mass selection for growth rate in Engelmann spruce. Silvae Genet. **38**, 6–12 (1989). — MITTON, J. B., LINHART, Y. B., DAVIS, M. L. and STURGEON, K. B.: Estimation of outcrossing in ponderosa pine, *Pinus ponderosa* LAWS, from patterns of segregation of protein polymorphisms and from frequencies of albino seedlings. Silvae Genet. **30**, 117–121 (1981). — MITTON, J. B., LINHART, Y. B., HAMRICK, J. L. and BECKMAN, J. S.: Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor. Appl. Genet. **7**, 5–13 (1977). — MITTON, J. B., LINHART, Y. B., STURGEON, K. B. and HAMRICK, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**, 86–89 (1979). — MORAN, G. F. and BROWN, A. H. D.: Temporal heterogeneity of outcrossing rates in alpine ash (*Eucalyptus delegatensis*). Theor. Appl. Genet. **57**, 101–105 (1980). — MUONA, O., HARJU, A. and KÄRKKÄINEN, K.: Genetic comparison of natural and nursery grown seedlings of *Pinus sylvestris* using allozymes. Scandinavian J. For. Res. **3**, 37–46 (1988). — MUONA, O., YAZDANI, R. and RUDIN, D.: Genetic change between life stages in *Pinus sylvestris*: allozyme variation in seeds and planted seedlings. Silvae Genet. **36**, 39–42 (1987). — NIEBLING, C. R. and CONKLE, M. T.: Diversity of Washoe pine and comparisons with allozymes of ponderosa pine races. Can. J. For. Res. **20**, 298–308 (1990). — PERRY, D. J. and DANCIK, B. P.: Mating system dynamics of lodgepole pine in Alberta, Canada. Silvae Genet. **35**, 190–195 (1986). — PERRY, D. J. and KNOWLES, P.: Evidence of high self-fertilization in natural populations of eastern white cedar (*Thuja occidentalis*). Can. J. Bot. **68**, 663–668 (1990). — PETERS, G. B., LONIE, J. S. and MORAN, G. F.: The breeding system, genetic diversity and pollen sterility in *Eucalyptus pulverulenta*, a rare species with small disjunct populations. Australian J. Bot. **38**: 559–570 (1990). — PLESSAS, M. E. and STRAUSS, S. H.: Allozyme differentiation among populations, stands and cohorts in Monterey pine. Can. J. For. Res. **16**, 1155–1164 (1986). — PRAT, D. and ARNAL, S. A.: Allozyme variation and mating system in three artificial stands of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) planted in Europe. Silvae Genet. **43**, 193–206 (1994). — RAO, C. R.: Linear statistical inference and its application. 2nd edition. John Wiley and Sons, New York (1973). — RITLAND, K.: Correlated matings in the partial selfer *Mimulus guttatus*. Evolution **43**, 848–859 (1989). — RITLAND, K.: A series of Fortran computer programs for estimating plant mating systems. J. Hered. **81**, 235–237 (1990). — RITLAND, K. and EL-KASSABY, Y. A.: The nature of inbreeding in a seed orchard of Douglas fir as shown by an efficient multilocus model. Theor. Appl. Genet. **71**, 375–384 (1985). — SHAW, D. V. and ALLARD, R. W.: Estimation of outcrossing rates in Douglas-fir using allozyme markers. Theor. Appl. Genet. **62**, 113–120 (1982a). — SHAW, D. V. and ALLARD, R. W.: Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. Silva Fennica **16**, 114–121 (1982b). — SORENSEN, F. C.: Embryonic genetic load in coastal Douglas fir, *Pseudotsuga menziesii* var. *menziesii*. Am. Nat. **103**, 389–398 (1969). — SORENSEN, F. C.: Frequency of seedlings from natural self-fertilization in Pacific Northwest ponderosa pine (*Pinus ponderosa* DOUGL. ex LAWS.). Silvae Genet. **43**, 100–108 (1994). — SORENSEN, F. C. and MILES, R. S.: Inbreeding depression in height, height growth, and survival of Douglas-fir, ponderosa pine, and noble fir to 10 years of age. For. Sci. **28**, 283–292 (1982). — SPROULE, A. T. and DANCIK, B. P.: The mating system of black spruce in North-Central Alberta, Canada. Silvae Genet. **45**, 159–164 (1996). — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey (1989). — TANAKA, K., KUROSAWA, Y., KUROKAWA, K. and OISHI, T.: Genetic polymorphism at erythrocyte esterase-D in pigs. Anim. Blood Groups Biochem. Genet. **11**, 193–197 (1980). — WORKMAN, P. L. and NISWANDER, J. D.: Population studies on Southwestern Indian tribes. II. Local differentiation in the Papago. Am. J. Hum. Genet. **22**, 24–49 (1970). — WRIGHT, S.: Evolution and the Genetics of Populations. Volume 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago. 511 pp. (1969). — YAZDANI, R., MUONA, O., RUDIN, D. and SZMIDT, A. E.: Genetic structure of a *Pinus sylvestris* L. seed-tree stand and naturally regenerated understory. For. Sci. **31**, 430–436 (1985). — YEH, F. C., KHALIL, M. A. K., EL-KASSABY, Y. A. and TRUST, D. C.: Allozyme variation in *Picea mariana* from Newfoundland: genetic diversity, population structure and analysis of differentiation. Can. J. For. Res. **16**, 713–720 (1986).

