The Pattern of Inbreeding in Washoe Pine and Survival of Inbred Progeny Under Optimal Environmental Conditions

By J. B. Mitton[1]), R. G. Latta[1]) and G. E. Rehfeldt[2])

(Received 17th March 1997)

Summary

Four enzyme polymorphisms were used to estimate the proportion of inbred offspring among seedlings of Washoe pine (*Pinus washoensis* Mason and Stockwell) grown in a shadehouse. Seeds from 10 trees in each of 4 natural populations were collected in the field, germinated in a green house, and grown for 2 years in a shade house at Moscow, ID. Progeny arrays were analyzed from each population. Among all populations pooled, the multilocus outcrossing rate estimate was t_m = 0.862. The multilocus outcrossing rate exceeded the average of the single locus outcrossing rate by about 0.07 in the pooled data, indicating some degree of mating between related individuals. In addition, the correlation of paternal parentage was $r = 0.07$, indicating approximately 14% of progeny pairs in an array were full sibs. Thus, the number of pollen parents in each array was limited. While the seedlings exhibited a significant degree of inbreeding, the genotypes inferred for the maternal seed trees had an inbreeding coefficient of $F_{is} = -0.037$, significantly lower than the inbreeding coefficient predicted from the mating system ($F_{is} = 0.11$). These results indicate that the inbred genotypes detected in the seedlings persist when trees are cultivated under optimal environmental conditions, but are eliminated in natural populations. Single-locus estimates of F in the pooled sample of seed trees were consistently negative, with a mean of $F = -0.10$, indicating that selection favored heterozygous trees in the pool of outcrossed genotypes.

Key words: mating system, inbreeding, Washoe pine, biparental inbreeding, correlation of paternity, allozyme variation.

FDC: 165.41; 232.13; 174.7 *Pinus washoensis;* (796).

Introduction

The high inbreeding depression reported for most conifers (Sorensen, 1969; Franklin, 1972; Sorensen and Miles, 1982) makes the fitnesses of seedlings highly dependent on the mating system. Conifers generally produce seed by outcrossing, but there are some important exceptions to this generalization (Mitton, 1992). While species with large and continuous distributions usually have high levels of outcrossing, species growing in small populations, and stands with very low densities, produce a substantial proportion of seed by selfing (Farris and Mitton, 1985). For example, outcrossing rates of tamarack, *Larix laricina*, eastern white cedar, *Thuja occidentalis*, and western red cedar, *Thuja plicata*, can be as low as $t = 0.53$ (Knowles et al., 1987), $t = 0.51$ (Perry and Knowles, 1990), and $t = 0.23$ (El-Kassaby et al., 1994), respectively. Each of these species occurs primarily as isolated clusters, or interspersed with other species. By contrast, the outcrossing rate in widespread species such as ponderosa pine is usually high and not different from $t = 1.0$ (Mitton et al., 1977, 1981), but in a stand with very low density, the outcrossing rate was approximately $t = 0.70$ (Farris and Mitton, 1985). In addition to selfing, biparental inbreeding could be relatively more important in small populations.

Washoe pine, *Pinus washoensis*, is restricted to 4 relatively small populations in the Sierra Nevada and the Warner Mountains (*Figure 1*; Niebling and Conkle, 1990), although individual trees may occur sporadically within forests dominated by ponderosa pine, *Pinus ponderosa*, and Jeffrey pine, *Pinus jeffreyii*. Thus, we might expect that Washoe pine will be more inbred (Peters et al., 1990; Sorensen, 1994; Hardner et al., 1996) than ponderosa pine either because of increased selfing, or biparental inbreeding. Inbred progeny suffer a fitness disadvantage (Charlesworth and Charlesworth, 1987) such that they are often eliminated by natural selection before reaching maturity, frequently at a very young age (e.g. Yazdani et al., 1985; Muona et al., 1987; Sproule and Dancik, 1996). This selective elimination of inbred progeny can be an important mechanism by which deleterious recessive mutations are purged from the populations. However, it is not clear whether this selection occurs in artificial environments.

This study began as a common garden, conservation genetics study of variation in a species limited to a few populations, some quite small. A substantial frequency of misshapen seedlings in the common garden shifted the immediate focus of the study to the mating system. In this study, we examine the mating system of Washoe pine, and test for biparental inbreeding and the proportion of full sibs in cones collected from natural populations. We also compare the genotypic distributions of mature trees in the field with shadehouse-grown seedlings to determine if selection removes inbred genotypes during the life cycle.

