with stand development because of the accumulation of microenvironmental effects and inter tree competition.

An increase of heritability might occur when supressed trees in a plot die and consequently the within-plot variance decreases. If it were so, the causes of difference of our tests' results with others' might lie in a species difference. Since *Abies sachalinensis* is a shade-tolerant species, supressed trees survive longer in the understory. Should intensive thinning be carried out or stands developed further till suppressed trees die, the within-plot variance might decrease.

Type B genetic correlation r_B is comparable to that in slash pine (Hodge and White, 1992) from 6 to 16 years but lower at 1 and 21 years. It is believed that relatively large GE interaction operates for height growth at all ages. This agrees with the result of the test for family variance as mentioned above.

Genetic correlations between height at various ages and height at age 21 in single sites are always higher than phenotypic correlation (*Table 3*). They reach more than 0.9 at age 11 on all sites. Genetic correlations estimated for the 4 sites together showed a high positive value from 6 years to 16 years, while negative correlation was observed at year 1.

Genetic gains in height at 21 years expected from selection at early stages on each site are comparable to that expected from selection at 21 years (Table 4). It is considered that early selection for Abies sachalinensis is possible in view of the genetic gain in height, similarly as was shown for other tree species in many researches (Souillace and Gansel, 1974; Franklin, 1979; Lambeth, 1980; FOSTER, 1986; COTTERILL and DEAN, 1988; REHFELDT, 1992; Eriksson et al., 1993). On the other hand, some researchers have reported the negative phenotypic juvenile-mature correlations for the more aged progeny tests and risks of early selection (Toda, 1972; GIERTYCH, 1974). In Abies sachalinensis, age 21 is about a half to one third of rotation age. There is a possibility that negative genetic age-age correlations are observed for our tests with further investigations continued. But in practice, precise estimation of genetic parameters for more aged stands will be difficult in our tests if plot error variance and withinplot variance become still larger.

In *Abies sachalinensis*, accerelated flowering is not developed as yet. Therefore it is impossible to shorten the breeing generation for the present. Nevertheless early

assessment data is useful for selection because the expected genetic gain is high. The best use of data at various ages to maximize the genetic gain needs to be examined.

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References

ARASHI, T., KUSABA, T. and HARA, M.: A model of eliminating effects of macroscopic environmental variations from measured individual growth values in a stand. J. Jap. For. Soc. 53: 396-399 - Becker, W. A.: Manual of Quantitative Genetics. Fourth edition. Academic Enterprises, Pullman, Washington (1994). Burdon, R. D.: Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. Silvae Genet. 26: 168-175 (1977). - Burdon, R. D.: Early selection in tree breeding: principles for applying index selection and inferring input parameters. Can. J. For. Res. 19: 499-504 (1989). -- COTTERLL. P. P. and DEAN, C. A.: Changes in the genetic control of growth of radiata pine to 16 years and efficiencies of early selection. Silvae Genet. 37: 138-146 (1988). - Eriksson, G., Jonsson, A., Dormling, I., Norell, L. and Sterner, L.-G.: Retrospective early tests of Pius sytvestris L. seedlings grown under five nutrient regimes. Forest - Foster, G. S.: Trends in genetic para-Sci. 39: 95-117 (1993). meters with stand development and their influence on early selection for volume growth in loblolly pine. Forest Sci. 32: 944-959 (1986). - FRANKLIN, E. C.: Model relating levels of genetic variance to stand development of four north American conifers. Silvae Genet. 28: 207-212 (1979). -- GIERTYCH, M.: Inadequacy of early tests for growth characters as evidenced by a 59-year old experiment. Proceedings of Joint IUFRO Meeting, Stockholm. 237-242 (1974). --- HATAKEYAMA, S.: Genetic and breeding studies on the regional differences of interprovenance variation in Abies sachalinensis Mast. Bull. Hokkaido For. Exp. Stat. - Hodge, G. R. and White, T. L.: Genetic para-19: 1-91 (1981). meter estimates for growth traits at different ages in slash pine and some implications for breeding. Silvae Genet. 41: 252-262 - JIANG, I. B.-J.: Early testing in forest tree breeding: a review. For. Tree Improv. 20: 45-78 (1987). -- LAMBETH, C. C .: Juvenile-mature correlations in Pinaceae and implications for early selecton. Forest Sci. 26: 571-580 (1980). - Namkoong, G. and CONKLE, M. T.: Time trends in genetic control of height growth in - REHFELDT, G. E .: ponderosa pine. Forest Sci. 22: 2-12 (1976). -Early selection in Pinus ponderosa: compromises between growth potential and growth rhythm in developing breeding strategies. Forest Sci. 38: 661-677 (1992). - SAS Institute Inc. : SAS/STAT user's guide. Version 6. 4th edition, volume 2. SAS Institute Inc., Cary. NC. (1989). — Squillace, A. E. and Gansel, C. R.: Juvenile: mature correlations in Slash Pine. Forest Sci. 20: 225-229 (1974). Toda, R.: Heritability problems in forest genetics. IUFRO-SABRAO Joint Symposia Proceedings, Tokyo, A-3(I), 1-9 (1972). YANAGISAWA, T.: Regional variation in shape of strobile of Abies sachalinensis, Forest breeding in Hokkaido 8: 8-25 (1965).

