Ciência Rural, Santa Maria, v.53:1, e20210176, 2023 http://doi.org/10.1590/0103-8478cr20210179

ISSNe 1678-4596
BIOLOGY

Genetic structure simulation for *Hancornia speciosa* populations in Northeast Brazil

Caetano Miguel Lemos Serrote[1,2] **Lia Rejane Silveira Reiniger**[1*]
Silvia Machado dos Santos Rabaiolli[1] **Charlene Moro Stefanel**[1]
Karol Buuron da Silva[1] **Ana Cristina da Fonseca Ziegler**[1]

[1]Programa de Pós-graduação em Engenharia Florestal, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: liarsr@ufsm.br. *Corresponding author.
[2]Faculdade de Ciências Agrárias, Universidade Lúrio (UNILURIO), Unango, Mozambique.

ABSTRACT: *Mangaba tree is a fruit tree species whose natural populations are fragmented by anthropic actions. For this reason, studies assessing the impact of fragmentation on the diversity and genetic structure of these populations are required in order to establish suitable conservation strategies. In our study, we used data from analyzes through microsatellite markers in computer simulations to estimate the rates of migration and selfing of six mangaba populations. The studied populations are located in the northeastern states of Ceará, Pernambuco and Sergipe. We tested different selfing and migration rates and selected the combination that showed values of observed and expected heterozygosity closest to those previously obtained with microsatellite markers. According to our simulations, selfing and migration were moderate. This may have led to an increase in inbreeding and genetic drift, resulting in low genetic diversity. We recommend expanding the area and reducing disturbance to promote the occurrence of pollinators, which play an important role in increasing genetic diversity.*

Key words: *Easypop, genetic diversity, habitat fragmentation, mangaba tree, microsatellites.*

Simulação da estrutura genética de populações de *Hancornia speciosa* no nordeste brasileiro

RESUMO: *A mangabeira é uma espécie frutífera cujas populações naturais se encontram fragmentadas por ações antrópicas. Desse modo, são necessários estudos sobre a avaliação do impacto da fragmentação sobre a diversidade e estrutura genética dessas populações para o estabelecimento de estratégias de conservação adequadas. No presente estudo, foram utilizados dados de análises com marcadores microssatélites em simulações computacionais para estimar as taxas de migração e autofecundação de seis populações de mangaba. As populações estudadas estão localizadas nos estados nordestinos do Ceará, Pernambuco e Sergipe. Foram testadas diferentes taxas de autofecundação e migração, e selecionada a combinação que apresentou valores de heterozigosidade observada e esperada mais próximos dos obtidos com marcadores microssatélites. Com base nas simulações, a autofecundação foi de 0,3 e a taxa de migração variou de 0,5 a 0,6, valores que podem ter conduzido ao aumento da endogamia e deriva genética, resultando em baixa diversidade genética. Recomenda-se a expansão da área e a redução de perturbações para promover a ocorrência de polinizadores, que desempenham um papel importante no aumento da diversidade genética.*

Palavras-chave: *Easypop, diversidade genética, fragmentação de habitat, mangabeira, microssatélites.*

INTRODUCTION

Habitat fragmentation is the process by which a large and continuous habitat is split into smaller and more isolated remnants, primarily by human disturbances such as land clearing and conversion of vegetation from one type to another. This phenomenon affects many ecosystems and species such as mangaba tree (*Hancornia speciosa* Gomes), a fruitful species native to Brazil (FRANKLIN et al., 2002; MOURA et al., 2005; SCHLAEPFER et al., 2018). It is a perennial species native to several regions and ecosystems in Brazil, extending along the coast, occurring naturally in marginal soils, subject to long periods of drought such as savannas and semi-arid areas in the Northeast. It is also reported throughout the Cerrado region of Central Brazil to the Pantanal biome (LEDERMAN et al., 2000). Among many consequences, habitat fragmentation in forest ecosystems affects the phenology, pollination patterns and reproductive success of species. The reduction in population size may reduce the density of reproductive trees, limit pollen availability and propitiate the occurrence of inbreeding and genetic drift effects. Besides the size reduction, the lack of connectivity can affect pollen and seed dispersal

Received 03.05.21 Approved 01.10.22 Returned by the author 04.08.22
CR-2021-0176.R3
Editors: Leandro Souza da Silva Maria do Céu Cruz

and limit gene flow among isolated fragments. These factors can lead to progressive loss of genetic diversity and, ultimately, to extinction (DUMINIL et al., 2016). For this reason, it is important to assess the impact of fragmentation on the genetic diversity and structure of forest tree populations in order to establish appropriate strategies for conservation.