# RAPD Diversity in Brazil Nut (*Bertholletia excelsa* HUMB. and BONPL., Lecythidaceae)

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**Abstract**

Estimates of randomly amplified polymorphic DNA (RAPD) variation within and between 5 provenances of *Bertholletia excelsa* (Brazil nut), a large South American rain forest tree, are presented. One hundred individuals of *B. excelsa* were screened for variation in 47 RAPD products, of which all but 6 (12.8%) of the products were polymorphic across the species. Estimates of the mean Shannon's phenotype diversity varied between 5.97 (Rio Branco) and 8.69 (Santarém) for each provenance and 10.580 for the species. An examination of the proportion of diversity present within and among provenances indicated that, on average, 68.7% of the variation occurred within provenances and only 31.3% of the observed variation resided between provenances.

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*Key words: Bertholletia,* Brazil nut, RAPD markers, genetic diversity.

*FDC:* 165.4; 165.5; 232.12; 176.1 *Bertholletia excelsa;* (81).

## Introduction

Genetic variation in natural populations is a resource for the survival and future evolution of a species, as well as a potential resource for improving its productivity (FRANKEL et al., 1995). Therefore, understanding genetic diversity and changes in diversity are essential for the effective management of a species (MILLAR and WESTFALL, 1992; SAVOLAINEN and KAERKKAEINEN, 1992).

In tropical ecosystems, such as the Amazonian rainforest, where rates of forest conversion into agricultural land are high (SIQUEIRA and SOUZA, 1990) and there is intensive logging pressure (YARED and BRIENZA, 1989), the diversity of tree species is particularly important since these are the structural components of the habitat. Furthermore, the persistence of evolutionarily viable populations is crucial to the preservation of the tropical forest habitats (LOVELESS, 1992). Central to the

understanding of tropical diversity at all levels, and its long term conservation, is the genetic organisation and breeding structure of tropical tree populations (BAWA et al., 1990; BAWA and KRUGMAN, 1991; LOVELESS, 1992).

*Bertholletia excelsa* HUMB. and BONPL. (Lecythidaceae) is a large tree, up to 50 m tall, distributed in the *terra firme* forests of the Guianas, Amazonian Colombia, Venezuela, Peru, Bolivia and Brazil (MORI and PRANCE, 1990). Across this range tree densities may be as low as 1 tree per 6 hectares and as high as 5 to 20 trees per hectare (SÁNCHEZ, 1973). Flowers of *B. excelsa* are visited, and presumably pollinated, by a variety of large-bodied bees (e.g. *Bombus, Centris* and *Xylocopa*; (NELSON et al., 1985)). The available evidence indicates that *B. excelsa* is largely outcrossed, but that a significant low level of inbreeding may occur (O'MALLEY et al., 1988). Seeds from the large indehiscent fruits of *B. excelsa* are apparently dispersed by agoutis (*Dasyprocta* spp.), a common neotropical rodent (HUBER, 1910). The species is known primarily for its 'nut' production (MORI and PRANCE, 1990), although recent data indicates that it may be a promising timber species for open plantations (KANASHIRO, 1992).

