American Journal of Botany 94(1): 111–118. 2007.

HIERARCHICAL PATTERNS OF PATERNITY WITHIN CROWNS OF *ALBIZIA JULIBRISSIN* (FABACEAE)[1]

ELEANOR A. PARDINI[2] AND J. L. HAMRICK

Department of Plant Biology, 2502 Miller Plant Science, University of Georgia, Athens, Georgia 30602 USA

The floral architecture and phenology of the tree species *Albizia julibrissin* (Fabaceae) offer the potential for flowers within inflorescences to share common pollen donors. Patterns of paternity within individual tree crowns may differ among isolated individuals and those in populations due to differences in pollinator foraging behavior. To determine how genetic diversity is partitioned within individual seed pools and whether these patterns differ among isolated and population trees, we obtained all fruits from three inflorescences from four clusters from three isolated trees and from three population trees in Athens, Georgia. We assayed 14 polymorphic allozymes to genotype all progeny within singly sired fruits to determine the multilocus genotype of each fruit's pollen donor. Inflorescences had multiple pollen donors, but simulation analyses revealed that redundancy of pollen donors tended to be more likely within inflorescences than randomly across the crown. Analysis of genetic and genotypic diversity indicated that individual maternal trees received pollen from many donors in uneven frequencies. Results suggest that isolated trees receive pollen from slightly fewer pollen donors and experience more within-plant pollinator movement than trees in populations. However, isolated trees receive qualitatively similar pollen from many sources, suggesting that these trees are not effectively isolated and that pollen moves long distances in this species.

Key words: *Albizia julibrissin*; correlated paternity; Fabaceae; mimosa; paternity analysis; pollen donor; seed pool.

Pollen movement plays a crucial role in structuring genetic diversity within plant populations. Examining the structure of plant pollen pools can provide insights into pollen movement at multiple spatial scales (Dyer and Sork, 2001) ranging from among populations or individuals to within individuals. The recent two-generation approach to the study of pollen movement quantifies differences among pollen pools sampled from maternal trees rather than among populations (Smouse et al., 2001; Dyer, 2005). These insights yield information about pollen movement among trees in stands with variable vegetative structure (Dyer and Sork, 2001), in fragmented landscapes (Dick et al., 2003) and under different silvicultural regimes (Sork et al., 2005). Less attention has been focused on patterns of pollen deposition within the canopies of individual trees. Heterogeneous pollen movement within tree canopies could represent a previously underestimated component of genetic structure that should be accounted for in estimates of pollen movement. Structuring of seed pools within plants could transmit preexisting genetic structure to the next generation in colonizing plants (e.g., high relatedness among seeds within fruits). Additionally, heterogeneity occurring within sectors of tree crowns or even within branches, inflorescences, or fruits, should be accounted for in collection schemes for the genetic analysis of pollen movement (Irwin et al., 2003; Hardy et al., 2004).

Previous work has shown that mating in wind-pollinated conifers is not random among maternal individuals or within sectors of individual tree crowns. Allele frequencies differ among trees (Dyer and Sork, 2001; Gibson and Hamrick, 1991), among crown levels (El-Kassaby, 1986), and among branches, and cones (Gibson and Hamrick, 1991). Higher outcrossing is often found within upper tree crowns than in lower tree crowns (Shen et al., 1981; Omi and Adams, 1986; Chaisurisri et al., 1994). Relatively less attention has focused on the breeding systems of temperate angiosperms (Godt and Hamrick, 1997). Animal pollinators likely move pollen in a more directed manner than does wind, and patterns of pollination are influenced by pollinator behavior and movement. It is reasonable then to expect even more heterogeneity among pollen donor arrays within crowns of animal-pollinated trees. A few studies have reported evidence for variation in outcrossing rates among inflorescences within plants (Smyth and Hamrick, 1984) or among flowers within inflorescences in aster species (Gibson, 2001). In tropical trees, redundancy of pollen donors has been documented within individual tree crowns (Apsit et al., 2001) and within clusters and inflorescences (Muona et al., 1991). The correlated breeding system of *Albizia julibrissin* Durazz., or mimosa, a tree widespread in the southeastern United States, presents the opportunity to investigate genetic structure within seed pools of an animal-pollinated, temperate tree species. In previous studies of *A. julibrissin,* low values for the effective number of fathers have been found ($N_{ep} = 2.05$ [single year]–2.87 [several years], Irwin et al., 2003; $N_{ep} = 2.1$–3.2, J. L. Hamrick, unpublished data), demonstrating that trees receive pollen in uneven amounts from different pollen donors. The fact that N_{ep} is small begs additional information about the dynamics of pollen donors within crowns. Unique features of the reproductive biology of mimosoid legumes such as *A. julibrissin* allow exceptionally detailed analyses of pollination patterns within tree crowns. Pollen is packed into large, 16-grain polyads that usually contain ample pollen grains to fertilize all the ovules within an ovary (Elias, 1980). Fruits are typically singly sired and progeny within fruits are full-siblings. Analysis of progeny arrays allows inference of the precise multilocus genotype of the pollen donor to each fruit, thus providing powerful resolution for analysis of patterns of paternity at very local spatial scales.