Genetics of Rubber Tree (Hevea brasiliensis (Willd. ex Adr. de Juss.) Müll. Arg.)

I. Genetic Variation in Natural Populations*)

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Summary

Electrophoretic analyses were effected in 2 populations of rubber trees (*Hevea brasiliensis*) for the enzymatic systems MDH, SKDH and LAP, with the objective of drawing inferences on the genetic structure of the populations. Four loci (LAP-1, LAP-2, SKDH and MDH-1), with 5, 4, 5, and 3 alleles/locus, respectively, were identified. The estimated variability parameters exhibit a high

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Figure 1. — Location of 2 natural rubber tree populations in the State of Acre, Brazil.

genetic variation for the 2 populations ($H_T=0.3356$) and the greater part of the variability is within the population (99.85 %). The parameters that measure genetic distance demonstrate that the 2 populations are similar. The results indicate that the collection of rubber tree germplasm in this region studied should be directed to 1 population, with a greater number of individual per population.

Key words: Natural population, rubber tree, isozymes, outcrossing and inbreeding.

FDC: 165.5; 228.8; 176.1 Hevea brasiliensis.

Zusammenfassung

Das Ziel der Arbeit war Rückschlüsse auf genetische Strukturen von Populationen ziehen zu können; dazu wurden elektrophoretische Untersuchungen der enzymatischen Systeme von MDH, SKDH und LAP innerhalb 2er Kautschukpopulationen (Hevea brasiliensis) durchgeführt. Vier Loci (LAP-1, LAP-2, SKDH und MDH-1) mit 5, 4, 5 und 3 Allelen pro locus wurden identifiziert. Die untersuchten Viabilitätsparameter deuten auf eine hohe genetische Variation der beiden Populationen hin ($H_T = 0,3356$); die Variabilität innerhalb ihrer Populationen beträgt 99,85 % und ist damit größer als zwischen den Populationen. Die Parameter, die den genetischen Abstand zwischen 2 Populationen messen, zeigten daß sie ähnlich sind. Die Ergebnisse weisen darauf hin, daß sich die Studien bei der Sammlung genetischen Materials von Kautschukbäumen auf eine Population konzentrieren sollten, anstatt mehrere Populationen unterschiedlicher Standorte zu wählen.

Introduction

The genus *Hevea* is a taxon characteristics of tropical rain forests and is distributed by 6°N and 15°S latitude and 46°E and 77°W longitude, covering a wide variation of ecological and bioclimatical regions (Wycherley, 1977).

The rational utilization of the genetic variability of natural populations has been modest, considering its potential, and entirely directed towards obtaining productive and disease resistant clones.

However, new information is being obtained on the form of organization, maintenance, and distribution of the genetic variability of tropical species. This leads to the hypothesis that the rational cultivation of a species in a tropical environment must necessarily exist in equilibrium with the biotic factors of the ecosystem; otherwise cultivation is doomed to failure.

The utilization of electrophoresis enables separating the products of the alleles of the same gene, regardless of the environment. The individuals may be characterized by their genotypes, composed of a sample of genes, which enables comparing individuals or groups of individuals, in the light of strictly genetic characteristics.