Some conservation programs focus on promoting gene flow among isolated fragments. Gene flow is an evolutionary force that enables the allele exchange, thus increasing the genetic diversity. Moreover, the reproductive system plays an important role in the genetic diversity since selfing limits the pollen and seed dispersion and the potential for recombination between alleles from different individuals, in contrast to crossing. As a result, it is expected that crossing species preserve higher genetic diversity than autogamous species (JULLIEN et al., 2019). In our study, we used data from microsatellite markers in computer simulations to estimate the selfing and migration rate of six *H. speciosa* populations from the Caatinga biome, covering the northeastern Brazilian states of Ceará, Pernambuco and Sergipe. The software used for the simulations was the Easypop 2.0.1 (BALLOUX, 2001).

MATERIALS AND METHODS

The Easypop 2.0.1 software 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), which were selected based on how well they could explain the genetic structure of six *H. speciosa* populations obtained by microsatellite markers from AMORIM et al. (2015). In the referred study, the average observed heterozygosity was 0.47, and the average expected heterozygosity was 0.60. The studied populations were: Reserva do Cajú (SE), Abaís (SE), Barrados Coqueiros (SE), Jacarecoara (CE), Tapera (CE) and Tamandaré (PE), all located in northeast Brazil, in the Caatinga biome (Table 1). A total of 94 indivíduals were collected, 59 from the state of Sergipe (SE), 20 from Ceará (CE), and 15 from Pernambuco (PE). According to the authors (AMORIM et al., 2015), in some cases, the total number of individuals was exactly the population size because these are remnant populations.

For the simulations, we considered diploid hermaphrodite species, with non-random mating system and non-clonal reproduction. According to the study by DARRAULT; SCHLINDWEIN (2006), it is self-incompatible, demanding different genotypes of the species and specific pollinators for crossing and fruit production to occur. The flowers have a complex pollination mechanism co-adapted to pollination by moths and butterflies. The fruits are dispersed by large and medium-sized mammals. A spatial migration model was considered, with coordinates based on the geographic location of the populations which were obtained from the geographical coordinates (latitude and longitude) according to the map. Regarding the mutation settings, we assumed nine loci evolving according to the single-step mutation model (SSM), which is assumed to be typical for microsatellites (VALDES et al., 1993), and considered a proportion of 0.1 K-allele model (KAM) events, under 42 possible allelic states, according to the data obtained by AMORIM et al. (2015). A mutation rate of 0.0001 mutations per locus and per generation was assumed. The genetic variability of the initial population was considered the maximum, and authors simulated 100 generations. Each combination of selfing

Table 1 - Sample sizes and geographic coordinates of the studied *Hancornia speciosa* populations used in the simulations.

Population	Sample size	Geographic coordinates
Reserva do Caju (SE)	19	11°11'6"S and 37°11'18"W
Abaís (SE)	20	11°18'18"S and 37°17'18"W
Barrados Coqueiros (SE)	20	10°49'10"S and 36°56'52"W
Jacarecoara (CE)	14	4°07'10"S and 38°10'34"W
Tapera (CE)	6	3°56'20"S and 38°20'18"W
Tamandaré (PE)	15	8°43'50"S and 35°6'10"W
Total	94	

Source: AMORIM et al. (2015).

and migration rates was replicated 100 times. The observed and expected heterozygosities from AMORIM et al. (2015) 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.

RESULTS AND DISCUSSION

According to our simulations, there is a clear trend of increasing heterozygosity (both H_O and H_E) with increasing migration rate and decreasing selfing (Figure 1). Heterozygosity is one of the parameters of genetic diversity. While gene flow increases the genetic diversity by enabling the allele exchange among populations (SMITH et al., 2020), selfing reduces genetic diversity by limiting the pollen and seed dispersion and; therefore, the potential for recombination (JULLIEN et al., 2019).

