

## ESTIMATION OF MATING SYSTEM PARAMETERS OF *ALBIZIA JULIBRISSIN* (FABACEAE)

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### ABSTRACT

Quantitative estimates of the mating system of *Albizia julibrissin*, an entomophilous mimosoid leguminous tree, were obtained using six polymorphic allozyme markers. Thirty-six progeny from 24 to 25 maternal families from each of three Georgia populations were analyzed. Despite life history characteristics and pollinator behavior that suggest a potential for inbreeding, mean single and multilocus outcrossing rates did not differ significantly from 1.0 for any population, or for any maternal family. These results strongly suggest that mimosa is self-incompatible. Biparental inbreeding was insignificant even though seed dispersal via wind-dispersed pods suggests that related individuals may establish in close proximity to one another. Significant heterogeneity was found among the pollen pools of maternal individuals belonging to the same population, indicating that mating does not occur at random within these populations.

**Key words:** *Albizia julibrissin*, mating system, outcrossing rate, allozymes

### INTRODUCTION

The mating system of a species is an important determinant of its genetic structure and evolution. Outcrossing species generally maintain more genetic diversity within their populations and exhibit less genetic differentiation among their populations than selfing species (BROWN 1979; HAMRICK & GODT 1989). However, biparental inbreeding (mating among relatives) can occur even within outcrossing populations (BROWN 1989), particularly if seed dispersal is local, as it is for many plant species.

Temperate forest trees have been the focus of a number of mating system studies. Most studies, however, have involved commercially valuable wind-pollinated conifers (*e.g.*, EL-KASSABY & RITLAND 1986; MORGANTE *et al.* 1991; EL-KASSABY *et al.* 1993), with relatively little attention being given to angiosperms, and less yet to insect-pollinated species. Most wind-pollinated temperate trees are highly outcrossed (MITTON 1992; BACILIERI *et al.* 1996; ROSSI *et al.* 1996), although a few exhibit significant levels of inbreeding (KNOWLES *et al.* 1987; PERRY & KNOWLES 1990). In contrast to the temperate tree flora, which is primarily wind-pollinated, most tropical trees are animal-pollinated. Controlled crossing experiments of 34 tropical hermaphroditic tree species indicated that 80% (27) were self-incompatible (BAWA *et al.* 1985). Consistent with these observations, high outcrossing rates have been found for most tropical tree species

(reviewed in LOVELESS 1992 and NASON & HAMRICK 1997; BOSHIER *et al.* 1995). Studies of the mating systems of additional temperate angiosperm species are needed to expand our knowledge of this important population process.

*Albizia julibrissin* Durazz (mimosa) is a small, attractive tree native to subtropical and temperate regions of Asia from Iran to China and Japan (ELIAS 1980). A popular flowering tree with a wide-spreading crown, mimosa is widely grown as an ornamental in warmer regions of the United States and Europe. Mimosa has become naturalized in the southern U. S., where it is typically found on forest edges, along roadsides and at abandoned homesites. Its range in North America extends from Maryland south through Florida and west to Texas and California (RADFORD *et al.* 1968). Mimosa trees flower prolifically in full sun, and numerous insects (including honeybees, bumblebees and butterflies) are attracted to the fragrant and showy pink inflorescences (MJWG, pers. obs.). Hummingbirds have also been observed visiting flowers (MJWG, pers. obs.). In Georgia flowering occurs over an extended period, encompassing nearly four months (May – August). Numerous (8 to 16) hard seeds are produced in thin, flat pods. Although the seeds have no specialized dispersal mechanism, mimosa pods are readily dispersed by wind (MJWG & JLH, pers. obs.).

This study is the first phase of a detailed investigation of the breeding system dynamics and population genetic structure of this mimosoid legume. Here our

objective was to characterize the mating system of several mimosa populations in Georgia. Several life-history features of mimosa suggest that the species has a high potential for inbreeding. First, mimosa is monoecious, and many perfect flowers open simultaneously on an individual. Field observations indicate that geitonogamous pollination may be common, since pollinators frequently move between flowers on the same tree. The coincident dispersal of seeds that are at least half-sibs by the movement of pods suggests that related individuals may often be recruited near one another. Because mimosa often flowers at a very small size, individuals derived from several annual seed cohorts and their parental trees may inter-mate.

