

OUTCROSSING RATES AND INBREEDING COEFFICIENTS IN RUBBER TREES (*Hevea brasiliensis* (WILLD. EX ADR. DE JUSS.) MÜELLER. ARG.)*

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ABSTRACT

Electrophoretic analyses were carried out in starch gel with young leaves of rubber trees of two native populations from the state of Acre, Brazil. The parameters evaluated in this study were obtained from the expected (\hat{H}_e) and observed (\hat{H}_o) heterozygote frequencies. Natural outcrossing rates (\hat{t}) and inbreeding coefficients in populations (\hat{f}) were estimated. Estimated \hat{t} was 19.30% and 21.34% for populations 1 and 2, with an average of 21.84% while estimated \hat{f} was 64.15%.

INTRODUCTION

The natural outcrossing rate of *Hevea brasiliensis* (rubber trees) has been little investigated despite its importance for the definition of breeding strategies and for the understanding of the genetic structure of natural populations.

Traditional methods for the evaluation of the reproductive system of a species are based on the observation of crosses, on the behavior of pollinating agents, on the examination of flower morphology, and on the results of controlled pollination experiments. These methods, though they provide indications about the reproductive

* Part of a thesis presented by J.R.P. to ESALQ/USP in partial fulfillment of the requirements for the Doctoral degree.

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system of a species, do not permit direct measurement of the reproductive success of populations and are inadequate for analysis at the population level.

The determination of outcrossing rates in natural populations has always been limited by factors such as irregular distance between plants - especially in arboreal species, natural barriers between plants, the difficulty in identifying a good genetic marker and the expression of dominance most often exhibited by these markers. Ritland and Jain (1981) have pointed out the strength and versatility of estimative processes when electrophoretic data are used, considering that many segregant loci with codominant alleles may frequently be detected in populations.

Electrophoresis permits the separation of alleles of the same gene, regardless of the environment. Individuals may be characterized by their genotypes, which consist of a gene sample, with the consequent possibility of comparing individuals or groups of individuals, in terms of strictly genetic characteristics.

We estimated the natural outcrossing rate and the degree of inbreeding existing in two natural rubber tree populations on the basis of expected and observed heterozygote frequencies obtained by electrophoresis.

MATERIAL AND METHODS

Plants were grown from seeds collected from native rubber tree populations existing in the Catuaba Forest Reserve at km 22, route BR 364, belonging to the Federal University of Acre (UFAC), and in the EMBRAPA/CPAF-Acre area located at km 14, route BR 364, both in the state of Acre (Figure 1).

Electrophoretic analysis were carried out on horizontal starch gel using fresh extracts of young leaves in foliar stage "B" (anthocyanin coloring, measuring 1.0 to 2.0 cm) as recommended by Lebrun and Chevallier (1988). The enzymatic systems studied were leucine aminopeptidase (LAP) - EC 3.4.11.1, malate dehydrogenase (MDH) - EC 1.1.1.37, and shikimate dehydrogenase (SKDH) - EC 1.1.1.25 (Table I).

One leaflet each from twenty seedlings per family were used for the enzyme source. A total of 26 and 27 families (open-pollinated progenies) for populations 1 and 2, respectively, were analysed.

The inbreeding coefficients within populations were estimated from expected (\hat{H}_e) and observed (\hat{H}_o) heterozygote frequencies, using the following equation:

$$\hat{f} = (\hat{H}_e - \hat{H}_o) / \hat{H}_e,$$

according to Li (1955) and Weir (1990), where:

$$\hat{H}_e = 1 - \sum p_u^2, p_u \text{ being the expected homozygote frequencies for the "u" allele;}$$

$$\hat{H}_o = 1 - \sum P_{uu}, P_{uu} \text{ being the observed homozygote frequency for the "u" allele.}$$