Thus, Chevallier (1988) utilized isozyme markers to compare the amount of genetic variability existing in germplasm collections collected in 1981 in the States of Acre, Rondônia, and Mato Grosso, and of clones originated from Wickham's collection. The results demonstrated a high genetic variability existint in the collections. A total of 31 alleles in 10 loci and 23 alleles in 9 loci, respectively, were observed for the collection collected in 1981 and Wickham's material, with a proportion of 3.1 and 2.55 alleles per polymorphic locus. The material from Acre, Rondônia and Mato Grosso are polymorphic in 82.5%, 73.3% and 97% of their loci, respectively.

The objective of this study was to draw inferences on the genetic structure in 2 natural rubber tree populations, through isozyme characterization.

Material and Methods

The material used in this study consisted of plants originated from rubber tree seeds collected in 2 natural populations located 8 km apart in the State of Acre, Brazil, in native rubber tree populations existing in the Catuaba Forest Reserve, located at km 22 of Highway BR 364,

Table 1. — Enzymatic systems, abbreviations, buffers, allozymes and relative mobility (RM) used in the study of 2 natural populations of rubber tree.

| ENZVME | ABBREVI | BUF | FE | | F10.4 | |
|----------------------|---------|-----------|-----|----------|----------|-----|
| ENZYME | ATIONS | ELECTRODE | GEL | STAINING | ALLOZYME | RM |
| .Malate dehy- | MDH-1 | А | В | С | 1 | 100 |
| drogenase | | | | | 2 | 90 |
| EC 1.1.1.37 | | | | | 3 | 80 |
| .Shikimic de- | SKDH | Α | В | c | 1 | 100 |
| hydrogenase | | | | | 2 | 115 |
| EC 1.1.1.25 | | | | | 3 | 98 |
| | | | | | 4 | 76 |
| | | | | | 5 | 56 |
| .Leucine am <u>i</u> | LAP-1 | A | В | D | 1 | 100 |
| nopeptidase | | | | | 2 | 107 |
| EC 3.4.11.1 | | | | | 3 | 116 |
| | | | | | 4 | 93 |
| | | | | | 5 | 87 |
| | LAP-2 | A | В | D | 1 | 100 |
| | | | | | 2 | 113 |
| | | | | | 3 | 91 |
| | | | | | 4 | 76 |

^{*)} A: Tris citrate pH 6.6; B: Histidine 0.05M pH 6.0; C; Tris HCl pH 8.5; D: Tris Maleate pH 5.4 (Lebrun and Chevaller, 1988).

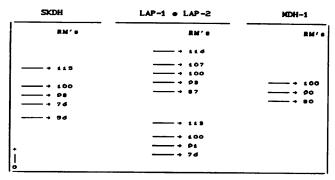


Figure 2. — Zimograms of electrophoretic patterns representing 17 alleles of 4 loci in 3 enzymatic systems of 2 natural rubber tree populations.

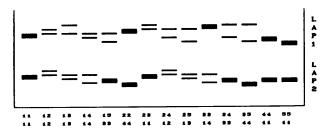


Figure 3. — Profiles of the isoenzyme bands found in the analysis of plants in 2 natural rubber tree populations.

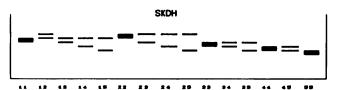


Figure 4. — Profiles of the isoenzyme bands found in the analysis of plants in 2 natural rubber tree populations.

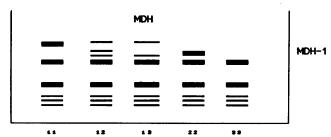


Figure 5. — Profiles of the isoenzyme bands found in the analysis of plants in 2 natural rubber tree populations.

belonging to the Federal University of Acre (UFAC), and in the EMBRAPA/UEPAE-Rio Branco area, located at km 14 of Highway BR 364 (Figure 1).

The electrophoretic analyses were effected in starch gel, with horizontal migration, utilizing fresh extract of young rubber tree leaves in foliar stage "B" (dropping leaves, anthocyanin coloration, with about 1.0 cm to 2.0 cm) according to Lebrun and Chevallier's (1988) recommendation. The enzymatic systems malate dehydrogenase (MDH) — EC 1.1.1.37, shikimic dehydrogenase (SKDH) — EC 1.1.1.25, and leucine aminopeptidase (LAP) — EC 3.4.11.1 were studied (Table 1).

One leaflet from each of 20 seedlings per family which were in a foliar stage adequate for the analyses were collected for each run. A total of 26 and 27 open-pollinated

families for population 1 and 2, respectively, with variable numbers of plants/progeny were analysed.