Early forest genetic research involved studies of the geographic patterns of quantitative genetic variation. However, such studies have several drawbacks for population genetics including: environmental influences on quantitative traits, polygenic inheritance of traits, expression of traits only after several years of growth (HAMRICK et al., 1992). With the advent of biochemical and molecular markers for evaluating variation in tree populations many of these drawbacks have been overcome, and now play central and critical roles in the assessment of genetic diversity in native forest ecosystems (CONKLE, 1992). For some tropical hardwoods, especially where breeding is not yet as advanced as for some *Pinus* and *Eucalyptus* species, activities such as testing potentially useful species and the characterisation of reproductive systems and provenance collections are urgent tasks and genetic marker studies can be used as part of a research programme to investigate these issues (HAINE, 1994).

On source of genetic markers is randomly amplified polymorphic DNA (RAPD). RAPDs are one of a family of techniques which produce arbitrary fragment length polymorphisms and utilise single, arbitrary, decamer DNA oligonucleotide primers to amplify regions of the genome using the polymerase chain reaction (PCR; HADRYS et al., 1992; NEWBURY and FORD-LLOYD, 1993; WELSH and MCCLELLAND, 1990; WILLIAMS et al., 1990, 1993). Priming sites are thought to be randomly distributed throughout a genome and polymorphism in these regions results in differing amplification products. This method has been widely used for the assessment of genetic diversity (e.g. CHALMERS et al., 1992, 1994; KAZAN et al., 1993; NESBITT et al., 1995; RUSSELL et al., 1993; WACHIRA et al., 1995) and the study of breeding and conservation strategies (KEIL and GRIFFIN, 1994; ROSSETTO et al., 1995). The aim of the present study is to understand the distribution of genetic diversity among 5 provenances of Brazil nut, widely sampled from Amazonian Brazil.

## Material and Methods

Fully expanded leaves were collected from a provenance trial established in 1982, at the Belterra Experimental Station (about 30 km south of Santarém, Pará, Brazil) and composed of 5 provenances of *Bertholletia excelsa* (*Table 1; Fig. 1*). The material was collected into plastic bags and dried with silica-gel (CHASE and HILLS, 1991) and kept at ambient temperature until it was returned to the laboratory and stored at –20°C.

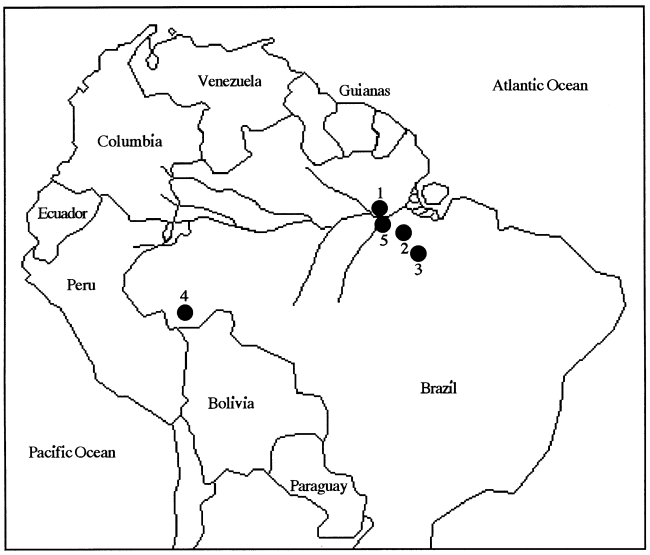


*Figure 1.* – Distribution of the 5 provenances of *Bertholletia excelsa* sampled for RAPD variation.

Seed collection, at the time of trial establishment, varied according to provenance due to the difficulties of reaching many of the populations in their natural habitats. The Santarém and Rio Branco provenances were collected from individual trees, whilst the Alenquer and Altamira provenances were sampled from bulk collections in local markets and the Marabá provenance was a bulk collection sampled from a 'nut' reception centre.

Intact, total DNA was extracted from dried material of individual plants according to the following protocol, modified from DOYLE and DOYLE (1987). A 9 mm diameter leaf disc was ground in a 1.5 ml Eppendorf tube using a disposable plastic pestle, 1 ml of 2 x CTAB extraction buffer (containing 1% PVP-40T) was added and the tubes incubated for 30 minutes at 65 °C. The extracts were treated twice with 200 µl chloroform:isoamyl alcohol (24:1) before precipitating the nucleic acid-CTAB complex, removing the CTAB and resuspending the DNA in 100 µl TE. Initially DNA concentration was determined by using BRL quantification markers as standards. However, since an examination of RAPD product profiles where

*Table 1.* – Locations and RAPD phenotype diversity ($H_{pop}$) of the 5 provenances of *Bertholletia excelsa* studied.