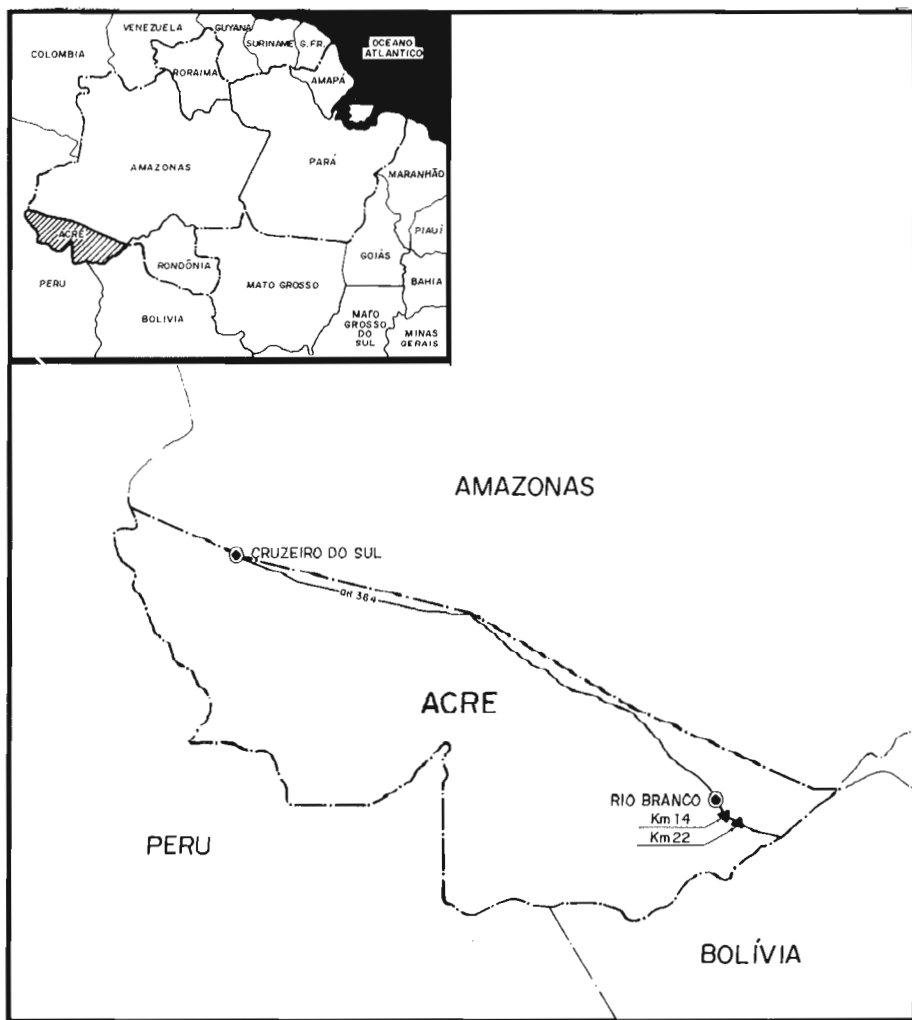


Figure 1 - Location of two native rubber tree populations in the State of Acre.

The outcrossing rate of the species was estimated for each allele:

$$\hat{t} = (1 - \hat{f}) / (1 + \hat{f}).$$

The inbreeding coefficient error and the outcrossing rate error were calculated:

$$\text{Var}(\hat{t}) = \frac{4}{(1 + \hat{f})^4} [\text{Var}(\hat{f})]; \text{ according to Weir (1990);}$$

Table I - Enzyme systems, buffers, allozymes and relative mobility (RM).

Enzyme	Loci	Buffers*			Allozyme	RM
		Electrode	Gel	Staining		
Leucine aminopeptidase EC 3.4.11.1	LAP-1	A	B	D	1	100
					2	107
					3	116
					4	93
					5	87
	LAP-2	A	B	D	1	100
					2	113
					3	91
					4	76
Shikimate dehydrogenase EC. 1.1.1.25	SKDH	A	B	C	1	100
					2	115
					3	98
					4	76
					5	56
Malate dehydrogenase EC. 1.1.1.37	MDH-1	A	B	C	1	100
					2	90
					3	80

* A: Tris-citrate, pH 6.6; B: 0.05 M Histidine, pH 6.0; C: Tris-HCl, pH 8.5; D: Tris-maleate, pH 5.4 (Lebrun and Chevallier, 1988).