In the analysis of the electrophoretic data, at the level of populations, the computer program BYOSYS-1 (Swofford, 1989) was used. This program provides estimates of the allelic frequencies and measurement of genetic variability, besides testing the deviations of genotypic frequencies in relation to the Hardy-Weinberg equilibrium, calculating Wright's (1978) F-statistics and Nei's D coefficient (Nei, 1972) between populations.

Results and Discussion

The allozyme variants are characterized according to their loci and, for this, the migration distance of a standard variant is considered.

Figure 2 is a schematic representation of the electrophoretic patterns which showed good activity, and whose loci were determined for each of the 3 systems studied.

In the leucine aminopeptidase (LAP) system 2 loci (LAP-1 and LAP-2) were identified. Five alleles were identified in LAP-1. By convention they were denoted as follows: the most common — allele 1; those which presented RM's (relative migration) higher than 1, alleles 2 and 3, respectively; and those which showed RM's lower than 1, alleles 4 and 5 (*Table 1*). In locus LAP-2, of slower migration, 4 alleles were identified, where allele 2 presented RM higher than 1 and the others lower values (*Figure 3*).

In the shikimic dehydrogenase (SKDH) system only 1 locus, with 5 alleles, was identified. Allele 2 presented RM higher than 1 (Figure 4).

The interpretation of the band pattern of all of the loci in the malate dehydrogenase (MDH) system proved to be difficult under the conditions in which the material in question was analysed. In this system, the most rapid

Table 2. — Frequency of 17 alleles in 2 natural rubber tree populations (POP 1 and POP 2) and number of individuals/locus (N).

| LOCI | ALLELES | POP 1 | POP 2 | MEAN |
|-------|---------|--------|--------|--------|
| | 1 | 0,7968 | 0,8527 | 0,8251 |
| | 2 | 0,0498 | 0,0417 | 0,0457 |
| LAP-1 | 3 | 0,0418 | 0,0465 | 0,0442 |
| | 4 | 0,0548 | 0.0233 | 0,0388 |
| | 5 | 0,0568 | 0,0359 | 0,0462 |
| | N | 502 | 516 | 1018 |
| | 1 | 0,8941 | 0,8586 | 0,8760 |
| | 2 | 0,0000 | 0,0020 | 0,0010 |
| LAP-2 | 3 | 0,0169 | 0,0154 | 0,0161 |
| | 4 | 0,0890 | 0,1240 | 0,1068 |
| | N | 472 | 488 | 960 |
| | 1 | 0,5619 | 0,5127 | 0,5370 |
| | 2 | 0,2560 | 0,2639 | 0,2600 |
| SKDH | 3 | 0,1381 | 0,1146 | 0,1262 |
| | 4 | 0,0393 | 0,0856 | 0.0628 |
| | 5 | 0,0048 | 0.0231 | 0,0141 |
| | N | 420 | 432 | 852 |
| | 1 | 0,9328 | 1,0000 | 0,9681 |
| MDH-1 | 2 | 0.0192 | 0,0000 | 0,0091 |
| | 3 | 0,0480 | 0.0000 | 0,0228 |
| | N | 469 | 519 | 988 |

locus, presenting 3 alleles, was identified and analysed. Allele 3, which was slower, overlapped the most rapid allele of another possible locus (Figure 5).

The monomeric structure of the SKDH and LAP enzymes is in agreement with the findings of IRCA (1986), Chevallier et al. (1984) and Chevallier (1988) and in disagreement in relation to the number of alleles per locus. The number of alleles per locus, for both systems, was higher than those analysed previously and, therefore, presented a higher genetic variability in these populations.

The frequency of alleles per locus in the 2 rubber tree populations studied presented similar values, with the exception of allele 2 of LAP-2, which was not detected in population 1 but present at a frequency of 0.02 % in population 2. Also, alleles 2 and 3 of the MDH-1 occured in frequencies of 1.92 % and 4.8 %, respectively in population 1 were surprisingly not present in population 2 (Table 2). Some differentiation is indicated.

The frequency percentage of generally rare alleles may vary according to the origin of specific populations. According to Chevalier (1988), percentages of 23 %, 41 % and 34 % were found for generally rare alleles in rubber tree populations from Mato Grosso, Acre, and Rondônia, respectively. However, no unique allele in any population was identified, only variations in their frequencies.

