



Simulating the reproductive system of *Hymenaea courbaril* populations in the Amazon forest

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Abstract - In this study we used simulation of genetic parameters based on microsatellite data to investigate the reproductive system of three *Hymenaea courbaril* L. species populations. Different selfing, migration and clonal reproduction rates were tested using the Easypop software. The results suggest the presence of both sexual and clonal reproduction in these populations. The simulated populations showed gradual decrease of the genetic diversity due the low gene flow caused by fragmentation. Due to connectivity importance, it is suggested expanding the area and replacing the explored trees using high genetic diversity germplasm in order to minimize the negative effects of fragmentation.

Simulação do sistema reprodutivo de populações de *Hymenaea courbaril* na Amazônia Sul-Occidental

Resumo - Neste estudo foram usadas simulações de parâmetros genéticos com base em dados de microssatélites para investigar o sistema reprodutivo de três populações de *Hymenaea courbaril* L. Foram testadas diferentes taxas de autofecundação, migração e reprodução clonal usando o software Easypop. Os resultados sugerem a presença de reprodução sexual e clonal nessas populações. Houve diminuição gradual da diversidade genética devido ao baixo fluxo gênico ocasionado pela fragmentação. Devido à importância da conectividade, sugere-se a expansão da área e substituição das árvores exploradas por germoplasma de elevada diversidade genética, visando a minimizar os efeitos negativos da fragmentação.

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The Amazon Forest is the largest continuous forest ecosystem in the world, characterized by high heterogeneity of habitats, soil, and micro-climates, resulting in a high biodiversity. This biodiversity has attracted worldwide attention regarding the sustainable exploitation and conservation of this ecosystem.

However, as deforestation of the Amazon advances at an alarming pace, sustainable exploitation requires research on the impact of forest management activities on the distribution, structure, genetic diversity and ecology (pollination and dispersion) of potential timber species (Werth & Avissar, 2002; Silva et al., 2014).

Hymenaea courbaril L. is a heavily exploited Amazon Forest timber species. Among several consequences, deforestation causes population fragmentation and size reduction. Small-sized populations are more susceptible to genetic drift and inbreeding effects, leading to a loss in genetic diversity, which is determinant for the sustainability of species (Silva et al., 2014; Matesanz et al., 2017). According to Charlesworth (2006), inbreeding increases homozygosity, making individuals to express recessive deleterious mutations and reducing their survival probabilities and fertility. Another effect of inbreeding is the decrease in effective population size, resulting in greater effects of genetic drift, which reduces the ability of natural selection to eliminate deleterious mutations from the population and increases the frequency of advantageous mutations. The genetic diversity of populations is affected by the reproductive system. According to the prevailing sexual reproduction mode, natural populations of forest species can be classified as autogamous (when selfing prevails), allogamous (when outcrossing prevails), or mixed. Outcrossing favors the increase of genetic diversity through the recombination of genes while selfing reduces the genetic diversity (Destro & Montalván, 1999). Clonal reproduction is other factor that reduces genetic diversity in trees although this reproduction system enhances population persistence via short range dispersal (Otálora et al., 2013).

Aspects related to the reproductive system should be considered in genetic diversity conservation programs, besides gene flow, which is important to reduce inbreeding. In this study, the Easypop program (Balloux, 2001) was used to perform simulations of genetic data obtained through microsatellite molecular markers by Silva et al. (2014), to estimate the rates of selfing, migration, and clonal reproduction of three *H. courbaril* populations in the Amazon Forest.

Easypop version 2.0.1 (Balloux, 2001) was used to simulate different rates of selfing (0.1, 0.3 or 0.5), migration rate (0.1, 0.3, 0.5, 0.7 or 0.9), and clonal reproduction (0.0, 0.2, 0.4 or 0.6) in order to select the combination that results in parameters close to those reported by Silva et al. (2014). These published data comprised genetic records of microsatellite markers from three populations of *H. courbaril* sampled from Lábrea, Amazonas State, Capixaba, Acre State and Porto Acre, Acre State, in the Brazilian Amazon Forest. The estimated mean observed heterozygosity was

$H_O = 0.6197$ and the mean expected heterozygosity was $H_E = 0.6963$ (Silva et al., 2014).