Materials and Methods

Material

Cones were collected in each of 4 natural populations (*Figure 1*): 2 on the lower slopes of Mount Rose, (Hidden Lake, elev. 2270 m and Grass Lake, elev. 2380 m) near Reno, NV; 1 near Babbitt Peak (elev. 2500 m) on the border between California and Nevada west of Reno; and 1 in the southern tip of the Warner Mountains (elev. 2290 m) in northeastern California. Five to 15 ripening cones were cut from each mother tree in 1991 and kept separate as open-pollinated progeny arrays by personnel at the Carson Ranger District, Toiyabe National Forest, Carson City, Nevada. While the population in the Warner Mountains is large, occupying about 4,000 ha, the cones were collected from a limited area of only about 10 ha. The population on Babbit Peak covers a sizable area, but the density of trees is low. The two populations on Mount Rose are small, each covering only a few ha.

Seedlings were sown in containers (45 cm^3) at Moscow, ID. Five stratified seeds were planted in each container, and after germination, the number of seedlings per container was thinned to one. Germination was high, above 90%, and uniform across families. A few albino and chlorophyll deficient mutants were thinned, but otherwise, thinning was random and the

---

[1]) Department of Environmental, Population and Organismic Biology, Campus Box 334, University of Colorado, Boulder, Colorado 80309, USA, tel.: 303-492-89 56; fax: 303-492-86 99; e-mail: Mitton@Colorado.edu

[2]) Intermountain Research Station, USDA Forest Service, 1221 South Main Street, Moscow, ID 83843, USA.

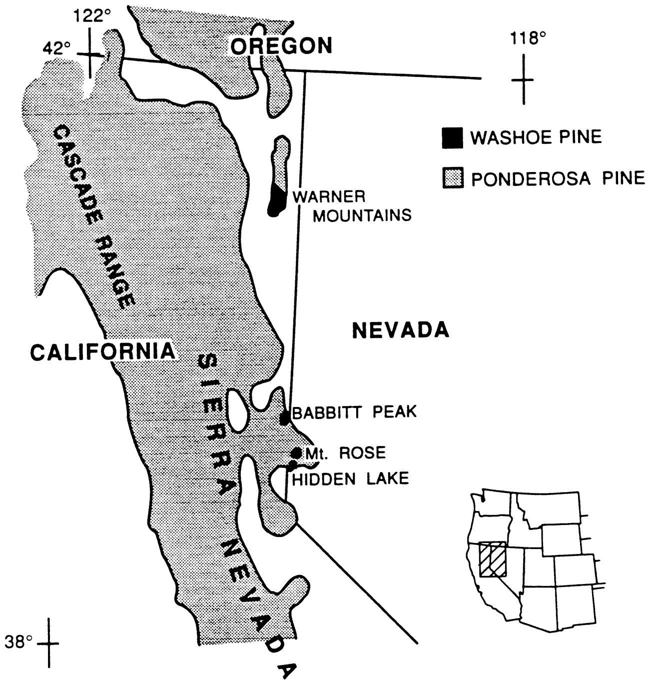

Figure 1. – The distribution of Washoe pine.

seedlings suffered no other postgermination mortality. Seedlings were kept in a green house for 2 months before being transferred to a shade house for the duration of the first growing season. The containers were returned to the green house for the winter months where they remained during the spring of the second growing season. After shoot elongation was completed, the containers were returned to the shade house for the duration of the second growing season.

Many seedlings exhibited abnormal development, and this caused us to shift our attention to the mating system. However, this shift in focus of the research was made after all the seeds were used, so we were unable to utilize megagametophytes for the analysis of the mating system.

Ten progeny arrays consisting of fifteen seedlings each were analyzed from each population, except Babbitt Peak from which only 9 progeny arrays were available (total: 39 progeny arrays, 585 seedlings). Tissue samples of mature needles were taken from the 2-year old seedlings, shipped to Boulder, CO, and prepared for electrophoresis by grinding them with the extraction buffer of MITTON et al. (1979). Megagametophytes were not available for estimates of the mating system, for the genetic material was taken from seedlings growing in a common garden. Protein polymorphisms were resolved on horizontal starch gels with the buffer system of TANAKA et al. (1980). The enzyme polymorphisms used in this study were shikimate dehydrogenase (Sdh), 6 phosphogluconate dehydrogenase (6Pgd), isocitrate dehydrogenase (Idh) and phosphoglucomutase (Pgm).