| Popl. No. | Provenance, State | Lat. (S) | Long. (W) | $H_{pop}$ |
|---|---|---|---|---|
| 1 | Alenquer, Pará | 01° 55' | 54° 46' | 7.88 |
| 2 | Altamira, Pará | 03° 32' | 52° 14' | 6.53 |
| 3 | Marabá, Pará | 05° 22' | 49° 06' | 7.29 |
| 4 | Rio Branco, Acre | 10° 00' | 67° 47' | 5.97 |
| 5 | Santarém, Pará | 02° 32' | 54° 45' | 8.69 |

$\bar{H}_{pop} = 7.272$, $H_{sp} = 10.58$, $(H_{sp} - \bar{H}_{pop})/H_{sp} = 0.313$, $\bar{H}_{pop}/H_{sp} = 0.687$

*Table 2.* – Characteristics of different primers and the total number of products scored in 5 populations of *Bertholletia excelsa.*

| Primer | Sequence (5' —› 3') | No. of Products |
|---|---|---|
| OPB-07 | GGTGACGCAG | 4 |
| OPB-11 | GTAGACCCGT | 14 |
| OPB-15 | GGAGGGTGTT | 6 |
| OPB-17 | AGGGAACGAG | 2 |
| OPB-18 | CCACAGCAGT | 12 |
| OPB-20 | GGACCCTTAC | 3 |
| OPE-03 | CCGAATGCAC | 2 |
| OPE-07 | AGATGCAGCC | 4 |

DNA concentrations were varied between 10 ng and 100 ng, revealed no differences in banding patterns, 5 µl of the DNA extract was routinely used in the RAPD reactions. Synthetic primers (OPB-01 – OPB-20; OPE-01 – OPE-20) were purchased from Operon Technologies Inc. (Alameda, California). Initially 40 primers were surveyed in a selection of the material and 8 primers were chosen (OPB-07; OPB-11; OPB-15; OPB-17; OPB-18; OPB-20; OPE-03; OPE-07; *Table 2*) for the complete analysis based on the ease of amplification and resolution of the products. Amplifications were done in 50 µl of reaction mixture containing: 17.5 µl distilled, deionised water; 5 µl 1mM dATP; 5 µl lmM dCTP; 5 µl lmM dGTP; 5 µl lmM dTTP; 5 µl 10 x Dynazyme™ buffer (100 mM Tris-HCl, pH 8.8; 15 mM $MgCl_2$; 500 mM KCl; 1% Triton-X-100); 2 µl 100 nM primer (Operon Technologies Inc., Alameda, California); 1 unit Dynazyme™ (Finnzymes OY; Flowgen Laboratories). The reaction mixture was subjected to amplification in either a Techne PHC-3 or a GeneE thermocycler for 45 cycles consisting of 1 minute at 92 °C, 3 minutes at 35 °C and 2 minutes at 72 °C. A final cycle of 3 minutes at 72 °C was used to complete extension of any remaining products. Products were stored at 4 °C prior to analysis (1 to 2 days). All amplification sets contained a negative control. Amplified fragments were resolved on 2% agarose gels in tris-acetate buffer containing 0.5 µg/ml ethidium bromide with a 123 bp ladder (Gibco-BRL). Banding patterns were visualised under UV light, recorded on Polaroid 667 film and then scored. Approximately half of the amplifications were repeated twice to check on the stability of amplification products.

RAPD data were scored as the presence (1) of absence (0) of amplification products; no account was taken of the product intensity. Similarities between accessions were calculated using the asymmetric similarity measure JACCARD's coefficient, $J_{xy} = C_{xy}/(n_x + n_y - C_{xy})$, where $C_{xy}$ is the number of positive matches between 2 individuals, $n_x$ is the number of products found in accession x and $n_y$ is the number of products found in accession y (JACCARD, 1908; LYNCH, 1990). Values in the similarity matrix were clustered using unweighted pair-group mean analysis (UPGMA; (SNEATH and SOKAL, 1973)) and analysed using principal coordinate analysis. Similarity, principal coordinate and clustering analyses were conducted using the R package (LEGENDRE and VAUDOR, 1991). SHANNON's information measure ($H = -\sum p_i \log_2 p_i$, where $p_i$ is the frequency of the ith RAPD product) was used to obtain an estimate of RAPD phenotype diversity in each population, and to partition the diversity between populations (KING and SCHAAL, 1989; LEWONTIN, 1972).