The simulations were performed for a diploid hermaphrodite species with non-random mating. A spatial migration model was considered, with coordinates based on the populations' geographic location (latitude and longitude). Regarding mutation, it was assumed 10 loci evolving according to the single-step mutation model (SSM), with a proportion of 0.1 *K*-allele model (KAM) events, under 107 possible allelic states (Silva et al., 2014). A mutation rate of 0.0001 mutations per locus per generation was assumed. The number of generations assumed in the simulations was 100. For each combination of selfing and migration rates, 100 replicates were simulated and we selected the combination that showed values of observed and expected heterozygosity closest to those previously obtained with microsatellite markers.

Values of heterozygosities (observed and expected) for different rates of selfing, migration and clonal reproduction are shown in Table 1. The closest values of H_O and H_E to those obtained using microsatellite markers ($H_O = 0.6197$ and $H_E = 0.6963$ respectively) were generated under the selfing of 0.1, the migration rate of 0.1 and the clonal reproduction rate of 0 to 0.2 (Table 1). These values were significantly different from the other rates, based on two independent sample t-tests at a 5% level of probability of error.

Our results showed a trend of increasing observed and expected heterozygosity as migration rate increased and selfing and clonal reproduction reduced (Table 1). Gene flow (migration) enables gene exchange among populations, thus increasing the genetic diversity (Madsen et al., 1995). On the other hand, selfing and clonal reproduction limit the potential for recombination, resulting in low genetic diversity (Loveless & Hamrick, 1987).

Our results suggest evidence of natural clonal reproduction in *H. courbaril*, although it has not yet been reported. Therefore, field trials on clonal reproduction of *H. courbaril* should be performed to confirm this finding considering the importance of this knowledge for management and conservation actions. Clonal reproduction was registered in the congener species *Hymenaea stigonocarpa* Mart. ex Hayne (Moreno, unpublished data) and it is considered common in natural forests (Silvertown, 2008).

Table 1. Estimates of observed (H_o) and expected (H_e) heterozygosities generated by simulations, for different rates of selfing, migration and clonal reproduction of three *Hymenaea courbaril* populations from the Brazilian Amazon Forest.

Selfing rates	Migration rates	Clonal reproduction rates							
		0		0.2		0.4		0.6	
		H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
0.1	0.1	0.654	0.693	0.653	0.693	0.656	0.691	0.650	0.681
	0.3	0.683	0.711	0.674	0.705	0.684	0.725	0.686	0.713
	0.5	0.689	0.721	0.685	0.724	0.685	0.721	0.689	0.722
	0.7	0.697	0.731	0.678	0.712	0.689	0.718	0.702	0.734
0.3	0.1	0.568	0.684	0.567	0.689	0.559	0.672	0.565	0.676
	0.3	0.630	0.711	0.563	0.686	0.585	0.695	0.550	0.669
	0.5	0.566	0.679	0.557	0.686	0.583	0.704	0.577	0.690
	0.7	0.572	0.697	0.581	0.700	0.580	0.692	0.588	0.707
0.5	0.1	0.418	0.628	0.417	0.621	0.445	0.657	0.423	0.616
	0.3	0.435	0.657	0.431	0.641	0.442	0.646	0.445	0.649
	0.5	0.436	0.656	0.442	0.655	0.443	0.658	0.452	0.668
	0.7	0.459	0.677	0.436	0.648	0.444	0.668	0.443	0.657

According to Destro & Montalván (1999), forest populations can be autogamous (outcross from 0 to 0.05), mixed (outcross from 0.05 to 0.95), or allogamous (outcross from 0.95 to 1). Outcrossing in *H. courbaril* was described by Bawa (1974). Based on our findings, the studied populations can be classified as mixed, with predominance of outcrossing in a rate of 0.9.