[1] Manuscript received 9 February 2006; revision accepted 16 November 2006.

The authors thank C. Deen and undergraduate workers in the Hamrick Lab for assistance with lab work, R. Pulliam for invaluable assistance with the simulation analyses and programming in MATLAB, and K. Parker, S. Chang, and three anonymous reviewers for valuable comments that improved this manuscript. Funding was provided by USDA NRICGP-2000-00882 to J.L.H. and M.J.W.G.

[2] Author for correspondence (e-mail: epardini@wustl.edu)

The spatial distribution of *A. julibrissin* presents an opportunity to examine differences in these patterns among isolated trees and those growing in populations. Plant density has been shown to affect pollinator behavior and breeding systems of animal pollinated trees. Isolated pasture individuals of several tropical tree species have been shown to experience increased selfing rates (Aldrich and Hamrick, 1998; Dick, 2001; Fuchs et al., 2003) or disrupted reproductive biology (Rocha and Aguilar, 2001). Increased spatial isolation can result in increased within-plant foraging time, which can have consequences for the degree of outcrossing and seed set. Ghazoul and McLeish (2001) documented greater within-plant pollinator foraging and an associated reduction in seed set in isolated individuals of a tropical tree.

This study seeks to analyze genetic diversity at a very local spatial scale by examining patterns of paternity within seed pools of individual tree crowns. The floral architecture and flowering phenology of *A. julibrissin* crowns offer the potential for flowers located within inflorescences to have a greater than random chance of sharing common pollen donors. *Albizia julibrissin* is self-incompatible, but isolated trees typically produce many fruits, suggesting they are routinely visited by pollinators and receive pollen from long distances. However, if isolated individuals experience less among-plant pollinator visitation and greater within-plant movement relative to trees in populations, progeny from these individuals may result from fewer sires. In this study, we used a hierarchical sampling scheme and genetic analyses to determine (1) whether flowers located within the same inflorescence have proportionally higher chances of sharing common pollen donors and (2) whether patterns of paternity differ between isolated trees and those growing in populations, as estimated by genetic and genotypic diversity of pollen pools.

MATERIALS AND METHODS

Albizia julibrissin is native to central Asia and has become naturalized throughout the southeastern United States. It is a small to medium tree with a large, flat crown. Flowers are clustered in dense inflorescences, which are grouped in numerous indeterminate clusters throughout the crown (Elias, 1980). *Albizia julibrissin* flowers from May through August in Athens, Georgia and attracts numerous generalist pollinators including honeybees, bumblebees, butterflies, and hummingbirds (Godt and Hamrick, 1997). Flowers within a single inflorescence generally open synchronously and last about 1 day, while inflorescences within the same cluster flower variably over several weeks. Flowers are perfect with numerous, showy, white to pink stamens. It is suggested that only the single, large, apical flower within an inflorescence is functionally ovulate (Elias, 1980), but three to nine fruits commonly mature within inflorescences (J. L. Hamrick and E. A. Pardini, personal observation). Singly sired fruits are dispersed by gravity and wind, thus full-sibling progeny are often dispersed in clumps.

Previous research revealed heterogeneity among pollen pools of individual trees within populations of *A. julibrissin* but little differentiation among pollen pools of different populations (Godt and Hamrick, 1997), suggesting that while trees within populations do not mate at random, there are routinely high levels of gene flow among populations across the local landscape. High outcrossing rates and genetic evidence suggest that *A. julibrissin* is self-incompatible despite the potential for within-inflorescence and within-plant pollinations (Godt and Hamrick, 1997). Multilocus outcrossing rates for four isolated trees in Athens, Georgia did not differ from 1.0 (J. L. Hamrick, unpublished data). High observed seed set on isolated trees suggests that isolated trees are a part of the breeding network.

