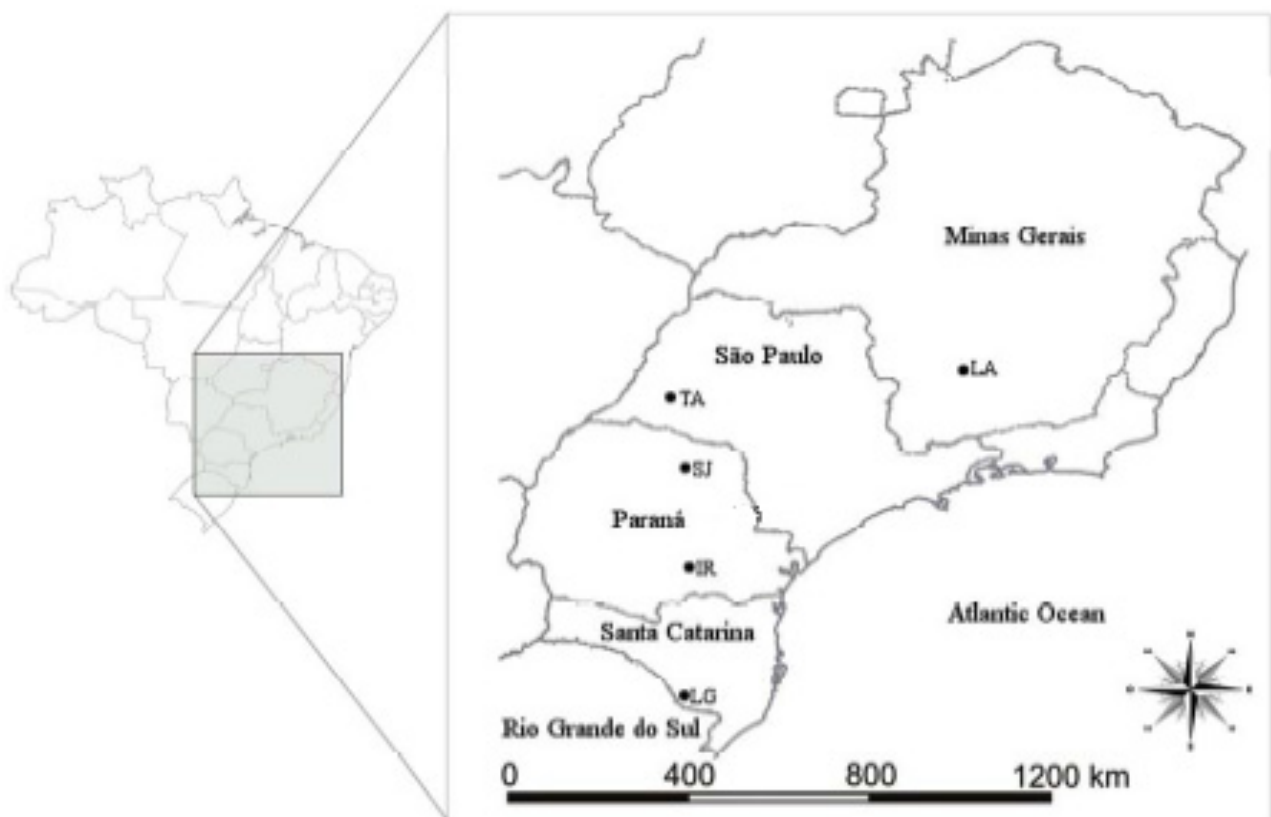
Fig. 1   Geographic location of the five *L. divaricata* populations used in molecular characterization and simulations [6].

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Table 1   Values of observed and expected heterozygosities from the five populations used in the study.

Population	State	A	N	Ho	He	F_{IS}
Lages (LG)	SC	2	32	0.64	0.64	-0.01
Irati (IR)	PR	1.3	30	0.65	0.67	0.03
São Jerônimo (SJ)	PR	2.5	30	0.54	0.63	0.15
Taciba (TA)	SP	6.2	28	0.49	0.7	0.31
Lavras (LA)	MG	7.5	30	0.43	0.71	0.40
Average				0.55	0.67	0.88

A: area size, in hectares; N: number of individuals; Ho: observed heterozygosity, obtained by the use of microsatellite markers; He: expected heterozygosity, obtained by the use of microsatellite markers; F_{IS}: inbreeding coefficient. Adapted from Ref. [6].

in parameters similar to those determined by Conson [6] when studying the genetic structure of nine *Luehea divaricata* populations in the Atlantic Forest, in Brazil. In the referred study, the average observed heterozygosity was 0.55, and the average expected heterozygosity was 0.67 (Table 1).