## Results

One hundred individuals of *Bertholletia excelsia*, from 5 provenances, were screened for variation in 47 RAPD products (*Table 3*). Assuming that each RAPD product represents a single locus, all but 6 (12.8%; OPB-15-A; OPB-15-C; OPB-15-E; OPB-17-B; OPB-20-C; OPE-03-A) of the loci were polymorphic across the species. The frequency of the products varied between 0 (absent) and 1 (monomorphic), with 49% of the frequencies greater than 0.801 and only 25.6% of the frequencies less than 0.601. The mean number of products per provenance (*Table 2*) varied between 35 (Marabá) and 41 (Santarém; mean for all provenances = 38.6). Seven products were restricted to individual populations (*Table 3*): Altamira (OPB-11-H; OPE-03-A); Marabá (OPB-20-C); Santarém (OPB-11-E; OPB-18-K; OPB-11-K; OPB-18-G).

Estimates of the mean SHANNON's phenotype diversity varied between 5.966 (Rio Branco) and 8.686 (Santarém) for each population and 10.580 for the species (*Table 1*). Furthermore, SHANNON's measure of phenotypic diversity was used to partition the diversity into within- and among-provenance components (*Table 1*). An examination of the proportion of diversity present within provenances ($H_{pop}/H_{sp}$) and among provenances [$(H_{sp} - H_{pop})/H_{sp}$] indicated that, on average, 68.7% of the variation occurred within provenances (*Table 1*) and only 31.3% of the observed variation resided between provenances.

Calculation of JACCARD's similarity indices revealed that within provenance estimates for similarity varied between 0.734 (Santarém) and 0.854 (Altamira), whilst between provenance estimates are summarised in *figure 2*. Three provenances (Alenquer, Altamira, Marabá) cluster together as a discrete group, which correlates with the geographic distribution of the populations. However, the Santarém provenance, which is geographically close to these provenances, is distinct from them, being more similar to the geographically isolated Rio Branco provenance.

## Discussion

*Bertholletia*, a monospecific genus, is apparently most closely related to some species of the genus *Lecythis* (e.g. *L. lurida*; MORI and PRANCE, 1990)). However, differentiation between the genera is so great that hybridisation between them is probably not possible, hence the search for germplasm for the improvement of either Brazil nut production or wood quality will have to come from the variation found within *B. excelsa*, rather than from closely related species in other genera (MORI and PRANCE, 1990). The understanding of the level of genetic variation, and the distribution of that variation, in *Bertholletia excelsa* is therefore crucial. The RAPD results reported here provide new information regarding the organisation of genetic variation in Brazil nut. Two major observations can be made from the RAPD data reported here: (i) the majority of variation is distributed within provenances and (ii) provenance clustering is independent of geographic location.

The partioning of RAPD variation in *Bertholletia excelsa* where 68.7% of the variation is distributed within provenances, agrees with findings reported for *Eucalyptus globulus* (NESBITT et al., 1995), *Camellia sinensis* (WACHIRA et al., 1995)

*Table 3.* – RAPD frequencies for 5 provenances of *Bertholletia excelsa*.