$$\text{Var}(\hat{f}) = \frac{1}{nH_c^4} \left\{ 2H_o^2 \left[\sum_u p_u^2 (p_u + P_{uu}) + \sum_{u < v} p_u p_v P_{uv} \right] - (4H_c H_o \cdot \right.$$

$$\left. \sum_u p_u P_{uu} \right) - (2H_o - H_o H_c - H_c)^2 + H_c^2 (1 - H_o) \Big\};$$

according to Vencovsky (personal communication), where: $\text{Var}(\hat{f})$ is the variance of the inbreeding coefficient, n is the number of trees studies, H_c is the expected heterozygote frequency in the population, H_o is the observed heterozygote frequency in the population, p_u is the expected homozygote frequency for the "u" allele, P_{uu} is the observed homozygote frequency for the "u" allele, p_v is the expected heterozygote frequency for the "v" allele, and P_{uv} is the observed heterozygote frequency for alleles "u" and "v".

RESULTS AND DISCUSSION

Two loci were identified in the LAP system (LAP-1 and LAP-2). Five alleles were identified in LAP-1, which were denoted by convention as allele 1 (the most common), alleles 2 and 3 (alleles with relative migration (RM) higher than 1), and 4 and 5 (alleles with RM lower than 1) (Table I). Four alleles were detected in the LAP-2 locus, of slower migration, which were identified as allele 1 (the most common), allele 2 (with RM higher than 1), and the remaining ones classified in decreasing RM order (Figures 2 and 3).

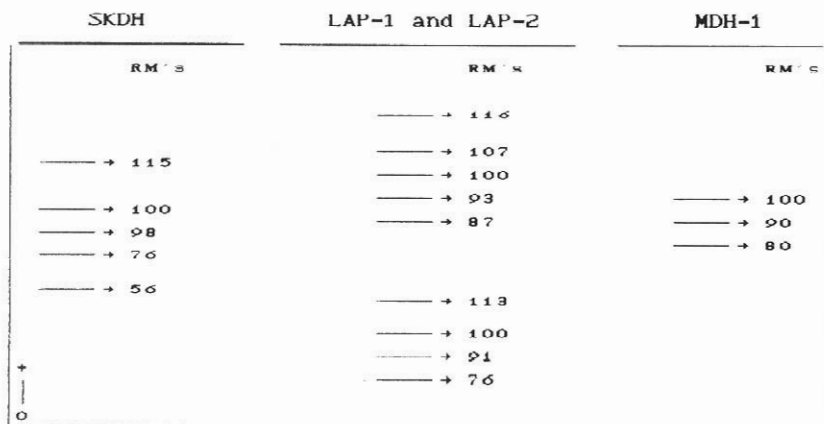


Figure 2 - Zymograms of the electrophoretic patterns representing seventeen alleles at four loci for three enzyme systems.

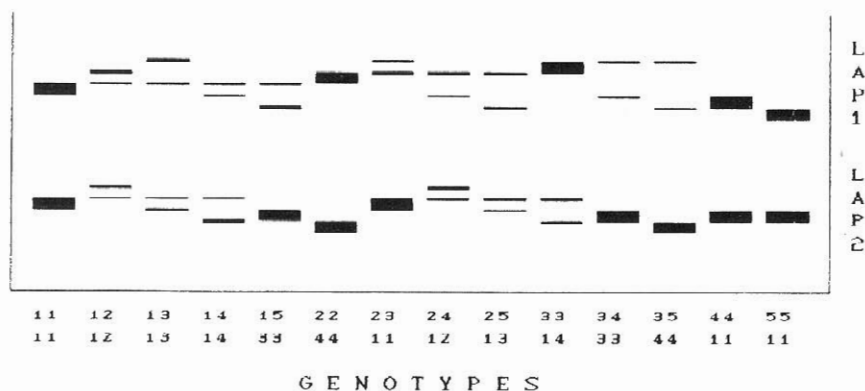


Figure 3 - Isozyme band patterns detected in the analysis of specimens from two natural rubber tree populations.