For the simulations, authors considered diploid hermaphrodite specie, with non-random mating and no clonal reproduction. A spatial migration model was considered, with coordinates based on the geographic location of the populations and where obtained from the real geographical coordinates (latitude and longitude) according to the map.

With respect to the mutation settings, authors assumed 10 loci evolving according to the single-step mutation model (SSM), with a proportion of 0.1 K-allele model (KAM) events, under 50 possible allelic states, according to the data obtained by Conson [6]. A mutation rate of 0.0001 mutations per locus per generation was assumed. The genetic variability of the initial population was considered the maximum, and authors simulated 400 generations. For each combination of selfing and migration rates, authors replicated 100 replicates.

The observed and expected heterozygosities (0.55 and 0.67, respectively), from Conson [6] were used to select the model settings that presented values closest to the field observations (Table 1). Authors used the two independent sample *t*-tests at a 5% level of probability to compare the values of observed and expected heterozygosities of the selected model with others.

2.3 FSTAT Analyses

Authors used FSTAT program [7] to analyze the output files of the selected model. Through the use of FSTAT, the forest populations, including allelic richness, genetic diversity, and indices of genetic differentiation among populations were characterized. Authors estimated gene flow by the average number of migrants per generation, based on the proportion of the total genetic variance in a subpopulation (R_{ST}). R_{ST} statistic was used in this study in place of the common F_{ST} statistic because authors used microsatellite loci data that evolve according to step-by-step mutations and is the most indicative estimate for genetic differentiation between populations.

The coefficient of inbreeding within the populations (F_{IS}) was used to estimate the rate of apparent outcrossing (*t*a), given by the equation of Nei and Syakudo [8]:

$$t_a = \frac{1 - Fis}{1 + Fis} \qquad (1)$$

In turn, authors estimated gene flow in terms of the average number of migrants per generation (*N*m) from the value of R_{ST} [9]:

$$Nm = \frac{1}{4}\left(\frac{1}{R_{ST}} - 1\right) \qquad (2)$$

3. Results and Discussion

3.1 Selection of Model Parameters

Values of observed (Ho) and expected (He) heterozygosities generated from EASYPOP simulations for different selfing and migration rates are

Table 2 Estimates of observed (H$_O$) and expected (He) heterozygosities generated by simulations, for different rates of selfing and migration

Migration rate	Rate of selfing					
	0.1		0.3		0.5	
	Ho	He	Ho	He	Ho	He
0.1	0.656	0.694	0.543	0.658	0.424	0.633
0.2	0.668	0.706	0.555	0.674	0.433	0.647
0.3	0.670	0.708	0.565	0.684	0.433	0.649
0.4	0.674	0.711	0.568	0.686	0.437	0.653
0.5	0.681	0.719	0.571	0.692	0.441	0.659
0.6	0.683	0.719	0.573	0.695	0.442	0.663
0.7	0.684	0.722	0.575	0.698	0.444	0.664
0.8	0.687	0.723	0.578	0.701	0.446	0.670
0.9	0.689	0.725	0.580	0.705	0.452	0.674

presented in Table 2. There is clear trend of increasing heterozygosity (both Ho and He) with increasing migration rate and reducing selfing. Heterozygosity is one of the parameters of genetic variability. According to Madsen et al. [10], gene flow enables the allele exchange between populations, thus increasing the genetic variability. In turn, selfing limits the pollen and seed dispersion and the potential for recombination between alleles from different individuals; as a result, autogam species tend to present low variability within populations and high variability between populations [11].

The closest values of Ho and He to those obtained through the use of microsatellite markers (0.55 and 0.67, respectively) were generated under the selfing of 0.3 and the migration rate of 0.2 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. This model enables to characterize the populations.
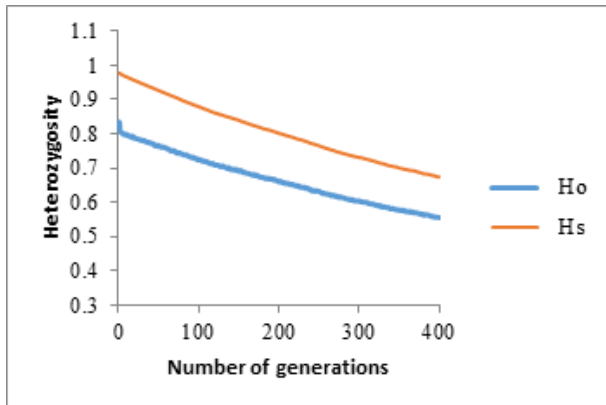
3.2 Genetic Structure of the Forest Populations