| Product | Alenquer | Altamira | Marabá | Rio Branco | Santarém |
|---|---|---|---|---|---|
| OPB-07-A | 0.188 | 0.400 | 0.529 | 0.842 | 0.647 |
| OPB-07-B | 1.000 | 0.857 | 1.000 | 0.579 | 1.000 |
| OPB-07-C | 0.125 | 0.429 | 0.625 | 0.842 | 1.000 |
| OPB-07-D | 1.000 | 0.857 | 0.824 | 0.579 | 0.294 |
| OPB-11-A | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 |
| OPB-11-B | 0.867 | 1.000 | 1.000 | 1.000 | 0.750 |
| OPB-11-C | 0.133 | 0.000 | 0.400 | 0.000 | 0.000 |
| OPB-11-D | 0.000 | 0.176 | 0.000 | 0.000 | 0.000 |
| OPB-11-E | 0.867 | 0.118 | 0.800 | 0.471 | 0.300 |
| OPB-11-F | 0.400 | 0.118 | 0.350 | 0.118 | 0.150 |
| OPB-11-G | 0.000 | 0.000 | 0.000 | 0.000 | 0.300 |
| OPB-11-H | 1.000 | 1.000 | 1.000 | 0.882 | 1.000 |
| OPB-11-I | 0.235 | 0.118 | 0.400 | 0.000 | 0.650 |
| OPB-11-J | 0.105 | 0.235 | 0.000 | 0.353 | 0.300 |
| OPB-11-K | 0.941 | 1.000 | 0.700 | 1.000 | 1.000 |
| OPB-11-L | 0.235 | 0.294 | 0.350 | 0.000 | 0.450 |
| OPB-11-M | 0.059 | 1.000 | 0.000 | 0.000 | 0.050 |
| OPB-11-N | 0.294 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-15-A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-15-B | 0.400 | 0.750 | 0.750 | 0.900 | 0.421 |
| OPB-15-C | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-15-D | 0.700 | 1.000 | 0.900 | 0.789 | 0.450 |
| OPB-15-E | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-15-F | 0.000 | 0.050 | 0.550 | 0.105 | 0.100 |
| OPB-17-A | 0.895 | 1.000 | 1.000 | 0.833 | 1.000 |
| OPB-17-B | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-18-A | 0.947 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPB-18-B | 0.474 | 0.250 | 0.600 | 0.526 | 1.000 |
| OPB-18-C | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 |
| OPB-18-D | 1.000 | 1.000 | 1.000 | 1.000 | 0.900 |
| OPB-18-E | 1.000 | 1.000 | 1.000 | 0.895 | 1.000 |
| OPB-18-F | 0.263 | 0.000 | 0.000 | 0.105 | 0.000 |
| OPB-18-G | 0.000 | 0.000 | 0.000 | 0.000 | 0.150 |
| OPB-18-H | 0.474 | 1.000 | 1.000 | 1.000 | 0.895 |
| OPB-18-I | 1.000 | 1.000 | 1.000 | 0.947 | 1.000 |
| OPB-18-J | 0.000 | 0.000 | 0.250 | 0.000 | 0.000 |
| OPB-18-K | 1.000 | 1.000 | 1.000 | 0.947 | 1.000 |
| OPB-18-L | 0.000 | 0.250 | 0.000 | 0.000 | 0.000 |
| OPB-20-A | 1.000 | 1.000 | 1.000 | 1.000 | 0.800 |
| OPB-20-B | 0.786 | 0.350 | 0.389 | 0.000 | 0.000 |
| OPB-20-C | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPE-03-A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| OPE-03-B | 0.824 | 1.000 | 0.800 | 0.889 | 0.789 |
| OPE-07-A | 1.000 | 1.000 | 0.438 | 1.000 | 0.722 |
| OPE-07-B | 1.000 | 1.000 | 0.813 | 1.000 | 0.500 |
| OPE-07-C | 1.000 | 1.000 | 1.000 | 0.300 | 0.778 |
| OPE-07-D | 0.550 | 0.250 | 0.000 | 0.750 | 0.944 |

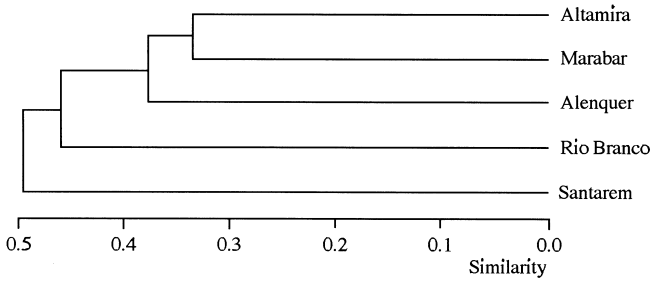


*Figure 2.* – UPGMA clustering of 5 provenances of *Bertholletia excelsa* based on JACCARD's similarity measure.

and *Grevillea scapigera* (ROSSETTO et al., 1995). A previous study, based on isozyme markers, of genetic variation in *Bertholletia excelsa* showed that for two populations, approximately 150 km apart, gave $D_{st}$ values of 0.0375 (BUCKLEY et al., 1988). In an attempt to compare genetic diversity in long-lived woody species with species representing other life forms, and to assess whether the levels and distribution of genetic diversity were related to life history and other ecological characteristics, HAMRICK (1992) reports that woody species maintain more variation within populations than among populations compared to other life forms. Furthermore, large geographic ranges, outcrossing breeding systems and either wind or animal-ingested seed dispersal patterns confer greater within, than among, population genetic diversity. BUCKLEY et al. (1988) used these factors to explain the very low levels of genetic variation found within *B. excelsa*, compared to other tropical species.