Study sites and sampling method—Paternity analyses using full-sibling progeny arrays provide exceptional resolution for fine-scale breeding patterns (within tree crowns) but the high number of progeny per maternal tree required for this approach limit the number of trees that can be analyzed. We selected six maternal trees (three isolated, three in populations) in Athens, Georgia for analysis. Population trees (designated ACC, AWP, KNO) were separated from each other by at least 3 km. Isolated trees (designated WLK, JFR, JML) were separated from each other by at least 5 km and were separated from the nearest populations by 225 m, 100 m, and 600 m, respectively. All study trees were reproductively mature adults with full crowns of similar size and shape. Numbers of inflorescences and fruits were counted for five clusters on each tree. A hierarchical sampling scheme was used to collect all mature fruits from three inflorescences in four clusters (12 inflorescences total) from each tree. Because we were interested in patterns of paternity among fruits within inflorescences, only inflorescences with at least two fruits were sampled. All fruits were collected in October and November 2001 and thus include pollinations that occurred throughout the entire flowering season that year.

Allozyme analysis—Seeds were extracted and kept separate by fruit. Seeds were soaked in concentrated sulfuric acid for 45 min to erode the seed coat and germinated in petri dishes. After 24 h, seeds were transplanted to flats and placed in the greenhouse. Leaf and root tissue from 2- to 4-week old seedlings of fruits with at least six seedlings were crushed with a mortar and pestle. Enzymes were extracted using the extraction buffer of Mitton et al. (1979) and adsorbed onto filter paper wicks that were stored in 96-well plates at −70°C. Enzymes were electrophoresed on 11% starch gels using five electrode buffer systems to resolve 14 polymorphic loci. Isocitrate dehydrogenase (*Idh*), 6-phosphogluconate dehydrogenase (*6Pgdh*), and phosphoglucoisomerase (*Pgi*) were resolved on buffer system 4; alcohol dehydrogenase (*Adh*) and menadione reductase (*Mnr-1* and *Mnr-2*) were resolved on buffer system 7; aspartate aminotransferase (*Aat-1* and *Aat-2*) and uridine diphosphoglucose pyrophosphorylase (*Ugpp*) were resolved on buffer system 10; phosphoglucomutase (*Pgm-1* and *Pgm-2*) and malate dehydrogenase (*Mdh*) were resolved on buffer system 11; and cathodal peroxidase (*Cper*) and fluorescent esterase (*Fe*) were resolved on buffer system 6. Buffer numbers refer to those in table 2 in Soltis et al. (1983).

Inference of parental genotypes—Progeny of 205 fruits from the six maternal study trees were analyzed. Multilocus maternal genotypes for the six study trees were inferred locus by locus by examining the genotypes of all progeny from each tree. Multilocus pollen donor genotypes of the singly sired fruits were then inferred by examining the genotypes of the progeny arrays from each fruit, given the known maternal genotype. Fruits for which unique paternal genotypes could not be inferred, because of either ambiguity from low germination or occasional multiple sirings, were not included in the analysis of paternity patterns.

Probability that the same genotype could occur by chance in a population—In any genotypic array there is some probability that identical genotypes could occur by chance. To evaluate the likelihood that identical genotypes among pollen donors within a sample from a single tree crown could occur by chance (vs. representing the same paternal individual), several statistics based on allelic diversity were calculated. The total number of possible genotypes (N_g) is the product of the number of genotypes possible at each locus:

$$N_g = \prod_{i=1}^{L} [a_i(a_i + 1)]/2,$$

where a_i is the number of alleles at locus i and L is the number of loci analyzed (Cheliak and Pitel, 1984; Parker and Hamrick, 1992). The probability of identity for a single locus (PI_S), assuming Hardy–Weinberg equilibrium, is:

$$PI_S = \sum_i x_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2,$$

where p_i and p_j are the frequencies of the ith and jth alleles (Paetkau and Strobeck, 1994). The overall multilocus exclusion probability (PI_M) is the product of the locus-specific probabilities. The likelihood of randomly drawing an individual with a different multilocus genotype from the focal individual within a sample of N individuals is $(1 - PI_M)^N$ (Parker et al., 1998). The probability of finding at least one individual with the same genotype as the focal individual in a population of size N, therefore, is one minus this quantity.