The closest values of H_O and H_E to those obtained through the use of microsatellites (0.47 and 0.60, respectively) were generated under the selfing of 0.3 and the migration rate of 0.5 to 0.6 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. Migration rates are higher than selfing rates, which suggested that spatial patterns may be more important than the degree of inbreeding for *H. speciosa.*

The rate of selfing of 0.3 generated from the simulations implies an outcrossing rate of 0.7 which enables us to classify the mode of reproduction of the population as mixed, with a predominance of outcrossing, according to the classification of DESTRO & MONTALVÁN (1999). This author classifies vegetal 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). Outcrossing in hermaphrodite plants, like *H. speciosa* is usually enforced by self-incompatibility. According to TAKAYAMA & ISOGAI, 2005), self-incompatibility is an important mechanism in flowering plants that prevents selfing and thereby generate and maintain genetic diversity within a species. The estimated rate of self-incompatibility among tropical tree species is 0.88 (SOBIERAJSKI et al., 2006). In studying the reproductive system of *Shorea congestiflora* and *S. trapezifolia* from Sri Lankan tropical rain forests, MURAWSKI et al. (1994) observed differences in the outcrossing rates between the two species due to differences in the degree of self-incompatibility. This finding ratifies the importance of self-incompatibility in promoting outcrossing; therefore, increasing genetic diversity.

Regarding the migration settings, the simulations derived a rate of 0.5 to 0.6. Migration in these populations occurs, probably, through seed

	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
	0,1		0,2		0,3		0,4		0,5		0,6		0,7		0,8		0,9	
Selfing 0.1	0,50	0,53	0,51	0,54	0,51	0,53	0,54	0,57	0,54	0,57	0,55	0,57	0,55	0,58	0,55	0,58	0,55	0,58
Selfing 0.3	0,41	0,51	0,42	0,52	0,43	0,52	0,43	0,52	0,47	0,56	0,47	0,56	0,46	0,56	0,46	0,56	0,46	0,56
Selfing 0.5	0,33	0,48	0,33	0,50	0,33	0,50	0,34	0,50	0,34	0,51	0,35	0,51	0,35	0,51	0,36	0,52	0,42	0,51

Figure 1 - Estimates of observed (H_O) and expected (He) heterozygosities generated by simulations, for different rates of selfing and migration.

dispersal, mediated by long-distance dispersers, considering the long distances among them. Gene flow is the exchange of alleles among populations, capable of altering the original gene composition. It can be translated as the number of migrants among populations each generation (SLATKIN, 1985). High levels of gene flow increase the genetic diversity in populations (BURCZYK et al., 2004), as observed by plotting the heterozygosity (observed and expected) and the migration rate (Figure 1).

Gene flow in plant populations is an evolutionary force that promotes the homogenization of gene frequencies between populations. Through gene flow, the allele frequencies between populations become more similar. It is opposite to genetic drift that promotes genetic differentiation between populations (ELLSTRAND, 1992). Some conservation programs focus on reducing barriers to gene flow in fragmented populations (KWAK et al., 1998; AULER et al., 2002; KAMM et al., 2010). Such barriers can be physical or distance barriers, often created by populations fragmented into small groups which are more susceptible to genetic drift (NEIGEL, 1997). In another study, BUSCHBOM et al. (2011) observed effective long-distance gene flow that contributed considerably to the genetic diversity of an oak stand at St Sibay east of the Ural Mountains. This result reinforces the importance of gene flow in the long-term persistence of this relict stand.

In our simulation, there was a gradual decrease in the genetic diversity parameters number of alleles, observed heterozygosity and expected heterozygosity over generations, probably due to anthropic activities (Figure 2). The number of alleles determines the probability for a population to survive over generations under adverse events such as forest fires, insect and disease outbreaks, drought, which result in the loss of part of the original genetic diversity. High genetic diversity increases the chance of existing pre-adapted genotypes to future perturbations. When populations are fragmented, there is a decrease in the number of alleles in each fragment (GAMFELDT & KÄLLSTRÖM, 2007). In analysing the genetic diversity of *Dalbergia odorifera*, LIU et al. (2019) obtained a higher number of alleles in larger populations.