### MATERIALS AND METHODS

Thirty to forty mimosa pods were collected from each of 24 to 25 trees in each of three populations located in Athens, Georgia during autumn 1987. These natural populations were at the intramural athletic fields (hereafter designated AF) on the University of Georgia campus, on Barnett Shoals Road (designated BSR), and along Research Road (designated RR). Mean distance between populations was 1.9 km (range 1.3 to 2.6 km). In the laboratory, seeds were extracted and bulked by maternal tree. To speed germination, seeds were soaked in concentrated sulfuric acid for twenty minutes to break the seed coat and placed in a moist Petri dish to imbibe water. After 24 hrs, the swollen seeds were planted in flats and placed in the greenhouse. Two- to four-week-old whole seedlings were crushed using a mortar and pestle. Seedling enzyme extracts were stabilized by the addition of an extraction buffer (MITTON *et al.* 1979) and adsorbed onto chromatography paper wicks that were stored at  $-70^{\circ}\text{C}$  until needed for electrophoresis.

Quantitative estimation of mating system param-

eters was accomplished through the use of genetic markers. We analyzed six polymorphic allozyme loci on horizontal starch gels for 36 seeds per maternal tree: cathodal peroxidase (*Cper-1*), fluorescent esterase (*Fe-1* and *Fe-2*), isocitrate dehydrogenase (*Idh*), 6-phosphogluconate dehydrogenase (*6Pgdh-2*), and phosphoglucoisomerase (*Pgi-2*). Stain recipes were taken from SOLTIS *et al.* (1983). *Idh*, *Pgi-2* and *6Pgdh-2* were resolved using Buffer 4 (buffer numbers refer to Table 1 in SOLTIS *et al.*, 1983), while *Cper-1*, *Fe-1* and *Fe-2* were resolved with Buffer 6. The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (WEEDEN & WENDEL 1989). Although no formal crosses were made, our studies indicate that progeny genotypes were consistent with maternal genotypes.

Mating system parameters, including single locus ( $t_s$ ) and multilocus outcrossing rates ( $t_m$ ) and pollen allele frequencies were estimated using a statistical program (RITLAND 1990) developed from the algorithms of RITLAND & JAIN (1981) which are based on the mixed-mating model of BROWN & ALLARD (1970). Assumptions of this model are given in CLEGG (1980) and SHAW *et al.* (1981). Maternal genotypes were inferred from the progeny arrays by the method of BROWN & ALLARD (1970). Standard errors for outcrossing estimates are based on 200 bootstraps. Heterogeneity of the pollen pools of maternal individuals within populations was examined by  $\chi^2$ -tests. The equation used was

$$\chi^2 = NG_{ST}(a-1) \text{ with df} = (a-1)(n-1)$$

where  $N$  is the total sample size in each population,  $G_{ST}$  is the proportion of genetic diversity found among the pollen pool of the maternal individuals in each population,  $a$  is the number of alleles, and  $n$  is the number of

**Table 1. Mating system estimates<sup>1</sup> and sampling parameters for three *Albizia julibrissin* populations [Athletic Fields (AF), Barnett Shoals Road (BSR), and Research Road (RR)] located in Athens, Georgia**

Population	No. of families	Total no. of progeny	$t_m$ (SE)	$t_s$ (SE)	$t_m - t_s$ (SE)
AF	25	900	0.967 (0.019)	0.933 (0.034)	0.034 (0.025)
BSR	25	900	1.003 (0.002)	1.004 (0.037)	--- <sup>2</sup>
RR	24	864	0.987 (0.021)	0.944 (0.037)	0.043 (0.025)
Mean	25	888	0.986 (0.014)	0.960 (0.022)	0.026 <sup>3</sup> (0.023)

<sup>1</sup>  $t_m$  is the multilocus outcrossing rate,  $t_s$  is the mean single locus outcrossing rate and SE is the standard error.