Statistical methods

Heterogeneity of allelic frequencies among populations and between pollen and ovule pools was analyzed using the Biosys program of SWOFFORD and SELANDER (1989). Significance of allelic frequency differences was tested with the X^2 test of WORKMAN and NISWANDER (1970).

Genetic data from progeny arrays were analyzed by the method of RITLAND (1990). This technique uses an iterative maximum log-likelihood estimation procedure that infers the maternal genotype from each array, along with allelic frequencies in the pollen and ovule pools, the rate of outcrossing (t) and the inbreeding coefficient (F) of the maternal trees. Since genotypes at several loci were available, both single and multilocus outcrossing rates were estimated. Multilocus estimators can more efficiently detect outcrossed progeny (RITLAND and EL KASSABY, 1985) such that the difference between single (t_s) and multilocus (t_m) outcrossing rate estimates give an indication of the level of mating between relatives. The method of RAO (1973) was used to test for heterogeneity among populations in the single locus estimates of outcrossing. We used RITLAND'S (1989) test for the frequency of correlated paternity to estimate the proportion of full sibs in the family arrays.

Results

Allelic frequencies were not significantly heterogeneous among populations for any of the 4 loci in either the pollen pool or the ovule pool. WRIGHT'S (1969) measure of population differentiation was F_{st} = 0.021 and F_{st} = 0.022 for the pollen and ovule pools, respectively. This level of differentiation is consistent with that reported by NIEBLING and CONKLE (1990 F_{st} = 0.016), and is consistent with high (Nm = 12.5) gene flow among populations. Since heterogeneity in allelic frequencies was slight, and sample sizes per population were small, parameters of the mating system were estimated in the pooled sample (*Table 1*). Analyses of the mating system were also conducted within each of the populations, and these results were similar to those from the pooled population. Due to small sample sizes, estimates of the mating system are more reliable in the pooled population.

The multilocus estimate of the outcrossing rate in the pooled populations was $t_m = 0.862 \pm 0.031$ (*Table 1*). This estimate was significantly less than 1.0, indicating that about 14% of the progeny were produced by self fertilization. Multilocus estimates of the outcrossing rate in each population ranged from 0.76 at Grass Lake to 0.92 at Babbitt Peak. While the rates of outcrossing at Hidden Lake and Grass Lake, the 2 smallest

Table 1. – Estimates of the mating system for 4 natural populations of Washoe pine.

Locality	t_m	±SE	t_s	±SE	t_m-t_s	±SE	F	±SE
Pooled	.862	.031	.791	.031	.071	.016	-.037	.085
Hidden Lake	.883	.045	.806	.040	.077	.035	-.263	
Grass Lake	.764	.060	.711	.064	.053	.023	-.061	
Warner Mountains	.883	.084	.823	.063	.060	.041	-.156	
Babbitt Peak	.923	.086	.834	.064	.089	.040	-.271	

tm, *ts*, and *F* are the multilocus estimate of outcrossing, the single locus estimate of outcrossing, and the inbreeding coefficient, respectively.

populations, were significantly lower than 1.0, differences among populations were not significantly different ($X^2 = 3.42$, $P > 0.05$). The outcrossing rates (*Table 1*) were not related to the areal extent of the population, the population size, or the density of the populations.

The single-locus estimate of outcrossing was $t_s = 0.791 \pm 0.031$ (*Table 1*). The single-locus estimates of outcrossing were consistently lower than the multilocus estimates by about 0.07 in each population. The difference between the multilocus estimate of outcrossing and the average of the single-locus estimates suggests that some of the inbreeding is from crosses among related individuals (Ritland and El-Kassaby, 1985). The genetic correlation of uniting gametes is $(t_m\text{-}t_s)/2$, which for our data suggests an inbreeding coefficient among non-self-fertilized seed of about 0.038. This suggests approximately 15% half sib mating or 30% first cousin mating, in addition to the 14% of seeds produced by self fertilization. With only 4 loci, it is possible that additional biparental inbreeding has gone undetected, so that the estimates of biparental inbreeding should be considered lower limits. The difference between t_m and t_s is similar for the pooled data and for populations analyzed separately. This, combined with the low differentiation among populations, indicates that biparental inbreeding is not an artifact of different pollen pools in each population. We calculated the degree of inbreeding, *F*, expected from the combined effect of selfing and biparental inbreeding as $F = (l\text{-}t_s)/(1 + t_s)$. This gave an estimate of total inbreeding of $F = 0.11$ in the 2 year old progeny.