MORI and PRANCE (1990) have criticised part of BUCKLEY et al.'s study due to the small sampled sizes and the possibility that one or both of the sample populations may have been derived from Amerindian intervention. Some groups of Amazonian Indians manipulate their environment such that the growth of useful plants is encouraged (BALÉE, 1989; POSEY, 1985) and it has been suggested that apparently natural stands of *B. excelsa* may be remnants of plantations started by pre-Columbian Amerindians (MÜLLER et al., 1980).

The present study, whilst increasing the number of provenances sampled and their geographic range (latitude 1° 55' to 10° 00' S; longitude 49° 06' to 67° 47' W; distances between populations vary between approximately 60 km and 2100 km), supports the conclusions of BUCKLEY et al. (1988) that the majority of *Bertholletia excelsa* RAPD variation lies within provenances.

One group of populations is particularly prominent, Alenquer, Altamira and Marabá, and correlates well with geographic locality. Whilst part of the clustering may be a reflection of the sampling method used to collect seed at the time of trial establishment, this cannot explain all the observed clustering patterns. Although the Santarém and Alenquer provenances are close to each other geographically (c. 60 km), they are distinct from each other on the basis of the RAPD data. On the other hand, the Santarém and Rio Branco provenances are at opposite extremes with respect to RAPDs diversity values (*Table 1*), and represent the best and the worst provenances respectively with the respect to silvicultural performance over 12 years (KANASHIRO, 1992).

The data presented here demonstrates that between provenance RAPD variation is low in *Bertholletia excelsa* across its vast Brazilian Amazonian range. Whilst it is not possible to say whether the overall levels of variation present in the species are high, compared to other rain forest species, the importance of assessing variation from across the range of the species has been demonstrated.