The chi-square values for the equilibrium deviations of HARDY-WEINBERG for each locus were obtained considering 3 classes of homozygotes and heterozygotes observed and expected. Class A embodies all of the homozygotes for the most common allele; class B, the heterozygotes between the common/rare alleles, and class C, the rare homozygotes and the other heterozygotes. These were all significant, with the exception of locus MDH-1 in population 2, which has a non-significant value (*Table 3*). This demonstrates that the 2 populations are not in equilibrium for most of the loci studied.

The lack of equilibrium in the populations may not reflect what occurs with the populations in the adult phase, since the native rubber trees produce many seeds annually, in the period from January to March. The seeds which escape predation by animals germinate normally, forming a veritable carpet around the mother plant. However, few seedlings reach the adult plant stage due to the fact that the rubber tree is a heliophyte species that requires the opening of a gap in the forest to meet its light requirements.

As may be deduced, the material for analysis consisted of seeds which, under natural conditions, will form seedlings, few of which will eventually reach the adult plant stage. Therefore, the disequilibrium manifested in the studied phase may not reflect the disequilibrium existing in adult plants. In addition, all of the estimates of the population parameters are subject to error.

The genetic variability measured by the mean number of alleles/locus (A) in the 2 populations studied, was maintained at a relatively high level, with 4.0 and 4.3, respectively, for populations 1 and 2 (*Table 4*). This was true when compared with the patterns presented in IRCA (1986) for the leucine aminopeptidase (LAP) system with 1 locus and 3 alleles, for the malate dehydrogenase (MDH-1), with 1 allele, and for the shikimic dehydrogenase, with 1 locus and 3 alleles.

In analysing material from samples of natural populations of rubber trees originated from native rubber tree populations in the States of Acre, Rondônia and Mato Grosso, Chevallier (1988) found values of the number of alleles per polymorphic locus varying from 2.4 to 3.1.

The proportion of polymorphic loci found in the 2 populations studied was also high in the 3 systems studied, utilizing the criterion that a locus is considered polymorphic when the frequency of the most common allele does not exceed 95%. In the present case, 100 % was found for population 1 and 75% for population 2 (*Table 4*). The measurement of polymorphic loci in plants representative of native rubber tree populations from the State of Acre, estimated by Chevallier (1988), showed values ranging from 70 % to 90 %.

Table 3. — Chi-square for Hardy-Weinberg equilibrium deviations considering the observed number (N_o) and the expected number (N_o) of 17 alleles in 4 loci of natural rubber tree populations.

| rocı | CLASSES' 1' | POPU | LATION 1 | POPULATION 2 | | |
|-------|-------------|----------------|----------|----------------|---------|--|
| | C21.0080 | N _o | N X2 | N _o | N X2 | |
| | A | 329.0 | 317,7 | 394,0 | 378.1 | |
| LAP-1 | В | 140,0 | 162,6 | 98,00 | 129,9 | |
| | c | 32,00 | 20,70 | 27,00 | 11,10 | |
| | | | 9,70 | | 31,4** | |
| | Α | 389,0 | 376,3 | 371,0 | 362,6 | |
| LAP-2 | В | 64,00 | 89,50 | 102,0 | 118.7 | |
| | С | 18.00 | 5,300 | 18,00 | 9,600 | |
| | | | 38,5 | | 9,81 | |
| | Α | 155,0 | 131,7 | 142,0 | 112.9 | |
| SKDH | В | 160,0 | 206,6 | 159,0 | 217,2 | |
| | С | 104,0 | 80,70 | 133,0 | 103,9 | |
| | | | 21,4 | | 31,2 | |
| MDH-1 | A | 414,0 | 408,1 | 497,0 | 497,0 | |
| | В | 47,00 | 53,80 | 10,00 | 9,900 - | |
| | c | 8,000 | 2,100 | 0,000 | 0,100 | |
| | | | 19,2 | | 0,04" | |

A — number of homozygotes for the most common allele; B number of heterozygotes between the common/rare alleles; C number of rare homozygotes and other heterozygotes.

ns) — non significant

Table 4. — Mean size of sample/locus (\mathbf{N}), mean number of alleles/locus (A), proportion of polymorphic loci (P), mean heterozygosity observed ($\mathbf{H_0}$) and expected ($\mathbf{H_e}$) in 2 natural rubber tree populations.

| POPULATION | N | A | b ₍₃ , | н° | н |
|------------|--------|-------|-------------------|---------|---------|
| 1 | 465,0 | 4,0 | 100,0 | 0,252 | 0,319 |
| | (17,0) | (0,6) | | (0,085) | (0,105) |
| 2 | 487.8 | 4,3 | 75,0 | 0,229 | 0,295 |
| | (18,8) | (0,5) | | (0,092) | (0,130) |

¹⁾ Values in parentheses correspond to standard deviations.

