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Genetics of Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.: Lecythidaceae)

2. Mating system

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Summary. Here we report the results of a mating system analysis of an Amazonian population of *Bertholletia excelsa,* a tropical rain forest canopy tree species. Using progeny data from 29 seed parents, two highly polymorphic isozymes were analyzed to derive single locus and multilocus estimates of outcrossing, based on a mixed mating model. The two single locus estimates were very similar, and both were somewhat smaller than the multilocus estimate, indicating the possibility that the populations are genetically structured. The multilocus outcrossing estimate ($t_m = 0.85 \pm 0.03$) reveals that outcrossing is prevalent, but that a significantly low level of inbreeding may be occurring. The high outcrossing rate indicates that even though dispersion of individuals is very low within populations of this tropical rain forest tree, pollen dispersal mechanisms appear to be adequate to enable crosses with a relatively large number of potential mates.

Key words: Outcrossing rate – *Bertholletia excelsa* – Brazil nut – Isozyme analysis – Tropical rain forest

Introduction

Mating systems are important determinants of the genetic composition of populations. The mating system regulates the distribution of genetic variability among the progeny of an individual, among individuals of a population, and among subdivisions of a population (Hamrick 1982). Mating systems have been studied in many temperate zone herbaceous plants (Clegg 1980 and references therein; Smyth and Hamrick 1984; Ennos 1985; Kesseli and Jain 1985), wind-pollinated conifers (Shaw and Allard 1982; Cheliak et al. 1985a; Cheliak et al. 1985b), and animal-pollinated *Eucalyptus* spp. (Brown et al. 1975; Moran and Brown 1980). These studies have revealed considerable spatial and temporal variation in outcrossing rates within species. As compared to the species investigated to date, tropical rain forest trees have low population densities, and conspecifics in many cases are widely scattered. Because dispersion is expected to influence gene flow and thereby the mating system, it is surprising that very little is known about mating systems in tropical rain forest trees, despite considerable interest in the role of breeding systems in the microevolution of tropical rain forest populations (Fedorov 1966; Ashton 1969; Bawa 1976). So far mating systems have been analyzed in only one species, *Pithecellobium pedicellare,* a large canopy tree in lowland rain forests of Costa Rica (O'Malley and Bawa 1987).

Here we report the results of an analysis of the mating system of *Bertholletia excelsa* (Brazil nut), a monotypic genus of large trees distributed on non-flooded ground in the tropical rain forests of the Guianas, Amazonian Colombia, Venezuela, Peru, Bolivia, and Brazil (Mori and Prance 1987). Trees are found in stands of 50–100 trees, with stands separated by a kilometer or more and with overall densities ranging from one individual per six hectares to 15–20 individuals per hectare (Mori and Prance 1988). Pollination is accomplished by large bees, and crossing experiments indicate that self-incompatibility is strong, though not necessarily complete (Muller et al. 1980; Moritz 1984; Mori and Prance 1988). The large, indehiscent fruits contain a thick pericarp and fall to the ground close to the maternal parent. The fruits are gnawed open by agoutis, which eat some seeds and cache the rest. Although agoutis have small ranges, some caches are pirated by neighbors who re-cache the seeds at greater distances from the maternal sporophyte. Seedling

Table 1. Observed and expected genotypic numbers, heterozygosity (h), fixation index (F), and Chi-square test for departure from Hardy-Weinberg proportions for parents (Gen_0) and offspring (Gen_1)

		Genotypes										
		1/1	1/2	2/2	1/3	3/3	2/3	Total	h	F	Chi-square	df
Fest3												
Gen_0	Obs.	7	16	6	0	0	0	29	0.499	−0.105	0.318	1
	Exp.	7.6	14.5	6.9	–	–	–					
Gen_1	Obs.	167	268	127	1	0	2	565	0.500	0.041	0.957	3
	Exp.	160.9	279.6	121.5	1.60	0	1.4					
Mdh1												
Gen_0	Obs.	0	12	7	3	0	7	29	0.580	−0.309	5.212	3
	Exp.	1.9	8.5	9.4	2.6	0.9	5.7					
Gen_1	Obs.	43	163	160	54	25	131	576	0.605	0.002	1.639	3
	Exp.	39.8	161.5	163.6	61.8	24.0	125.3					