<sup>2</sup> Negative values for biparental inbreeding are not biologically meaningful.

<sup>3</sup> Since negative values of biparental inbreeding are meaningless, the mean has been calculated considering the value for BSR to be zero.

Table 2. Variation among pollen pools of (a) maternal individuals within *Albizia julibrissin* populations and (b) *A. julibrissin* populations. ( $G_{ST}$  values were significantly different from zero ( $P < 0.05$ ) except for *Fe-2* among populations)

Locus	Overall $H_T$	Proportion of genetic variation among pollen pools			
		Among families within populations ( $G_{ST}$ )			Among populations $G_{ST}$
		AF	BSR	RR	
<i>Cper-1</i>	0.408	0.115	0.186	0.150	0.058
<i>Fe-1</i>	0.516	0.230	0.065	0.150	0.032
<i>Fe-2</i>	0.490	0.124	0.124	0.119	0.000
<i>ldh</i>	0.382	0.123	0.116	0.287	0.036
<i>6Pgdh-2</i>	0.063	0.064	0.079	0.305	0.018
<i>Pgi-2</i>	0.791	0.205	0.149	0.175	0.004
Mean	0.442	0.144	0.120	0.198	0.025

maternal individuals (WORKMAN & NISWANDER 1970). In an analogous manner, we also tested for allele frequency differences among the pollen pools of the three populations.

The average inbreeding coefficient (Wright's  $F$ ; WRIGHT 1965) was calculated for maternal individuals via RITLAND & JAIN's (1981) mating system program. The hypothesis that the mean  $F$  values were equal to zero was tested by the equation  $\chi^2 = NF^2(a-1)$  with degrees of freedom =  $a(a-1)/2$  where  $N$  is the number of individuals in the population and  $a$  is the number of alleles per locus (LI & HORVITZ 1953). Since  $F$  is a mean value we used  $a = 2$ , which provides a conservative  $\chi^2$  estimate. The expected fixation index at inbreeding equilibrium was calculated from the multilocus outcrossing rate (FYFE & BAILEY 1951) by the equation  $F_e = (1 - t_m)/(1 + t_m)$ .

## RESULTS AND DISCUSSION

Mean single-locus outcrossing estimates for the three populations ranged from 0.933 to 1.004, whereas multilocus estimates ranged from 0.967 to 1.003 (Table 1). No population outcrossing estimate was significantly different from 1.0. Family outcrossing estimates were calculated simultaneously solving for the outcrossing rate and pollen allele frequencies of the progeny arrays. Most families had outcrossing rates of 1.0 or above (rates  $> 1.0$  are statistically valid although they are not biologically meaningful). Several families (3 in RR; 5 in AF and 5 in BSR) had outcrossing rates less than, but not significantly different from, 1.0. (These latter family estimates ranged from  $t_m = 0.81$  to 0.99, with a mean outcrossing rate of 0.91.)

These high outcrossing rates, coupled with the potential for geitonogamous pollination (between-flower, within-plant pollination) and autogamy (within-

flower fertilization) strongly suggests that mimosa is self-incompatible. High outcrossing rates and self-incompatibility systems are common among tropical angiosperm trees (BAWA 1974; LOVELESS 1992). Of the tropical tree species for which quantitative mating system estimates are available only a few [e.g., *Cavanillesia platanifolia* (H. & B.) H.B.K. (MURAWSKI & HAMRICK 1990), *Ceiba pentandra* (L.) Gaertn. (MURAWSKI & HAMRICK 1992), *Eucalyptus pellita* F. Muell. (HOUSE & BELL 1996) and *Shorea trapezifolia* (Thw.) Ashton (MURAWSKI *et al.* 1994)] have clearly exhibited mixed-mating systems ( $t_m < 0.80$ ). Within the Fabaceae self-incompatibility has been documented for several tropical trees (e.g., *Enterolobium cyclocarpum* (Jacq.) Griseb., *Pterocarpus rohrii* Vahlare and *Pithecelobium saman* (Jacq.) Bentham; BAWA 1974). Few studies of entomophilous temperate trees are available; a paternity analysis study of the insect-pollinated, weedy leguminous tree *Gleditsia triacanthos* L. (honey locust) found no self-fertilization, but this species is mostly dioecious (SCHNABEL & HAMRICK 1995). However, a mating system analysis of three *Liriodendron tulipifera* L. populations (a north temperate entomophilous tree in the Magnoliaceae) found outcrossing rates ranging from 0.55 to 0.86 (BROT-SCHOL *et al.* 1986).