Table 5. — Total genetic diversity (H_T) , within the population (H_S) , between populations (D_{ST}) , and proportion of diversity in relation to total (G_{ST}) for 4 loci in 2 natural rubber tree populations.

| roci n _e | | H _T | н _ж | G _{s T} | Dst | |
|---------------------|------|----------------|----------------|------------------|--------|--|
| LAP-1 | 1020 | 0,3109 | 0,3096 | 0,0042 | 0,0013 | |
| LAP-2 | 962 | 0,2211 | 0,2206 | 0,0023 | 0,0005 | |
| SKDH | 853 | 0,6253 | 0,6239 | 0,0022 | 0,0014 | |
| MDH-1 | 976 | 0,0726 | 0,0712 | 0,0193 | 0,0014 | |
| MEAN | ** | 0,3356 | 0,3351 | 0,0003 | 0,0001 | |

^{*) -} Total number of plants analysed in the 2 populations.

^{**):} p < 0.01

²⁾ A locus is considered polymorphic if the frequency of the most common allele does not excede 0.95.

Table 6. — Wright's Fixation Index within the population (\mathbf{F}_{IS}) and for the species (\mathbf{F}_{IT}) and measure of differentiation between populations $((\mathbf{F}_{ST})$, for each allele/locus in 2 natural rubber tree populations.

| 1.001 | Ê | | ALLELES | | | | |
|-------|----|-------|---------|-------|-------|-------|-------|
| roci | | 1 | 2 | 3 | 4 | 5 | MEAN |
| | IS | 0,185 | 0,247 | 0,280 | 0,005 | 0,016 | 0,161 |
| LAP-1 | IT | 0,189 | 0,247 | 0,281 | 0,012 | 0,019 | 0,164 |
| | ST | 0,006 | 0,000 | 0,000 | 0,007 | 0,003 | 0,004 |
| | IS | 0,203 | -0,002 | 0,355 | 0,211 | - | 0,217 |
| LAP-2 | ΙT | 0,206 | -0,001 | 0,355 | 0,213 | - | 0,219 |
| | ST | 0,003 | 0,001 | 0,000 | 0,003 | - | 0,00 |
| SKDH | IS | 0,246 | 0,283 | 0,185 | 0,244 | 0,222 | 0,24 |
| | ΙT | 0,248 | 0,283 | 0,186 | 0,251 | 0,227 | 0,24 |
| | ST | 0,003 | 0.000 | 0,001 | 0,009 | 0,007 | 0,00 |
| MDH-1 | IS | 0,172 | 0,304 | 0,131 | - | - | 0,18 |
| | IT | 0,190 | 0,306 | 0,150 | - | - | 0,19 |
| | ST | 0,022 | 0,002 | 0,022 | - | - | 0,01 |

The values obtained of mean heterozygosity observed (H_0) and expected (H_e) , estimated for the 2 populations $(H_0=0.252 \text{ and } 0.229; H_e=0.319 \text{ and } 0.295, respectively for population 1 and 2) were lower than those found by Chevaller (1988) for 102 plants representing 5 municipalities in the State of Acre <math>(H_e=0.324 \text{ and } H_0=0.299)$.

Hamrick (1989) reports that the measurements most commonly utilized to estimate the levels of intrapopulational variation when working with isozymes are the percentage of polymorphic loci, the number of alleles per locus and the mean heterozygosity. He also presents values for 16 taxa of tropical species, where the percentage of polymorphic loci was 60.9% and the mean heterozygosity was 0.211. Therefore, the genetic variability found in the 2 rubber tree populations was higher than the average for other tropical species.

The results obtained for genetic diversity in the population, measured by Nei's (1973) index, through the partition of total genetic diversity ($H_{\rm T}$) in the components of diversity within populations ($H_{\rm S}$) and between populations ($D_{\rm ST}$), showed a relatively high value for total diversity. The $H_{\rm T}=0.3356$ value (Table~5) is higher than the patterns found by ($H_{\rm AMRICK}$ (1983) in 122 studies involving 91 plant species, 47 genera and 19 families, which ranged from 0.172 to 0.712 for 20 types of natural populations, with $H_{\rm T}=0.3039$.