Effect of flower location on patterns of paternity—The chance that fruits within an inflorescence share common pollen donors is heavily dependent upon

the number of fruits within the inflorescence. Because sample sizes per inflorescence were low and chances of repeat observations of pollen donors within inflorescences were uneven, we could not use simple proportions or chi-square analyses to address this question. Instead, we developed a simulation analysis to randomize pollen donor genotypes over all sampled flower positions to determine how often the empirical pattern would be observed by chance. If it was rare to observe the empirical or a more extreme value, the empirical observation was not likely to occur by chance, suggesting some biological driving factor (i.e., floral position).

The statistic of interest was the proportion of pollen donor genotypes occurring once within an inflorescence (proportion of singlets). A low proportion of singlets is thus equivalent to more repeat pollen donors within inflorescences (doublets, triplets, etc.). The pattern of singlets is heavily dependent upon the number of fruits in each inflorescence and the number of inflorescences within a tree crown. For example, it might be more likely for repeat pollen donors to occur in a tree that has one or more inflorescences with six or eight fruits as opposed to a tree with many inflorescences consisting of only two or three fruits. To simulate pollination for each tree crown, pollen donor genotypes were randomized over all possible flower positions 100 000 times. Recalling a low proportion of singlets is equivalent to more repeat pollen donors within-inflorescences, the *P* value represents the proportion of simulated pollinations for which the proportion of singlets was less than or equal to the empirically observed data value. Because flowers within inflorescences open synchronously but inflorescences within clusters open asynchronously, we chose to analyze this probability only at the inflorescence level. Details of the MATLAB (2002) simulation program can be obtained upon request from the corresponding author.

Differences among isolated and population trees: genetic and genotypic diversity—Treating the collection of pollen donor genotypes for each maternal tree as a pollen pool "population" allowed comparisons among trees using standard genetic and genotypic diversity parameters. Genetic diversity statistics were estimated for each pollen pool population as described in Hedrick (2005) using the computer program LYNSPROG (M. D. Loveless, A. Schnabel, and J. L. Hamrick, University of Georgia, unpublished program). These measures included the percentage of polymorphic loci (*P*), the mean number of alleles per polymorphic locus (AP), the effective number of alleles per locus (A_e), and observed (H_o) and expected (H_e) heterozygosity. Deviations from Hardy–Weinberg expectations for each tree were estimated for each polymorphic locus with Wright's fixation index (Wright, 1922). Fixation indices were tested for significant deviation from expectation using chi-square tests (Li and Horovitz, 1953). The proportion of genetic diversity partitioned among populations was evaluated using Nei's (1973, 1977) genetic diversity statistic, G_{ST}, calculated for each polymorphic locus and averaged across polymorphic loci. Heterogeneity among pollen pools of the six maternal trees was tested using chi-square tests (Workman and Niswander, 1970). Additionally, inflorescences were pooled for each tree, and analysis of molecular variance (AMOVA) was used to partition genetic variance into components within inflorescences, among inflorescences within tree crowns, and among trees (Excoffier et al., 1992; Excoffier, 2001). Sample sizes were too low, however, to estimate the component of genetic diversity among inflorescences within clusters.

Because multilocus, diploid genotypes of pollen donors to each maternal tree crown were available, rather than just pollen pool allele frequencies, additional analyses at the genotype level were performed for each maternal tree. For each tree crown, we present the total number of fruits for which a paternal genotype could be inferred (*N*), the number of distinguishable pollen donor genotypes (*G*), the proportion of distinguishable genotypes (*G*/*N*) (Ellstrand and Roose, 1987), and the proportion of total pollen donor genotypes that were unique (*U*). The effective number of pollen donor genotypes (N_{eg}), was calculated as

$$N_{eg} = 1/\sum g_i^2,$$

where g_i is the frequency of the *i*th pollen donor genotype. Because the number of fruits available for genotypic analysis varied widely (ranging from 15 to 45), the effective number of genotypes was standardized by the sample size (N_{eg}/N) for comparisons among trees. Genotypic diversity measured by Simpson's index (*D*), adjusted for finite sample size (Pielou, 1969; Peet, 1974), was estimated as

$$D = 1 - \sum([n_i(n_i - 1)]/[N(N - 1)]),$$

where n_i is the number of individuals of genotype *i* and *N* is the total sample size. The null hypothesis that two pollen pools come from assemblages with the same diversity was tested using the following test statistic:

$$t = (D_1 - D_2)/\sqrt{s_1^2 + s_2^2},$$

where

$$s^2 = 4\left[\sum p_i^3 - \left(\sum p_i^2\right)^2\right]/N,$$

and where $p_i = n_i/N$, or, the proportion of the total number of individuals of genotype *i* (Brower and Zar, 1977). Genotypic diversity was compared among pairs of trees with $t_{\alpha = 0.01,\infty}$ and using a Bonferroni correction for multiple comparisons. (Fager's) evenness (*E*) was estimated by

$$E = N(G + 1)/2 - \sum R_i n_i,$$

where *N* is the total sample size, *G* is the total number of genotypes, R_i is the rank order of genotype *i*, and n_i is the number of individuals of genotype *i* Fager, 1972. For each of the genotypic diversity measures, the means and standard deviations for the population trees ($N = 3$) and the isolated trees ($N = 3$) were calculated, and two sample *t* tests were used to compare means between the two groups of trees.