$h = 1 - p_i^2$; $F = 1 - h_0/h_e$

Table 2. Maximum likelihood estimates of pollen and ovule gene frequencies, single and multilocus outcrossing rate with standard errors, and Chi-square tests for agreement with mixed mating model

Locus	Allele	Pollen	Ovules	t	S.E.	Chi-square	d.f.
Fest2	1	0.560	0.517	0.794*	0.055*	3.56	1
	2	0.434	0.483				
	3	0.006	0.000				
Mdh1	1	0.233	0.259	0.803*	0.043*	22.53	9
	2	0.512	0.569				
	3	0.255	0.172				
Multilocus				0.849	0.033		

* Denotes single locus estimate

establishment results from unrecovered caches (Huber 1910; Smythe 1978; Mori and Prance 1988).

Our objectives were (1) to estimate the rate of outcrossing in a natural population and, (2) to assess whether the population may be genetically structured by inbreeding other than selfing. The outcrossing rate was estimated by fitting the parameters of the mixed mating model to genotypic data derived from two polymorphic allozymes. Other polymorphic allozymes identified in this species (Buchley et al. 1988) departed strongly from the assumptions of the mixed mating model and were not utilized in this analysis.

Materials and methods

The *Bertholletia excelsa* (Brazil nut) seeds analyzed in this investigation were collected from a population located at Acre, Brazil (10°45′S, 68°10′W, Buckley et al. 1987). The electrophoretic methods used to resolve allozyme polymorphisms in *Mdh1* and *Fest3* are described in Marty et al. (1984). Both polymorphisms involve dimeric isozymes with exceptionally clear electrophoretic phenotypes. A single locus is assumed to encode the variation for each allozyme polymorphism (i.e., *Fest3* and *Mdh1*).

Results

Nearly complete genotypic data was obtained for 578 seeds from one population of *Bertholletia excelsa* for two highly variable loci, *Fest3* and *Mdh1* (Table 1). Seed parent genotypes were determined from progeny arrays following the method of Brown and Allard (1970), as implemented in the computer program of Ritland and Jain (1981). All *Fest3* heterozygous parents were confirmed by the observation of at least one homozygous offspring for each of the two alleles in the parental genotype. For *Mdh1*, 18 of 22 progeny arrays assigned heterozygous genotypes were also confirmed in this way. Observed genotypic numbers did not depart significantly from random mating proportions for both generations at both loci. For both loci, the fixation index for the parents was smaller than that of the progeny.

The parameters of the mixed mating model (pollen and ovule gene frequencies and outcrossing rate, *t*) were fitted to the *Bertholletia excelsa* genotypic data (Table 2) by the multilocus maximum likelihood method of Ritland and Jain (1981). The two single locus estimates of *t* closely agreed (*Fest3:* 0.794 and *Mdh1:* 0.803), but the multilocus estimate was somewhat larger (0.849). All estimates were significantly less than 1.0, based upon confidence intervals. The gene frequencies in the pollen and ovule pools for *Fest3* were homogeneous, but departed significantly from homogeneity for *Mdh1*. A Chi-square

Table 3. Chi-square tests for homogeneity of numbers of heterozygous offspring among identical homozygous seed parents

*Fest3** 1/1 seed parents:				*Fest2* 2/2 seed parents:				*Mdh1* 2/2 seed parents:			
Family	1/1	1/2	Total	Family	1/2	2/2	Total	Family	1/2	2/2	Total
1	13	6	19	8	3	7	10	1	4	16	20
11	13	7	20	18	5	15	20	5	6	14	20
15	11	9	20	24	11	9	20	11	9	11	20
17	13	7	20	25	12	8	20	14	10	10	20
22	13	7	20	26	14	6	20	18	2	18	20
27	14	6	20	30	6	14	20	29	13	7	20
								30	5	15	20
Total	77	42	119	Total	51	59	110	Total	49	91	140
Chi-square = 1.188, 5 d.f. (n.s.)				Chi-square = 13.490, 5 d.f. ($P < 0.05$)				Chi-square = 19.341, 6 d.f. ($P < 0.01$)			

* (note: 2/3 heterozygotes were combined with 1/2 heterozygotes)

Table 4. Contingency table analysis for independence of *Fest3*[a] and *Mdh1* genotypes

	Mdh1 genotypes						
	1/1	1/2	2/2	1/3	3/3	2/3	Total
Fest3							
1/1	13	51	49	15	6	31	165
1/2	20	77	65	28	10	68	268
2/2	10	33	43	9	9	25	129
Total	43	161	157	52	25	124	562

Chi-square = 9.676, 10 d.f. (n.s.)