The highest proportion of genetic variability detected for the 2 populations of rubber trees is contained within populations, which presented the value of ${\rm H_S}=0.3351$ (Table 5), corresponding to 99.85 % of total variation, while the variation between populations corresponded to only 0.03 % of total variation (${\rm G_{ST}}=0.0003$). Such results show that there was a gene flow between the 2 groups of plants, since, in spite of the apparent discontinuity between the 2 groups, which are 8 km apart, the differentiation process was shown to be incipient.

The high proportion observed for variability contained within the rubber tree populations indicates that the collection of rubber trees for conservation of genetic resources may be directed towards 1 or few populations in 1 collecting region and a higher number of individuals per population. Using this procedure a greater genetic rep-

resentativeness of the species can be collected at a lower cost per region.

Undoubtedly, the concentration of efforts in genetic conservation of tree species should be determined by the relative magnitude of the genetic variation between and within populations, in such a manner as to preserve maximum variability of the natural populations.

In the case of this study, the collection of rubber trees effected in only 1 of the populations studied would represent the genetic variation existing in both, in view of the existing genetic similarity.

The proportion of genetic diversity between populations in relation to total genetic diversity ($D_{\rm ST}/H_{\rm T}$), defined as $G_{\rm ST}$, was shown by NEI (1977) as a parameter equivalent to WRIGHT's (1965) $F_{\rm ST}$. The mean values of $F_{\rm ST}$ per locus found in this study ranged from 0.002 to 0.018 (*Table 6*) evidencing a small genetic diversity between the populations.

The estimate of Nei's (1972) genetic standardized identity index for the 2 rubber tree populations studied is 0.997, and the genetic distance is 0.003, which indicates that the 2 populations are very close genetically.

The genetic diversity between the 2 populations, estimated by the $D_{\rm ST}$ parameter (NeI, 1973), for the average of the 4 loci in the 2 populations, was also at the very low value of 0.0001 (*Table 5*), which corresponds to 0.03 % of the total genetic diversity.

As may be observed, the estimations which assess the genetic differences between populations, utilizing different methodologies, provide low but similar values, leading to the conclusion that the two native rubber tree populations studied are genetically similar. This, in a certain manner, was expected, considering the geographic distance between the 2 rubber tree populations and also because they were located in an up-land area that, compared with low-land rubber tree populations, are more similar.

The native rubber tree populations from which the materials were collected for analysis are located alongside Highway BR 364, which connects the cities of Rio Branco (AC) and Porto Velho (RO), and are separated by a distance of about 8 km one from another (Figure 1). The vegetation existing between the 2 rubber tree populations alongside the highway is not the original one, due to implementation of agro-livestock activities. It is possible that this discontinuity in the original vegetation interrupted the gene flow between the plants of the rubber tree populations, initiating a differentiation process between the 2 plant groups, which might end up forming 2 genetically different populations.

With regard to the low value of Nei's coefficient of genetic diversity between populations Buckley et al. (1988), utilizing the electrophoresis technique, analysed Brazil nut (Bertholletia excelsa) plants sampled in 2 natural populations, 1 in the State of Acre and the other in the State of Amazonas. The low level of genetic differentiation found between the populations — about 3.75 % in relation to total diversity — was a surprise, in view of the geographic distance between the populations, the type of distribution of the plants in the population and the seed dispersal. To explain such results the cited authors raised the hypothesis that the 2 populations originated from a common ancestral population or even by anthropic action.

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Literature cited

Buckley, D. P., O'Malley, D. M., Apsit, V. S., France, G. T. and Bawa, K. S.: Genetics of Brazil nut (Bertholletia excelsa, Hums. and Bonpl.; Lecythidaceae). 1. Genetic variation in natural populations. Theor. Appl. Genet. 76: 923—928 (1988). — Chevaller, M. H.: Genetic variability of Hevea brasiliensis germplasm using isozyme markers. II. Rubb. Res. Inst. of Malaysia 3: 42—53 (1988). — Chevaller, M. H., Lebrun, P. and Normand, F.: Approach to the genetic variability of germplasm using enzymatic markers. In: Colloque Explotation-Physiologie et Amelioration de L'Hevea. Montpellier, 1984. Compte Rendu. Montpellier, ICRA/GERDAT. p. 376 (1984). — Hamrick, J. L.: Isozymes and analysis of genetic structure in plants populations. In: Soltis, D. E. and Soltis, P. S. (ed.): Isozymes in plant biology. Portland, Dioscorides Press.