RESULTS

The average number of inflorescences per cluster was 14.9 and ranged from 11.8 (JML) to 20.8 (KNO). The average number of fruits per cluster was 25.4 and ranged from 13.8 (JML) to 37.6 (ACC). The average number of fruits per inflorescence was 1.7 and ranged from 1.2 (JML) to 2.3 (ACC). Fifty-eight percent of the inflorescences contained a single fruit, 27% contained two fruits, and 15% contained three to seven fruits.

Probability the same genotype could occur by chance in a population—The number of multilocus paternal genotypes possible (N_g) in the pollen pools for the six study trees sampled ranged from 7.086×10^6 to 212.576×10^6 . The probability of two pollen donors sharing the same genotype by chance in the sample of *N* individuals in a pollen pool ranged from 0.001 (AWP) to 0.011 (KNO). It is unlikely that repeat observations of pollen donor genotypes could be drawn by chance from the population samples, suggesting it is reasonable to assume multiple occurrences of a genotype represent a common pollen donor.

Effect of flower location on patterns of paternity—Low germination reduced the number of fruits in some inflorescences to one (Fig. 1). These inflorescences therefore did not allow repeat pollen donors to occur. The number of times distinguishable pollen donor genotypes were observed within a single inflorescence ranged from one to five (Table 1). Results of the simulation analyses indicated the empirically observed values of proportion of singlets were generally very rare and fell well below the simulated values. Of the six trees, differences between observed and expected values in JFR and WLK are highly significant ($P < 0.0001$), in AWP are significant ($P < 0.01$), in ACC are nearly significant ($P = 0.06$), and in KNO and JML are not significant (Fig. 2). These results suggest that neither a low proportion of singlets nor high numbers of repeat pollen donors within inflorescences are likely to occur by chance. Thus flowers within an inflorescence have a higher than random chance of sharing common pollen donors.

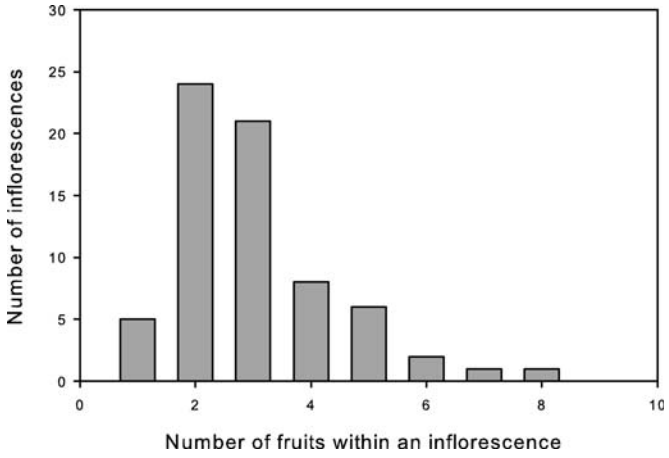

Fig. 1. Summary of the number of inflorescences with a particular number of mature fruits used for the hierarchical analysis. Inflorescences with two or more fruits represent opportunities for repeat pollen donors. Because our study was designed to test for repeat pollen donors within inflorescences, we collected inflorescences with at least two fruits. Poor germination of seeds from some fruits resulted in a few inflorescences with just a single fruit.

Isolated tree JML was unusual because several inflorescences contained just one fruit due to low seed germination, and only one pollen donor genotype occurred more than once. There were overall fewer opportunities for repeated pollen donors within a single inflorescence. In the simulation, both occurrences of the single repeated genotype usually fell in different inflorescences, resulting in all singlets, but occasionally fell within an inflorescence containing two fruits, resulting in the unique frequency distribution of simulations for this tree.

Differences among isolated and population trees: genetic and genotypic diversity—Genetic diversity was high within all six pollen pool populations (Table 2). Significant deviations from Hardy–Weinberg were found for 10 of 84 tests; eight were negative and two were positive. These locus-specific deviations, as well as the overall fixation index, $F_{IS} = -0.157$ (Wright, 1965), indicate a slight excess of heterozygotes.