The correlation of paternal parentage was $r = 0.07$, indicating approximately 14% full sibs in the progeny arrays. Although neither the correlation nor the proportion of full sib progeny reached statistical significance ($p < 0.1$), our sample size are modest, and these values may have biological, if not statistical, significance. A moderate number of full sibs in the progeny arrays of open pollinated conifers suggests each maternal tree received pollen from a limited number of paternal parents.

If the inbreeding coefficient were in equilibrium with the estimated outcrossing rate plus biparental inbreeding, the maternal trees would exhibit an *F* of 0.11. Despite significant levels of inbreeding, the values of the inbreeding coefficient, *F*, estimated for the mature trees pooled across populations was –0.037, which is not significantly different from zero. However, *F* in the mature trees is significantly less than in the progeny. The extremely negative values for *F* estimated from individual populations (*Table 1*) are probably not reliable, as the estimation algorithm tends to overestimate the number of heterozygous parents when population sizes are small (K. M. Ritland, pers comm). Inferred maternal genotypes did not differ significantly from Hardy-Weinberg expectations for any of the populations. Because allelic frequencies were homogeneous among our population samples, we pooled seed trees into a single sample (*Table 2*). For the calculation of *F* and X^2 (*Table 2*), alleles 1 and 3 were pooled for each polymorphism, to maximize the number of genotypes in each cell. While none of these distribution deviates significantly from Hardy-Weinberg expectations, the values of *F* are consistently negative. The values of *F* in *Table 2* represent total inbreeding (F_{it}). Combined with the estimate of F_{st} given above, a rough estimate of –0.05 to –0.10 seems plausible for inbreeding within populations (F_{is}). Thus there is a slight but non-significant excess of heterozygotes among the trees producing cones in these populations.

Discussion

Mating patterns in Washoe pine

The outcrossing rate measured for the pooled populations ($t_m = 0.862 \pm 0.03$) indicated that the seedlings carried some degree of inbreeding, and the disparity between t_m and t_s indicates that a high proportion of the inbred seed are produced by biparental inbreeding (15% half-sib mating or 30% first cousin mating, in addition to the 14% of seed produced by selfing). In addition, our estimate of correlated paternity suggests that wind pollinated progeny arrays from natural stands contain a small proportion of full sibs (approximately 14%).

Because progeny pairs showed some correlation of paternity, the number of paternal parents is limited. If n pollen parents contribute equally to the seed of a given maternal tree, then the probability that two seeds are sired by the same father is

$$n\,\frac{1}{n^2} = \frac{1}{n},$$

and the number of sires can be estimated as the reciprocal of this probability. Thus, approximately 1/0.14 = 7 sires on average mate with each maternal tree. If pollen parents do not contribute equally, then more sires per maternal tree are consistent with the proportion of full sibs, but a few of those sires will dominate the pollen pool. This restricted number of pollen parents may reflect the small population size at some of our collecting sites, or restricted pollen dispersal within populations. It may be that in Washoe pine, as in ponderosa pine (Linhart et al., 1981), clustered individuals are related, perhaps sharing their maternal parent, and therfore if pollen dispersal is limited, matings between relatives will occur giving the high level of biparental inbreeding we observed.

The disparate population sizes and histories of Washoe pine and ponderosa pine invite a comparison to understand the influence of population size on the mating system. Of the pines in the subsection ponderosae of the genus *Pinus* (Little and Critchfield, 1969), ponderosa pine has the greatest geographic distribution, while Washoe pine has the smallest. This disparity results largely from antithetical evolutionary histories during the Wisconsin era: while Washoe pine flourished in the Sierra Nevada (Critchfield, 1984; Niebling and Conkle, 1990) ponderosa pine occurred sporadically as an insignificant component of the Pleistocene flora of western United States (Betancourt et al., 1990). A warming climate

Table 2. – Genotypic and allelic frequencies and the inbreeding coefficient for 4 protein polymorphisms in 39 mature Washoe pine.