pp. 87-105 (1989). - Institute de Recherches sur le Caoutchouc: Laboratoire d'électrophorèse. In: Rapport Annuel: 1985. Paris, - LEBRUN, P. and CHEVALLIER, M. H.: Starch IRCA. p. 33-35 (1986). and polyacrylamide gel electrophoresis of Hevea brasiliensis: a laboratory manual. Montpellier, IRCA/CIRAD. 44p. (1988). -NEI, M.: Genetic distance between population. Am. Nat. 106: 283-- NEI, M.: Analysis of gene diversity in subdivided 292 (1972). populations. Proc. Natl. Acad. Sci. 70: 3321-3323 (1973). -The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420 (1965). Wright, S.: Evolution and genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago (1978). — Wycherley, P. R.: The genus Hevea. In: Worksshop in International Collaboration Hevea Breeding and the Collection an Establishment of Materials from the Neotrophic. 1977. Kuala Lumpur. 12 p. (1977).

Modulo Tile Constructions for Systematic Seed Orchard Designs

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Summary

Seed orchard designs purport to maximize outcrossing by having each clone as a near neighbour of every other clone, and to miniminze inbreeding by maintaining maximum separation between ramets of the same clone. This paper initially presents quantitative descriptors that measure how well a design achieves these aims. Modulo tile designs are subsequently introduced. These designs are new generalizations of the conventional systematic designs, but have superior performance with regard to the design descriptors. Modulo tile designs have well-defined properties and are applicable to any sufficiently large number of clones. Also incorporated are 2 examples of how clone numbering may be altered on the basis of known flowering phenology in order to achieve special effects with the modulo tile designs.

Key words: seed orchard designs, systematic designs, monoecious species, panmixia, inbreeding depression.

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Introduction

The physical arrangement of clones in seed orchards is designed to minimize inbreeding and maximize outcrossing. For monoecious species, these aims may be achieved by ensuring that ramets of any particular clone are well separated by other clones, and by surrounding each clone with as many genetically diverse neighbours as possible.

It is not a trivial matter to generate a seed orchard design which possesses these desirable properties. Computer programs which may use sophisticated search techniques are sometimes deployed, e.g. La Bastide (1967), Bell and Fletcher (1978), Marsh (1985), Vanclay (1986, 1991), Chakravarty and Bagchi (1993). Sometimes standard statistical designs have been adapted by controlling planting positions within blocks, in particular the balanced incomplete block designs of Dyson and Freeman (1968), and Free-

MAN (1967, 1969). Languer (1953) suggested the use of systematic designs and the properties of these designs were extensively investigated by Giertych (1965, 1971). Miscellaneous other designs such as the shifting-clone design of Malac (1962) have also been proposed. Giertych (1975) provides a good review of known seed orchard design constructions.

This paper introduces some quantitative descriptors which a plant breeder can use to evaluate and compare alternative seed orchard designs. It then proceeds to expound on modulo tile constructions, which are a generalization of conventional systematic designs. In these new constructions each ramet of a particular clone may be surrounded by one of several different sets of near neighbours. Thus it is possible to surround a clone with up to 36 different neighbouring clones, whereas in the usual systematic designs each clone can be surrounded by only 8 different clones.

A plant breeder who wishes to implement one of these modulo tile constructions and who has no desire to follow the theoretical development need only refer to the 2 sections marked with asterisks.

Assumptions used in constructing seed orchard designs

Table 1 depicts a small seed orchard design with 25 clones in a square planting arrangement. Clone number 1 at position row 4, column 4 has clones 2, 3, 4, 5 as nearest neighbours and clones 6, 7, 8, 9 as diagonal neighbours. In this paper the nearest and diagonal neighbours of any plant position are collectively called the first order square neighbourhood, or more briefly, the first neighbourhood. By collecting arrangements of plants in ever increasing squares about a subject plant, it is possible to obtain second order square neighbourhoods, third order square neighbourhoods, and so on. Table 1 displays the complete first, second and third neighbourhoods of a subject plant.