Low levels of differentiation were found among the six pollen pool populations ($G_{ST} = 0.047$). Levels of heterozygosity and genetic differentiation did not differ when population and isolated trees were analyzed separately (Table 2). Significant allele frequency heterogeneity ($P < 0.05$) occurred among pollen pools of the maternal trees for 10 of the 14 loci. Loci that did not have significant heterogeneity tended to have low genetic diversity, necessarily resulting in little allele frequency heterogeneity among populations. Analysis of molecular variance indicated that most genetic diversity (92.0%) was partitioned within inflorescences. Only 6.0% and 2.0% of the genetic diversity was partitioned among inflorescences within trees and among trees.

The number of fruits from the six study trees ranged from 15 to 45, for which 14–38 distinguishable pollen donor genotypes were recorded (Table 3). The effective number of pollen donor genotypes varied from 13.2 to 33.2, indicating some pollen donors were represented more than once in the pollen pools. There were no significant differences in means among population and isolated trees for any of the measures of genotypic diversity (*t* tests, see Table 3 for *P* values), but there was generally more variability among isolated trees than among population trees for *N*, *G*/*N*, *U*, N_{eg}/N, *D*, and *E*, and isolated trees tended to have slightly lower values for several of these measures.

TABLE 1. Summary of the number of times pollen donor genotypes were empirically observed within single inflorescences, presented for each of the six *Albizia julibrissin* study trees in Athens, Georgia. ACC, AWP, and KNO refer to trees in populations; WLK, JFR, and JML refer to isolated trees.

No. observations within a single inflorescence	No. pollen donor genotypes					
	ACC	AWP	KNO	WLK	JFR	JML
1	39	24	40	16	25	13
2	3	2	–	4	5	1
3	–	—	1	2	—	—
4	—	—	—	—	1	—
5	—	1	—	—	—	—

DISCUSSION

Our results demonstrate that patterns of mating are heterogeneous within individual trees of *A. julibrissin*. Many inflorescences contain fruits pollinated by several donors, but where there is redundancy of pollen donors, it is more likely to occur within inflorescences than randomly across the crown. Furthermore, our results show that all trees receive pollen from diverse pollen pools. Results suggest a trend for isolated trees to receive pollen from somewhat fewer donors and experience more within-plant pollinator movement than population trees. However, seed pools of isolated trees are qualitatively similar to those of trees growing in populations; they, too, receive pollen from many sources.

It is clear that some pollen donors contribute to multiple pollination events within the same maternal tree, evidenced by the result that the effective number of genotypes was consistently smaller than the total number of genotypes per crown. Simulation analyses showed a consistent trend, that was in the same direction for all trees and was significant for four trees, for fruits within the same inflorescence to have a much greater than random chance of sharing a common pollen donor. These results are consistent with the flowering phenology of this species. Within an inflorescence, flowers open synchronously and usually last about a day. It is likely that pollinators foraging within an inflorescence deposit multiple polyads from the same pollen donor on several stigmas within the inflorescence. A few other researchers have documented variation in outcrossing rates and genotypic composition among neighboring individuals and within individuals of animal-pollinated species. Individual heads within single maternal plants of *Carduus nutans* L. have different numbers of outcrossed progeny, suggesting that animal pollinators distribute pollen in nonrandom patterns (Smyth and Hamrick, 1984). Different levels of genetic diversity, structuring, and/or inbreeding have been reported for ray vs. disc flowers in the herb *Prionopsis ciliata* (Nutt.) Nutt. (Gibson, 2001) but not for the herb *Heterotheca subaxillaris* (Lam.) (Gibson and Tomlinson, 2002). Heterogeneity of pollen pools among individuals within populations has been observed in animal-