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### References


BALÉE, W.: The adaptation to culture in Amazonia. Adv. Econ. Bot. **7**: 1–21 (1989). — BAWA, K. S., ASHTON, P. S. and NOR, S. M.: Reproductive ecology of tropical forest plants: management issues. In: BAWA, K. S. and HADLEY, M. (Ed): Reproductive ecology of tropical forest plants. Man and Biosphere Series, pp. 3–13 (1990). — BAWA, K. S. and KRUGMAN, S. L.: Reproductive biology and genetics of tropical trees in relation to conservation and management. In: GOMEZ-POMPA, A., WHITMORE, T. C. and HADLEY, M. (Ed): Rain forest regeneration and management. Man and Biosphere Series, pp. 119–136 (1991). — BUCKLEY, D. P., O'MALLEY, D. M., APSIT, V., PRANCE, G. T. and BAWA, K. S.: Genetics of Brazil nut (*Bertholletia excelsa* HUMB. & BONPL.: Lecythidaceae). 1. Genetic variation in natural populations. Theor. Appl. Genet. **76**: 923–928 (1988). — CHALMERS, K. J., NEWTON, A. C., WAUGH, R., WILSON, J. and POWELL, W.: Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers. Theor. Appl. Genet. **89**: 504–508 (1994). — CHALMERS, K. J., WAUGH, R., SPRENT, J. I., SIMONS, A. J. and POWELL, W.: Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity **69**: 465–472 (1992). — CHASE, M. W. and HILLS, H. H.: Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon **40**: 215–220 (1991). — CONKLE, M. T.: Genetic diversity-seeing the forest through the trees. New Forests **6**: 5–22 (1922). — DOYLE, J. J. and DOYLE, J. L.: A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. **19**: 11–15 (1987). — FRANKEL, O. H., BROWN, A. H. and BURDON, J. J.: The conservation of plant biodiversity. Cambridge University Press, Cambridge (1995). — HADRYS, H., BALICK, M. and SCHIERWATER, B.: Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology **1**: 55–63 (1992). — HAINES, R.: Biotechnology in forest tree improvement. FAO, Rome (1994). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HUBER, J.: Mattas e madieras amazônicas. Bol. Mus. Paraense Hist. Nat. **6**: 91–225 (1910). — JACCARD, P.: Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**: 223–270 (1908). — KANASHIRO, M.: Genética e melhoramento de essências florestais nativas: aspectos conceituais e práticos. Revista do Instituto Florestal **4**: 1168–1178 (1992). — KAZAN, K., MANNERS, J. M. and CAMERON, D. F.: Genetic variation in agronomically important species of *Stylosanthes* determined using random amplified polymorphic DNA markers. Theor. Appl. Genet. **85**: 882–888 (1993). — KEIL, M. and GRIFFIN, A. R.: Use of randomly amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theor. Appl. Genet. **89**: 442–450 (1994). — KING, L. M. and SCHAAL, B. A.: Ribosomal DNA variation and distribution in *Rudbeckia missouriensis*. Evol. **43**: 1117–1119 (1989). — LEGENDRE, P. and VAUDOR, A.: The R Package: Multidimensional analysis, spatial analysis. Département de sciences biologiques, Université de Montréal (1991). — LEWONTIN, R.: The apportionment of human diversity. Evol. Biol. **6**: 381–398 (1972). — LOVELESS, M. D.: Isozyme variation in tropical trees: patterns of genetic organisation. New Forests **6**: 67–94 (1992). — LYNCH, M.: The similarity index and DNA fingerprinting. Mol. Biol. Evol. **7**: 478–484 (1990). — MILLAR, C. I. and WESTFALL, R. D.: Allozyme markers in forest genetic conservation. New Forests **6**: 347–371 (1992). — MORI, S. A. and PRANCE, G. T.: Taxonomy, ecology, and economic botany of the Brazil nut (*Bertholletia excelsa* HUMB. & BONPL.: Lecythidaceae). Adv. Econ. Bot. **8**: 130–150 (1990). — MÜLLER, I. A., RODRIGUES, A. A., MÜLLER, A. A. and MÜLLER, N. R. M.: Castahnha-do-Brasil. Resultados de pesquisa. EMBRAPA, Centro do Pesuisas Agropecuário do Trópico Umido (1980). — NELSON, B. W., ABSY, M. L., BARBOSA, E. M. and PRANCE, G. T.: Observation on flower visitors to *Bertholletia excelsa* H. B. K. and *Couratari tenuicarpa* A. C. SM. (Lecythidaceae). Acta Amazônica **15** Suppl.: 225–234 (1985). — NESBITT, K. A., POTTS, B. M., VAILLENCOURT, R. E., WEST, A. K. and REID, J. B.: Partitioning and distribution of RAPD variations in a forest tree species, *Eucalyptus globulus* (Myrtaceae). Heredity **74**: 628–637 (1995). — NEWBURY, H. J. and FORD-LLOYD, B. V.: The use of RAPD for assessing variation in plants. Plant Growth Regulation **12**: 43–51 (1993). — O'MALLEY, D. M., BUCKLEY, D. P., PRANCE, G. T. and BAWA, K. S.: Genetics of Brazil nut (*Bertholletia excelsa* HUMB. & BONPL.: Lecythidaceae). 2. Mating system. Theor. Appl. Genet. **76**: 929–932 (1988). — POSEY, D. A.: Indigenous management of tropical forest ecosystems: The case of the Kayapó Indians of the Brazilian Amazon. Agroforestry Systems **3**: 139–158 (1985). — ROSSETTO, M., WEAVER, P. K. and DIXON, K. W.: Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Mol. Ecol. **4**: 321–329 (1995). — RUSSELL, J. R. HOSEIN, F., JOHNSON, E., WAUGH, R. and POWELL, W.: Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. Molecular ecology **2**: 89–97 (1993). — SÁNCHEZ, J. S.: Explotación y comercialización de la castaña en Madre de Dios. Ministerio de Agricultura, Dirección General de Forestal y Caza, Lima, Peru (1973). — SAVOLAINEN, O. and KAERKKAEINEN, K.: Effect of forest management on gene pools. New Forests **6**: 329–345 (1992). — SIQUEIRA, J. D. and SOUZA, M. F.: Os sistemas de manejo florestal para a Amazonia. In: (Ed). 6° Congresso Florestal Brasileiro, Campos do Jordao-SP. pp. 79–84 (1990). — SNEATH, P. H. A. and SOKAL, R. R.: Numerical taxonomy. Freeman, San Francisco (1973). — WACHIRA, F. N., WAUGH, R., HACKETT, C. A. and POWELL, W.: Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome **38**: 201–210 (1995). — WELSH, J. and MCCLELLAND, M.: Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18**: 7213–7218 (1990). — WILLIAMS, J. G. K., HANAFEY, M. K., RAFALSKI, J. A. and TINGEY, S. V.: Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol. **218**: 704–740 (1993). — WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. and TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535 (1990). — YARED, J. A. G. and BRIENZA, S.: A atividade florestal e o desenvovimento da Amazonia. Para Desenvolvimento **25**: 60–63 (1989).