Despite the species having a mixed reproductive system, the results suggest a high reproductive dependence of the species by cross-pollination, which is carried out by biotic vectors. Bats are the main pollinators of this species, in addition to some daytime visitors such as hymenopterans, dipterans and hummingbirds (Bawa, 1974). According to the same author, although the species does not present a system of genetic incompatibility, it may present dichogamy or herkogamy that compromises self-fertilization rates. Due to the fragmentation process over time, there was gradual loss of alleles (Figure 1) and heterozygosity (Figure 2) in the *H. courbaril* populations. Once fragmentation turns large populations into smaller groups, connectivity of these groups through gene flow is crucial to avoid the loss of genetic diversity. *H. courbaril* has animal-mediated fruits and seeds dispersal (Silva et al., 2014), however, distances among fragments can possibly limit gene flow, such as observed in *Samanea saman* (Jacq.) Merr. in Costa Rica (Cascante et al., 2002).

In small-sized populations, there is an increased loss of alleles by genetic drift and decreased heterozygosity through increasing inbreeding levels (Ellstrand & Elam, 1993). Simulations carried out by Stefenon & Costa (2012), using the Easypop program indicated that smaller populations had a greater increase in the inbreeding coefficient over generations, resulting in less genetic variability, compared to larger populations.

Another effect of fragmentation is the deviation of genotype frequencies from the expected under the Hardy-Weinberg equilibrium, where observed heterozygosity equals expected heterozygosity (Ellstrand & Elam, 1993). Nevertheless, inbreeding reduces observed heterozygosity as shown in Figure 2.

For this reason, gene flow, an opposite force to genetic drift and inbreeding, has been explored in programs that prioritize genetic diversity such as restoration and conservation. The effect of two extreme migration rates (0.1 and 0.9) on the genetic diversity of *Cabralea canjerana* (Vell.) Mart. fragments was simulated by Serrote et al. (2019). The high migration rate homogenized the fragments, decreased the genetic differentiation coefficient among the fragments, and increased the genetic diversity, in contrast to the low migration rate that increased inbreeding, increased genetic differentiation, and reduced genetic diversity.

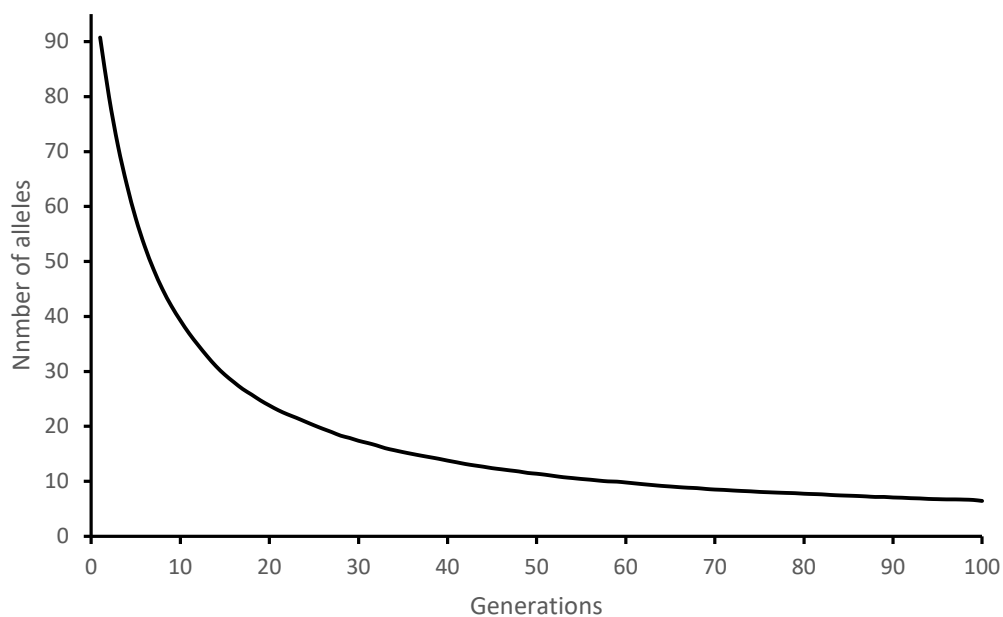


Figure 1. Simulation of number of alleles heterozygosities across 100 generations in three *Hymenaea courbaril* populations from the Brazilian Amazon Forest.