The heterozygosity of a population is defined as the probability that two randomly sampled gene copies are different, being a good measure of genetic diversity (NEI, 1978). The possibilities of combining different alleles rely on the number of alleles in the population. Therefore, the decrease in the number of alleles contributes to reducing the heterozygosity. It can be confirmed by the similar behavior of graphs for these parameters (Figure 2). In addition, when the lost alleles include self-incompatibility alleles, it leads to an increase in selfing, increasing the proportion of homozygosity (VINSON et al., 2015).

Our results reinforced the importance of conserving high genetic diversity in forest populations in order to ensure the sustainability of this important natural resource. The studied populations of *H. speciosa* result from a long fragmentation process by anthropic activities. Furthermore, the genetic erosion of this species can also be explained by

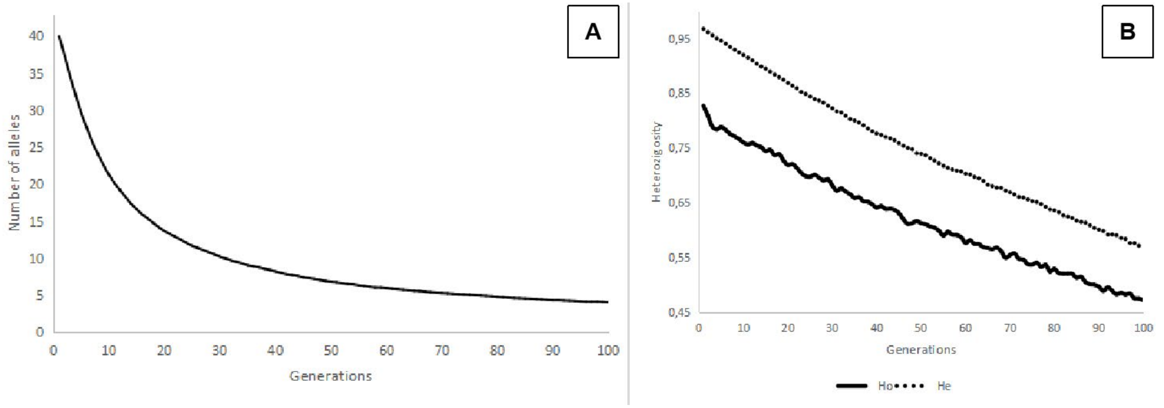

Figure 2 - Evolution of the number of alleles (A) and heterozygosity (B) over time. H_O: observed heterozygosity; He: expected heterozygosity.

human selection throughout domestication due to the real estate expansion and the intensification of agriculture in areas of natural occurrence (SOARES et al., 2019). According to the simulations, the genetic diversity of this species will diminish in the long-running. The long distances that separate these populations today limit the connectivity through gene flow. Therefore, local mutations are the unique source for new alleles. which are recombined through crossing, contributing to a level of genetic diversity in these populations. Thus, local strategies must be adopted to contain genetic erosion. We recommend enriching the area with high genetic diversity germplasm and minimizing disturbance to promote the occurrence of pollinators.

CONCLUSION

The *H. speciosa* populations have a selfing rate of 0.3 and a migration rate of 0.5 to 0.6. The observed selfing and migration rates could be the result of genetic drift and isolation, respectively, reducing the genetic diversity. Strategies to contain the loss of genetic diversity must be adopted locally. We suggest expanding the area and reducing the disturbance to promote the occurrence of pollinators, which are responsible for increasing the genetic diversity by promoting crossing.

ACKNOWLEDGMENTS

This study was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

AMORIM, J.A.E. et al. Diversity and genetic structure of mangaba remnants in states of northeastern Brazil. **Genetics and Molecular Research**, v.14, n.1, p.823-833, 2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/25730022/>. Accessed: Mar. 14, 2020. doi: 10.4238/2015.February.2.7.