The rate of selfing of 0.3 generated from simulations implies an outcrossing rate of 0.7. This value is in accordance with the apparent outcrossing (*t*a) of 0.696 obtained by using Eq. (2), given the intra-population inbreeding coefficient (F_{IS}) of 0.179 generated from the FSTAT analysis. This finding enables us to classify the mode of reproduction of the

population as mixed, with a predominance of outcrossing, according to the classification of Destro and Montalván [12]. This author classifies populations as autogamous (outcross from 0 to 0.05), mixed (outcross from 0.05 to 0.95) and allogamous (outcross from 0.95 to 1.0).

Mixed reproduction system in hermaphrodite vegetal species, like *Luehea divaricata*, suggests the presence of self-incompatibility mechanism in vegetal species that reduce, but do not completely prevent selfing [13]. According to Bawa [14], self-incompatibility is frequently used by angiosperms to prevent the adverse effects of inbreeding and the subsequent loss of genetic variability. Studying *Ceiba aesculifolia* populations, Quesada, et al. [15] observed outcrossing rates close to 1.0 because of a self-incompatibility mechanism present in this species.

With respect to the migration settings, the simulations derived a rate of 0.2 between populations, which is enough to prevent high genetic differentiation between (R_{ST} = 0.0062, from the FSTAT analyze). For gene flow, in terms of *Nm*, the number of migrants per generation, determined through the use of Eq. (3), was 40. This is a high value according to the scale set by Govindaraju [16], which distinguishes the following levels for gene flow: high (*Nm* > 1), intermediate (0.25 < *Nm* < 0.99), and low (*Nm* < 0.25).

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Fig. 2   Simulation of observed (Ho) and expected (Hs) heterozygosities across 400 generations in five populations of *Luehea divaricata*.

Gene flow is the movement of genes (gametes, propagules, and individuals) in populations that effectively changes the spatial distributions thereof. High rates of gene flow result in low genetic differentiation between populations by opposing the effects of evolutionary forces (inbreeding and genetic drift) [17]. A study regarding genetic structure for *Luehea divaricata* in Brazil [9] showed high genetic differentiation (F_{ST} = 0.22) between populations, due to low gene flow. According to Neigel [17], gene flow is an evolutionary force opposite to genetic drift, acting toward the homogenizing allele frequencies between populations, thus resulting in low genetic differentiation between them.

The behavior of observed and expected heterozygosity were plotted across the 400 generations simulated (Fig. 2). High levels of heterozygosities were observed (values close to one) in the beginning, with a progressive loss generation by generation.

Diversity loss may occur due to inbreeding, despite the self-incompatibility mechanisms present in this species. As a result of genetic drift effects, some alleles may lose every generation, including those for self-incompatibility. Inbreeding can also occur from biparental crossings when population size is small. According to Ridley [18], while a great habitat area may have sustained a single great population, it is possible that none of its fragments is able to sustain enough a subpopulation for a long time.

3.3 Implications for Conservation

Forest habitats have been destroyed and fragmented due to anthropic activities that turn a great and continuous area into two or more small sized fragments. In this condition, ecologic and genetic processes may occur and lead to disastrous consequences [1, 2]. Forest fragmentation affects the phenology, pollination patterns and reproductive success of species [2]. The reduction on the population size may reduce the density of reproductive trees and limit pollen availability, and propitiate the occurrence of inbreeding and genetic drift effects that lead to progressive loss of genetic variability and, ultimately, the population extinction.

The findings in this study suggest five genetically stable populations with moderate losses of variability due to high rates of gene flow between them despite high selfing rates. Gene flow enables sharing alleles from different populations and increases the heterozygosity. In order to maintain the present scenario in the future, authors suggest keeping monitoring the genetic variability as a guarantee for the persistence of these populations.

4. Conclusion

The five populations of *Luehea divaricata* present moderate levels of inbreeding as a result of the reproductive mode in this species. However, high rates of gene flow between populations minimize the losses of genetic variability.

This study stressed the importance of using computer simulations to investigate ecologic, reproductive and genetic patterns for forestry populations, thus enabling the application of suitable measures for conservation.

Acknowledgments

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