[a] *Fest* alleles 2 and 3 were combined for analysis here

approach based upon maximum likelihood estimates of *Mdh1* gene frequencies in the pollen and ovules yields a statistic testing homogeneity of 11.91 (2 degrees of freedom, $P < 0.01$). The departure from homogeneity is due primarily to differences involving allele 3.

Significant departures from the mixed mating model were detected at *Mdh1* by the Chi-square method of El-Kassaby and Ritland (1987), which tests differences between predicted and observed numbers of offspring of each genotype for each maternal genotype (Table 3). The departures indicate an excess of heterozygous offspring from heterozygous seed parents. A similar pattern is apparent, though not significant, in the *Fest3* genotypic data. Significant departure from the assumption of homogeneity of the pollen pool over all seed parents was detected in two of three tests involving both loci. No evidence of association was found between *Fest3* and *Mdh1* genotypes (Table 4).

Discussion

The results show that the progeny genotypic distributions of a population of *Bertholletia excelsa* could be explained by a mixed mating model with an outcrossing rate (multilocus) of 0.85 ± 0.03. This level of outcrossing implies that the mating system of Brazil nut produces predominantly outcrossed seed, but that a significant low level of selfing may be occurring. Departures from random mating are not detected directly from the genotypic distributions, but the power of the Chi-square test for this purpose is low (Ward and Sing 1970). These results are in agreement with those of Brazilian workers whose experimental crosses indicate that *Bertholletia* is predominantly or completely self-incompatible (Muller et al. 1980; Moritz 1984; Mori and Prance 1988). Similar outcrossing rates have been reported for *Eucalyptus*, a genus of tropical forest trees. The outcrossing rate of *Pithecellobium pedicellare*, a tropical forest leguminous tree, was reported to be nearly 1.0 (O'Malley and Bawa 1987).

The high outcrossing rates that were observed in these tropical tree species indicate that low population densities are compensated for by dispersal mechanisms that enable mating opportunities with relatively large numbers of individuals. These results are in contrast with the assumption that low population densities in these speciose communities have resulted in strong inbreeding, which has been proposed as a cause of high speciation rates and high species diversity (Fedorov 1966). Levels of gene flow among tropical tree populations have yet to be assessed, however a preliminary comparison of two Amazonian *Bertholletia* populations indicates that level of differentiation among widely separated populations may be very low (Buckley et al. 1988).

The fit of the *Bertholletia excelsa* data to a mixed mating model was not precise, thus the statistics reported here must be interpreted with caution. Pollen pool gene frequencies over maternal parents departed significantly from homogeneity, a basic assumption of the mixed mating model. The pollen and ovule gene frequencies strongly differed for one allele. Departures from the expected numbers of offspring of each genotypic category for each maternal category revealed a significant excess of heterozygous offspring from heterozygous moms for *Mdh1*. A similar trend was apparent for *Fest3*. This departure suggests that heterozygotes may have a higher level of outcrossing than homozygotes. These results are consistent with Ritland's "open-mating" model, which implicates population patchiness with respect to levels of inbreeding (Ritland 1985). By incorporating more levels of relationship than the mixed mating model, the open-mating model may prove valuable in resolving the mating systems of tropical trees, which characteristically occur at low population densities in highly diverse forests. The finding of lower single outcrossing rate estimates relative to the multilocus estimates in *Bertholletia* may be attributable to consanguineous mating, although we cannot exclude the possibility that we have underestimated the true outcrossing rate due to patchiness in outcrossing rates in our sample (Shaw and Allard 1982; Ennos and Clegg 1982; Ellstrand and Foster 1983).

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