Locus	11	12	22	13	23	33	F(1)	±SE	F(2)	±SE	F(3)	±SE	F	X^2
SDH	0	4	24	0	10	1	.05	.025	.80	.046	.15	.041	-.10	0.2
6PGD	0	4	8	2	17	8	.08	.030	.47	.057	.45	.056	-.08	0.2
IDH	0	1	13	0	20	5	.01	.013	.60	.055	.38	.055	-.12	0.6
PGM	1	7	20	1	9	1	.13	.038	.72	.051	.15	.041	-.11	0.2

F(1), F(2), and F(3) are the allelic frequencies of alleles 1, 2, and 3. SE is the standard error of the allelic frequencies. *F* and X^2 describe the fit of genotypic frequencies to Hardy-Weinberg expectations, after pooling alleles 1 and 3.

allowed the distribution of ponderosa pine to explode while relegating Washoe pine to a minor position at high elevations in the northern Sierra Nevada and Warner Mountains. Despite such radically different evolutionary histories, these closely related pines have remarkably similar mating systems. Except under unusually low stand densities (Farris and Mitton, 1985), the proportion of selfed seeds in ponderosa pine range from 0.04 (Mitton et al., 1981) to 0.11 (Sorensen and Miles, 1974). The mating system of Washoe pine is only slightly more inbred than that of ponderosa. There is little evidence, therefore, that inbreeding in the relatively isolated populations of Washoe pine is contributing to either erosion of genetic variability or genetic drift any more than in the broadly distributed populations of ponderosa pine.

Inbreeding depression

Despite the significant levels of inbreeding among 2 year old progeny, the genotypes inferred for the maternal seed trees growing in the natural habitats revealed no indication of inbreeding, for all of the estimated values of F were negative. Thus, inbred trees have survived in the common garden (at least up to age 2) that would not survive in nature to produce cones. Inbreeding depression tends to be severe for conifers; selfed seeds rarely mature and those that do tend have poor viability. Because conifers generally exhibit inbreeding depression for growth (e.g., Sorensen and Miles, 1982), selection is expected to eliminate inbred individuals between the seed and adult life stages. Numerous studies have shown an apparent increase in the outcrossing rate with the age of the material assayed (e.g., Moran and Brown, 1980; Shaw and Allard, 1982a, b; Hamrick and Schnabel, 1985; Cheliak et al., 1985; Yazdani et al., 1985; Boyle and Morgenstern, 1986; Perry and Dancik, 1986; Yeh et al., 1986; Brotschol et al., 1986; Mitton and Jeffers, 1989; Kanashiro, 1990; Prat and Arnal, 1994; Kertadikara et al., 1995). Thus it is not uncommon for adult trees to exhibit a lower inbreeding coefficient than their progeny.

The shadehouse, however, is less stressful than the natural environment (Libby et al., 1981; Muona et al., 1988); inter-tree competition is minimized, and density-dependent selection may not occur. Consequently, our Washoe inbred genotypes survived to age 2 even though inbreeding depression for survival usually acts early in the life cycle of conifers (*Table 2* in Charlesworth and Charlesworth, 1987). We do not know the level of inbreeding that persists in 2-yr old Washoe pine seedlings grown under field conditions. It is possible that inbreeding depression acts later in the life cycle than the 2 yr old seedlings we sampled. However, data from other species suggest that inbred genotypes are usually lost very early in the life cycle, especially in species for which inbreeding depression is severe (Husband and Schemske, 1996). For example, most inbreeding is removed by age 7 in Monterey pine, *Pinus radiata*, (Plessas and Strauss, 1986), and by age 3 in Scots pine, *Pinus sylvestris* (Muona et al., 1987). In either case, the high proportion of biparentally inbred offspring may contribute to the survivorship of inbred offspring. Biparentally inbred offspring are homozygous at fewer loci than selfed progeny, and consequently express fewer deleterious mutations, such that they can survive longer. However, such biparental inbreds will express some inbreeding depression, possibly manifested as reduced growth, and fecundity.

Practical implications of these results center on the persistence of inbred individuals when cultured under conditions that limit environmental stresses. Regardless of whether inbreeding normally persists to age 2 on Washoe pine or was permitted by benign greenhouse conditions, our results show that approximately 14% of the seedlings resulted from self fertilization, with an additional 15% to 30% resulting from matings between relatives. Apparently none of these inbred individuals reach maturity under natural conditions. On the one hand, the presence of such individuals should have little or no effect on forest management practices as long as planting densities exceed the desired density of the mature population by a great enough amount that failure of the inbreds to survive will not affect final stand density. To be sure, nursery practices that eliminate as many inbred individuals as possible would help assure a lack of effect on forest productivity. On the other hand, when native plants are used for landscaping, density dependent selection does not occur, while slow growing individuals are favored. Even though individuals planted as landscape specimens ordinarily are exposed to an environment much more benign than the natural, they are nevertheless subjected to the vicissitudes of climate. Our results suggest that as many as 45% could be in jeopardy.