Only one locus with five alleles was identified in the SKDH system. Again, allele 1 was the most common, allele 2 had RM higher than 1 and the remaining ones were classified in decreasing RM order (Figures 2 and 4).

The MDH system presented a band pattern of difficult interpretation in terms of all loci. The most rapid locus in the system, with three alleles, was identified and analyzed. Allele 3, which was slower, was superimposed on the most rapid allele of another possible locus (Figures 2 and 5).

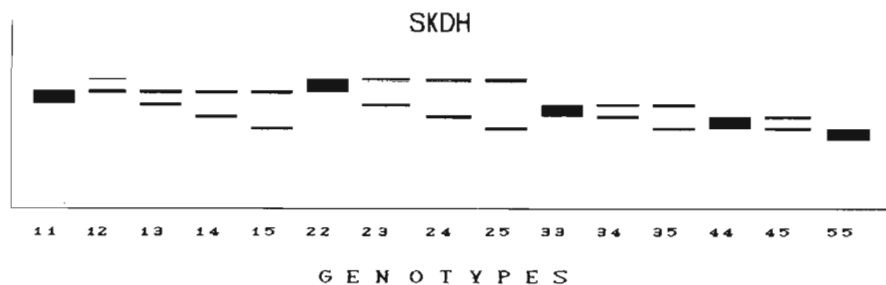


Figure 4 - Isozyme band patterns detected in the analysis of specimens from two natural rubber tree populations.

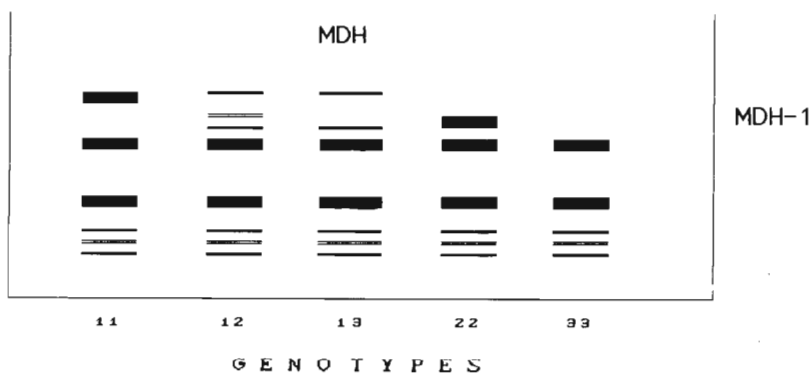


Figure 5 - Isozyme band patterns detected in the analysis of specimens from two natural rubber tree populations.

The inbreeding coefficient for the population (\hat{f}) and the outcrossing rate (\hat{t}) estimated from expected (\hat{H}_c) and observed (\hat{H}_o) heterozygote frequencies for the 17 alleles detected at the loci studied in the two populations are presented in Table II.

Table II - Estimates of inbreeding coefficient (\hat{F}) and outcrossing rate (\hat{t}) calculated from expected (\hat{H}_e) and observed (\hat{H}_o) heterozygote frequencies for four loci in two natural rubber tree populations.

Populations	Parameters	Loci*				Mean
		LAP-1	LAP-2	SKDH	MDH-1	
1	\hat{H}_e	0.3550	0.1928	0.5988	0.1271	-
	\hat{H}_o	0.3053	0.1359	0.4702	0.1003	-
	\hat{f}	0.1400	0.2951	0.2148	0.2108	0.1930
		(0.0374)	(0.0648)	(0.0374)	(0.0721)	-
	\hat{t}	0.7544	0.5443	0.6464	0.6518	0.6764
	(0.0574)	(0.0775)	(0.0510)	(0.0985)		
2	\hat{H}_e	0.2658	0.2463	0.6478	0.0195	-
	\hat{H}_o	0.2197	0.2077	0.4702	0.0197	-
	\hat{f}	0.1734	0.1567	0.2742	-0.0103	0.2134
		(0.0400)	(0.0548)	(0.0436)	(0.8193)	-
	\hat{t}	0.7044	0.7296	0.5696	1.0208	0.6483
	(0.0283)	(0.0412)	(0.0538)	(1.6729)	-	
Mean	\hat{H}_e	0.3109	0.2207	0.6252	0.0726	-
	\hat{H}_o	0.2598	0.1726	0.4701	0.0584	-
	\hat{f}	0.1644	0.2179	0.2481	0.1956	0.2184
		(0.0288)	(0.0412)	(0.0257)	(0.0755)	-
	\hat{t}	0.7176	0.6422	0.6024	0.6728	0.6415
	(0.0424)	(0.0557)	(0.0173)	(0.1058)	-	