AULER, N. M. F. et al. The genetics and conservation of *Araucaria angustifolia*: I. Genetic structure and diversity of natural populations by means of nonadaptive variation in the State of Santa Catarina, Brazil. **Genetics and Molecular Biology**, v.25, p.329–338, 2002. Available from: <https://www.scielo.br/j/gmb/a/wSv5Rs7wXpnVQ4NYmh35LfG/?lang=en>. Accessed: Jul. 02, 2021. doi: 10.1590/S1415-47572002000300014.

BALLOUX, F. "EASYPOP (Version 1.7): A Computer Program for the Simulation of Population Genetics. **Journal of Heredity**, v.92, n.3, p.301-302, 2001. Available from: <https://academic.oup.com/jhered/article/92/3/301/2187330>. Accessed: Mar. 14, 2019. doi: 10.1093/jhered/92.3.301.

BUSCHBOM, J. et al. Efficient long-distance gene flow into an isolated relict oak stand. **Journal of Heredity**, v.102, n.4, p.464-472, 2011. Available from: <https://academic.oup.com/jhered/article/102/4/464/899137>. Accessed: Jul. 03, 2021. doi: 10.1093/jhered/esr023.

BURCZYK, J. et al. Gene flow in forest trees: how far do genes really travel. **Forest Genetic**, v.11, p.1-14, 2004. Available from: <http://www.as.wvu.edu/~sdifazio/peer8.pdf>. Accessed: Out. 03, 2020.

DESTRO D.; MONTALVÁN, R. **Melhoramento Genético de Plantas**. Londrina: Ed. UEL, 1999. 820p.

DARRAULT, R. O.; SCHLINDWEIN, C. Polinização. In: SILVA JUNIOR, J. F.; LEDO, A. S. (Org.). **A cultura da mangabeira**. Aracaju: Embrapa Tabuleiros Costeiros, 2006. p. 43-56.

DUMINIL et al. Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests. **Heredity**, v.116, p.295–303, 2016. Available from: <https://www.nature.com/articles/hdy2015101#citeas>. Accessed: Jul. 04, 2021. doi: 10.1038/hdy.2015.101.

ELLSTRAND, N. C. Gene flow among seed plant populations. In: ADAMS, W.T. et al. **Population genetics of forest trees**. Dordrecht: Forestry Sciences, Springer, v.42, 1992, Available from: <https://link.springer.com/chapter/10.1007%2F978-94-011-2815-5_13>. Accessed: Jul. 03, 2021. doi: 10.1007/978-94-011-2815-5_13.

GAMFELDT, L.; KÄLLSTRÖM, B. Increasing intraspecific diversity increases predictability in population survival in the face of perturbations. **Oikos**, v.116, n.4, p.700-705, 2007. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.0030-1299.2007.15382.x>. Accessed: Out. 07, 2020. doi: 10.1111/j.0030-1299.2007.15382x.

JULLIEN, M. et al. Structure of multilocus genetic diversity in predominantly selfing populations. **Heredity,** v.123, p.176–191, 2019. Available from: <https://www.nature.com/articles/s41437-019-0182-6#citeas>. Accessed: Jul. 04, 2021. doi: 10.1038/s41437-019-0182-6.

KAMM, U. et al. Open areas in a landscape enhance pollen-mediated gene flow of a tree species: evidence from northern Switzerland. **Landscape Ecology**, v.25, p.903-911, 2010. Available from: <https://link.springer.com/article/10.1007/s10980-010-9468-z>. Accessed: Jul. 03, 2021. doi: 10.1007/s10980-010-9468-z.

KWAK, M. M. et al. Pollen and gene flow in fragmented habitats. **Applied Vegetation Science**, v.1, n.1, p.37–54, 1998. Available from: <https://www.jstor.org/stable/1479084>. Accessed: Jul. 03, 2021. doi: 10.2307/1479084.

LEDERMAN, I. E. et al. Mangaba (*Hancornia speciosa* Gomez). In: LENDERMAN, I. E. **Série Frutas Nativas**. Jaboticabal: Funep, 2000. 35p.