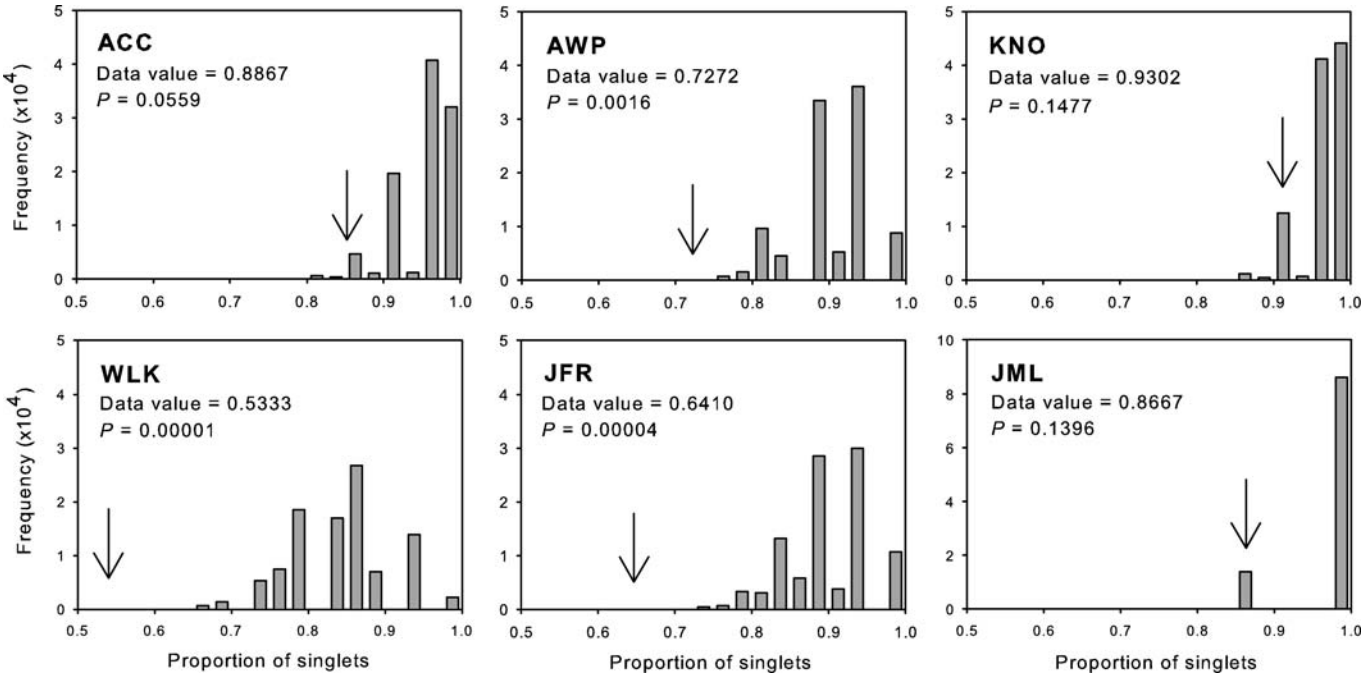

Fig. 2. Frequency histograms of the simulation analyses for each of the six maternal trees. Population trees (ACC, AWP, and KNO) are presented in the top three panels; isolated trees (WLK, JFR, and JML) are presented in the bottom three panels. The null distribution of the proportion of singlets for 100 000 simulations of pollen donor genotypes randomized over all sampled flower positions is shown. The data value is the empirically observed proportion of pollen donors that occurred once within an inflorescence (proportion of singlets) and is indicated by an arrow. The *P* value represents the proportion of simulations for which the proportion of singlets was less than or equal to the observed data value just by chance.

pollinated, tropical trees (Murawski and Hamrick, 1991; Boshier et al., 1995). An uneven contribution of many pollen donors to individual trees was found in *Enterolobium cyclocarpum* (Jacq.) Griseb.: in samples of 15 fruits per maternal tree most pollen donors sired a single fruit, but a few sired two or three fruits (Apsit et al., 2001). The probability of a common pollen source for a pair of fruit pods was higher within clusters and inflorescences than across the tree at random in the mimosaceous tree, *Acacia melanoxylon* R. Br. ex Ait. f. (Muona et al., 1991). Our study is unique in that singly sired fruits and our hierarchical sampling scheme allowed documentation not just of allele frequencies, but of diploid pollen donor genotypes.

Our results show that the vast majority of fruits have different pollen donors, but where redundancy of pollen donors occurs, it tends to be within the same inflorescence. Such

TABLE 2. Summary of genetic diversity within and among pollen pools of the six *Albizia julibrissin* study trees in Athens, Georgia. Genetic diversity was averaged and pooled across population trees, isolated trees, and all trees; pooling across all trees represents species level diversity within the region sampled. Standard deviations are indicated in parentheses.