* The values in parentheses correspond to the standard deviations.

The \hat{f} values varied considerably (Table II) indicating variation in the rates of fixed homozygotes in the populations. The negative \hat{f} value may indicate that the real value is close to zero or that a larger sample should be used to analyze this locus.

In a previous study (Paiva *et al.*, in press), we obtained similar inbreeding coefficient estimates within each population (\hat{f}) and for the mean of two populations (\hat{F}), using a different method based on analysis of variance of allele frequencies in the populations. The values obtained for the mean of 17 alleles were 21.6% and 21.8%.

The mean inbreeding rate for natural rubber tree populations estimated by two different methods was always above 20%, with all estimates presenting high and coherent

values. It cannot be concluded that inbreeding was due to selfing of plants since crossing between related individuals can also cause an increase in \hat{f} value.

Simmonds (1989) concluded that studies on cross pollination in rubber tree plantations apparently have not been well understood because of the occurrence of dwarf trees possibly caused by selfing of plants in seed gardens. This investigator also pointed out that the selfing rate ranges from 16% to 28%.

Estimates of the \hat{t} value from the expected and observed heterozygote frequencies were quite variable among loci, while the mean rate per locus in the two populations was more constant (Table II). The mean error maintained relatively low levels. The mean outcrossing rate for all loci in the two populations is low for a species assumed to be allogamous (Table II).

O'Malley *et al.* (1988), using the multilocus method, estimated an outcrossing rate of $\hat{t} = 0.85 \pm 0.03$ for Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.), a tropical rain forest canopy tree species, and pointed out that outbreeding is predominant in this species, though they did not rule out the possible occurrence of low inbreeding levels.

There is no doubt that tropical ecosystems have a high diversity of species, with great complexity in the interrelationships between organisms. The individuals of a species somehow exist isolated among individuals of other species. Plants and animals exist in an interdependent manner and are well coadapted, with consequent integration and stability of the ecosystem (Pianka, 1983). Thus, we propose that the natural outcrossing rate estimated for rubber trees, as well as the degree of inbreeding are biological strategies developed by the species so as to maintain a level of variability in the population, compatible with the diversity of the ecosystem.

ACKNOWLEDGMENTS

The authors are grateful to FAPESP for financial support, to the Federal University of Acre (UFAC) and to EMBRAPA/CPAF-Acre for logistic support for material collection, and to CENARGEN for partial support with laboratory material.

Publication supported by FAPESP.

RESUMO

O conhecimento da taxa de cruzamento natural da seringueira (*Hevea brasiliensis*) é de grande importância na definição de estratégias de melhoramento e no entendimento da estrutura genética das populações. Com o objetivo de estimar a taxa de cruzamento natural (\hat{t}) e os coeficientes de endogamia dentro de populações (\hat{f}), foram feitas análises de eletroforese em gel de amido com folhas jovens de plântulas de seringueira, originadas de 2 populações nativas do Estado do Acre. Os parâmetros foram estimados a partir das frequências esperadas (\hat{f}_e) e observadas (\hat{f}_o) de heterozigotos. As estimativas de \hat{f} foram de 19,30%, 21,34%

e 21,84%, respectivamente para as populações 1 e 2 e para a média delas. O valor médio de \hat{t} para todos os alelos foi de 64,15%. Discute-se a importância biológica desses parâmetros no equilíbrio das populações naturais.

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(Received July 16, 1992)