LIU, F. et al. Genetic diversity of the endangered *Dalbergia odorifera* revealed by SSR markers. Available from: <https://www.mdpi.com/1999-4907/10/3/225>. Accessed: Jan. 15, 2021. **Forests**, v.10, n.225, p.1-17, 2019. doi: 10.3390/f10030225.

MOURA, N. F. et al. Seleção de marcadores RAPD para o estudo da estrutura genética de populações de *Hancornia speciosa* Gomez. **Bioscience Journal**, v.21, p.119-125, 2005. Available from: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/6615>. Accessed: Nov. 25, 2020.

MURAWSKI, D. A. et al. Outcrossing rates of two endemic Shorea species from Sri Lankan tropical rain forests. **Biotropica**, v.26, n.1, p.23-29, 1994. Available from: <https://www.cabi.org/isc/abstract/19936793200>. Accessed: Dec. 20, 2020. doi: 10.2307/2389107.

NEI, M. **Molecular evolutionary genetics**. New York: Columbia University Press, 1978. 512p.

NEIGEL, J. E. A comparison of alternative strategies for estimating gene flow from genetic markers. **Annual Review of Ecology and Systematics**, v.28, n.1, p:105-28, 1997. Available from: <https://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.28.1.105>. Accessed: Jan. 29, 2020. doi: 10.1146/annurev.ecolsys.28.1.105.

SCHLAEPFER, D.R. et al. Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. **Ecosphere**, v.9, n.10, e02488, 2018. Available from: <https://esajournals.onlinelibrary.wiley.com/doi/full/10.1002/ecs2.2488>. Accessed: Jan. 23, 2021. doi: 10.1002/ecs2.2488.

SLATKIN, M. Gene flow in natural populations. **Annual Review of Ecology and Systematics**, v.16, p.393–430, 1985. Available from: <https://www.annualreviews.org/doi/abs/10.1146/annurev.es.16.110185.002141>. Accessed: Jul. 04, 2021. doi: 10.1146/annurev.es.16.110185.002141.

SMITH, A.L et al. Global gene flow releases invasive plants from environmental constraints on genetic diversity. **PNAS**, v.117, n.8, p.4218–4227, 2020. Available from: <https://www.pnas.org/content/pnas/117/8/4218.full.pdf>. Accessed: Jul. 04, 2021. doi: 10.1073/pnas.1915848117.

SOARES, L. A. S. S. et al. Anthropogenic disturbances eroding the genetic diversity of a threatened Palm tree: A multiscale approach. **Frontiers in Genetics**, v.10, p.1-12, 2019. Available from: <https://www.frontiersin.org/articles/10.3389/fgene.2019.01090/full>. Accessed: Jun. 05, 2021. doi: 10.3389/fgene.2019.01090.

SOBIERAJSKI, G. R. et al. Sistema de reprodução em nove populações de *Mimosa scabrella* Bentham (*Leguminosaceae*). **Scientia Forestalis**, Piracicaba, n.71, p.37-49, 2006. Available from: <https://www.ipef.br/publicacoes/scientia/nr71/cap04.pdf>. Accessed: Jan. 23, 2021.

TAKAYAMA, S.; ISOGAI, A. Self-incompatibility in plants. **The Annual Review of Plant Biology**, v.56, p.467–89, 2005. doi: 10.1146/annurev.arplant.56.032604.144249. Available from: <https://pdfs.semanticscholar.org/725d/beaeff1bb6b5441887253ca8c4de71f3b5d3.pdf>. Accessed: Feb. 14, 2021. doi: 10.1146/ annurev.arplant.56.032604.144249.

VALDES, A. M. et al. Allele frequencies at microsatellite *loci*: the stepwise mutation model revisited. **Genetics**, v.133, n.3, p.737-749, 1993. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1205356/>. Accessed: May, 10, 2021.

VINSON, C. C. et al. Impacts of selective logging on inbreeding and gene flow in two Amazonian timber species with contrasting ecological and reproductive characteristics. **Molecular Ecology**, v.24, n.1, p.38-53, 2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/25402015/>. Accessed: Feb. 10, 2021. doi: 10.1111/mec.13002.