Significant heterogeneity occurred ( $P < 0.05$ ) among the pollen pools of maternal trees for all loci in each mimosa population, indicating that trees did not mate at random (Table 2). Such observations are common among trees (e.g., GIBSON & HAMRICK 1991; LOVELESS 1992, ROSS *et al.* 1996) and can be attributed to temporal variation in phenology or spatial position within populations. For entomophilous plants variation in the outcrossed pollen received by maternal individuals is highly likely to be influenced by the presence of nearby individuals, because most insect

pollinators tend to fly to the nearest flowering individual when foraging (LEVIN & KERSTER 1974). The occurrence of significant variation among pollen pool allele frequencies of maternal trees within populations suggests that local genetic substructuring should occur in this species if seed dispersal and recruitment are geographically limited. Further studies are needed to explore the relatedness of trees across space within populations.

Despite tree-to-tree substructuring of the pollen pool, pollen pool allele frequencies varied relatively little among the three Athens, Georgia populations (Table 2). The proportion of total genetic variation at the six loci found among pollen pools of maternal individuals within populations ranged from 0.064 to 0.305, with a mean of 0.153 ( $SD = 0.068$ ), whereas the proportion of genetic variation found among pollen pools of the three populations ranged from 0.000 to 0.058, with a mean of 0.024 ( $SD = 0.022$ ). The relatively small proportion of genetic divergence among pollen pools of the three Athens populations is likely due to gene flow among populations. High seed set on isolated (by 1 km or more) mimosa trees suggests that long-distance pollen movement is routine (MJWG & JLH, pers. obs.). Further, the ready establishment of mimosa in many disturbed environments suggests that seed dispersal is high in this species. Analyses of pollen and seed flow have been initiated to further explore the influence of gene flow on genetic structure in this species.

Equilibrium inbreeding coefficients and observed fixation indices are expected to be similar if the mating system is the sole factor determining genotype frequencies within populations. Equilibrium inbreeding coefficients were close to zero (Table 3) as expected for an outcrossing species. The observed mean fixation indices in all populations were negative (Table 3), indicating an excess of heterozygotes among the adults, but none of these values was significantly different from zero.

The evolutionary dynamics of mating systems and the ecological factors that affect them are poorly

**Table 3.** Mean observed fixation index ( $F$ ) and equilibrium fixation index ( $F_c$ ) for three *Albizia julibrissin* populations

Population	$F$ (SE)	$F_c$
AF	-0.300 (0.042)	0.017
BSR	-0.300 (0.042)	0.000
RR	-0.190 (0.059)	0.007
Mean	-0.263 (0.037)	0.008

understood for most species. Temperate angiosperm trees have been especially poorly represented in the plant mating system literature. Although most tropical angiosperm trees are highly outcrossed (NASON & HAMRICK 1997), no generalizations can yet be made for temperate angiosperm trees. On the basis of these mating system results, we predict that mimosa's genetic structure will be similar to wind-pollinated and wind-dispersed temperate conifers, with most genetic diversity being found within populations, and a small proportion partitioned among them. Further investigations of the breeding system dynamics of this weedy tree species are underway and include studies of temporal heterogeneity in the pollen pool, comparisons of gene flow rates into isolated and clustered trees and the effect of changing stand density on paternity and gene flow.

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