Acknowledgements

We acknowledge support from a USDA Forest Service Collaborative Agreement to provide funding for the genetic analyses. Amanda Brinnand, Carson district, Toiyabe National Forest, supervised the collection of cones. Pat Wells managed the seeds and cultured seedlings. Gene Namkoong and Yousry El-Kassaby contributed helpful comments. Kermit Ritland contributed both the program for estimation of parameters of the mating system, and statistical advice.

Literature

Betancourt, J. L., Van Devender, T. R. and Martin, P. S.: Synthesis and prospectus. pp. 431–447. In: Betancourt, J. L., Van Devender, T. R. and Martin, P. S. (eds.). Packrat Middens. The Last 40,000 years of Biotic Chance. University of Arizona Press, Tucson, AZ (1990). — Boyle, T. J. B. and Morgenstern, E. K.: Estimates of outcrossing rates in six populations of black spruce in central New Brunswick. Silvae Genet. **35**, 102–106 (1986). — Brotschol, J. V., Roberds, J. H. and Namkoong, G.: Allozyme variation among North Carolina populations of *Liriodendron tulipifera*. Silvae Genet. **35**, 131–138 (1986). — Charlesworth, D. and Charlesworth, B.: Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. **18**, 237–268 (1987). — Cheliak, W. M., Dancik, B. P., Morgan, K., Yeh, F. C. H. and Strobeck, C.: Temporal variation and the mating system in a natural population of jack pine. Genetics **109**, 569–584 (1985). — Critchfield, W. B.: Crossability and relationships of Washoe pine. Madrono **31**, 144–170 (1984). — El-Kassaby, Y. A., Russel, J. and Ritland, K.: Mixed mating in an experimental population of western red cedar, *Thuja plicata*. Heredity **85**, 227–231 (1994). — Farris, M. A. and Mitton, J. B.: Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. Evolution **38**, 1151–1154 (1984). — Franklin, E. C.: Genetic load in loblolly pine. Am. Nat. **106**, 262–265 (1972). — Hamrick, J. L. and Schnabel, A.: Understanding the genetic structure of plant populations: some old problems and a new approach. pp. 50–70. In: H. R. Gregorius (ed.). Lecture Notes in Biomathematics 60: Population Genetics in Forestry. Springer Verlag, Berlin (1985). — Hardner, C. M., Vaillancourt, R. E. and Potts, B. M.: Stand density influences outcrossing rate and growth of open-pollinated families of *Eucalyptus globulus*. Silvae Genet. **45**, 226–228 (1996). — Husband, B. C. and Schemske, D. W.: Evolution of the timing and magnitude of inbreeding depression in plants. Evolution **50**, 54–70 (1996). — Kanashiro, M.: The demographic genetics of an Appalachian stand of *Liriodendron tulipifera* L. Ph. D. Thesis, North Carolina State University, Raleigh (1990). — Kertadikara, A. W. S. and Prat, D.: Genetic structure and mating system in teak (*Tectona grandis* L. f.) provenances. Silvae Genet. **44**, 104–110 (1995). — Knowles, P., Furnier, G. R., Aleksiuk, M. A. and Perry, D. J.: Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. **65**, 1087–1091 (1987). — Libby, W. J., McCutchan, B. G. and Millar, C. I.: Inbreeding depression in selfs of redwood. Silvae Genet. **30**, 15–25 (1981). — Linhart, Y. B., Mitton, J. B., Sturgeon, K. B. and Davis, M. L.: Genetic variation in space and time in a population of ponderosa pine. Heredity **46**, 407–426 (1981). — Little, E. L., Jr and Critchfield, W. B.: Subdivisions of the genus *Pinus* (pines). U.S.D.A. Misc. Publ. 1144, 51 p. (1969). — Mitton, J. B.: The dynamic mating systems of conifers. New Forests **6**, 197–216. Also: pp. 197–216, in: Adams, W. T., Strauss, S. H., Copes, D. L. and Griffin, A. R. (eds.). Population Genetics of Forest Trees. Kluwer Academic Publishers, Dordrecht (1992). — Mitton, J. B. and Jeffers, R. M.: The genetic consequences of 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)

By M. Kanashiro[1], S. A. Harris[2][4] and A. Simons[3]

(Received 7th May 1997)

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.

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

---

[1] EMBRAPA/CPATU, C.P. 48, CEP 66.095-100, Bélem, Pará, Brazil.

[2] Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK.

[3] International Centre for Research in Agroforestry – ICRAF, P.O. Box 30677, Nairobi, Kenya.

[4] Author for correspondance.