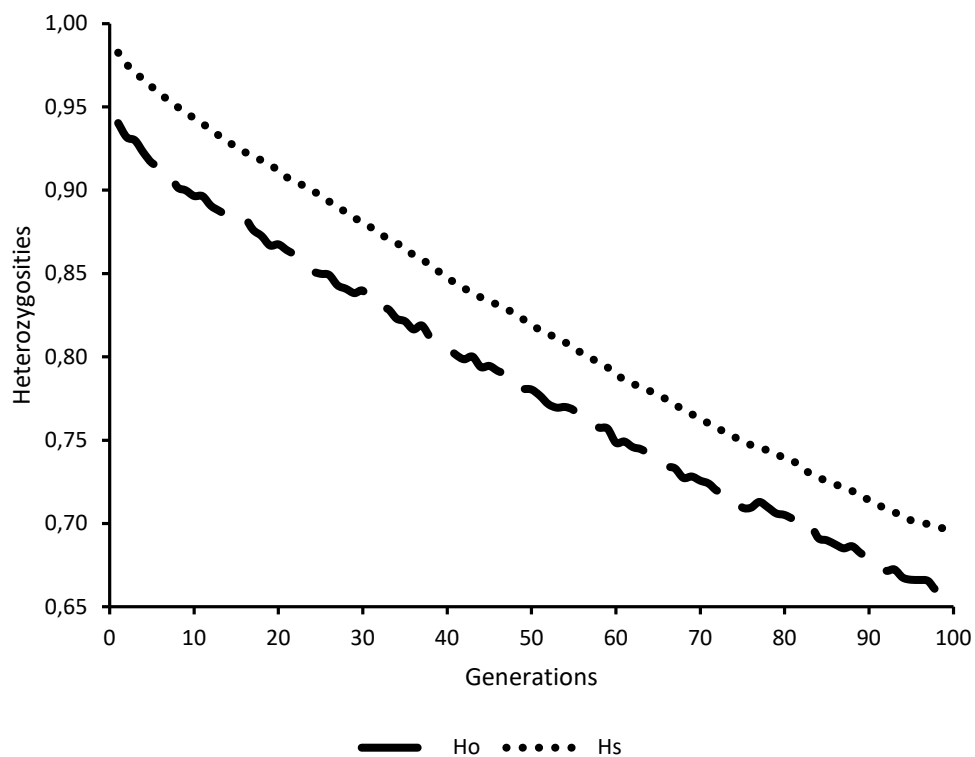


Figure 2. Simulation of observed (HO) and expected (HS) heterozygosities across 100 generations in three *Hymenaea courbaril* populations from the Brazilian Amazon Forest

Conclusions

The studied *Hymenaea H. courbaril* populations result from a long fragmentation process by anthropic activities, which likely resulted in a gradual loss of genetic diversity. The distances that currently separate these populations limit the interpopulation gene flow due to the distances covered by pollinators and fruit and seed dispersers. Our simulation study suggests that *H. courbaril* presents both sexual and clonal reproduction. The main mode of sexual reproduction is through outcrossing (90%). Our simulations suggest that populations may have experienced a gradual decrease in genetic diversity due to low gene flow (migration rate equal to 0.1). For this reason, strategies to prevent the loss of genetic diversity should be local, for each population. One strategy could be expanding the area of the populations and replacing the explored trees using high genetic diversity germplasm.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' Contributions

Caetano Miguel Lemos Serrote: Conceptualization, formal analysis, methodology, writing – original draft

Lia Rejane Silveira Reiniger: Conceptualization, investigation, methodology, supervision and writing – review & editing

Valdir Marcos Stefenon: Formal analysis, methodology, supervision and writing – review & editing

Charlene Moro Stefanel: Writing – original draft

Karol Buuron da Silva: Investigation and writing – review & editing

Ana Cristina da Fonseca Ziegler: Investigation

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