Tree, by group	*P*	AP	*A*	A_e	H_o	H_e	G_{ST}
Population							
ACC	100	2.62	2.62	1.65	0.407 (−0.057)	0.326 (−0.062)	
AWP	85.7	2.42	2.21	1.69	0.409 (−0.070)	0.331 (−0.060)	
KNO	92.9	2.46	2.36	1.54	0.281 (−0.055)	0.269 (−0.064)	
Pooled	100	2.64	2.64	1.68	—	0.322	0.044
Mean (SD)	92.7 (3.9)	2.5 (0.1)	2.4 (0.2)	1.6 (0.1)	0.365 (0.035)	0.309 (0.036)	—
Isolated							
WLK	100	2.38	2.38	1.70	0.426 (−0.060)	0.304 (−0.072)	
JFR	92.9	2.38	2.29	1.55	0.320 (−0.062)	0.275 (−0.059)	
JML	92.3	2.25	2.15	1.65	0.389 (−0.103)	0.313 (−0.061)	
Pooled	100	2.43	2.43	1.68	—	0.317	0.036
Mean (SD)	95.1 (3.4)	2.3 (0.1)	2.3(0.1)	1.6 (0.1)	0.378 (0.045)	0.297 (0.037)	—
All trees							
Pooled	100	2.64	2.64	1.68	—	0.321	0.047
Mean (SD)	94.0 (2.6)	2.4 (0.1)	2.3 (0.2)	1.6 (0.1)	0.372 (0.029)	0.303 (0.026)	—

Notes: P = Percentage of polymorphic loci; AP = Mean no. of alleles per polymorphic locus; A_e = Effective number of alleles per locus; H_o = Observed heterozygosity; H_e = Expected heterozygosity; G_{ST} = Proportion of genetic diversity partitioned among populations.

TABLE 3. Summary of genotypic diversity estimated by allozymes of pollen pools of the six *Albizia julibrissin* study trees in Athens, Georgia. Two-sample *t* tests were used to compare the population trees and the isolated trees; *P* values are given at the bottom of table.

Tree, by group	*N*	*G*	*G*/*N*	*U*	N_{eg}	N_{eg}/N	*D*	*E*
Population								
ACC	45	38	0.84	0.71	33.2	0.74	0.992	114.5
AWP	33	27	0.82	0.73	19.1	0.60	0.997	75
KNO	43	38	0.88	0.81	32.4	0.75	0.992	88.5
Mean (SD)	40.3 (6.4)	34.3 (6.4)	0.85 (0.03)	0.75 (0.05)	28.2 (7.9)	0.69 (0.10)	0.987 (0.009)	92.7 (20.1)
Isolated								
WLK	30	21	0.70	0.53	13.2	0.44	0.956	80
JFR	39	29	0.74	0.56	22.7	0.58	0.981	118
JML	15	14	0.93	0.87	13.2	0.88	0.990	6.5
Mean (SD)	28.0 (12.1)	21.3 (7.5)	0.79 (0.12)	0.66 (0.18)	16.4 (5.5)	0.64 (0.23)	0.976 (0.018)	68.2 (56.7)
All trees								
Mean (SD)	34.2 (11.0)	27.8 (9.5)	0.82 (0.09)	0.70 (0.13)	22.3 (8.9)	0.66 (0.16)	0.981 (0.014)	80.4 (40.3)
P value	0.195	0.084	0.489	0.436	0.374	0.436	0.519	0.1

Notes: N = No. fruits (pollen donors); G = No. distinguishable pollen donor genotypes; G/N = Proportion of distinguishable pollen donor genotypes per crown; U = Proportion of pollen donor genotypes represented once in a crown; N_{eg} = Effective no. pollen donor genotypes; N_{eg}/N = Effective no. pollen donor genotypes per crown; D = Shannon's genotypic diversity; E = Genotypic evenness.

correlated paternity within inflorescences and among progeny within fruits could lead to genetic structuring of individual seed pools in this species. Full-sibling progeny are usually dispersed within fruits, and if fruits from the same inflorescence share common pollen donors and tend to disperse locally, preexisting genetic structure could be transmitted to the next generation. There may be high levels of relatedness among near neighbors, especially in small or actively colonizing populations. Presumably, long-distance pollination and self-incompatibility prevent inbreeding in populations of *A. julibrissin*, and indeed, no biparental inbreeding has been detected (Godt and Hamrick, 1997). However, additional correlated paternity within inflorescences likely has little effective impact on spatial genetic structure in addition to that of correlated paternity within fruits because this species produces hundreds to thousands of fruits per tree and individual seed pools contain high levels of genetic diversity.

Overall, genetic and genotypic diversity were high in the pollen pools of both isolated and population trees. All pollen pools had similarly high levels of heterozygosity and genotypic diversity indicating that individual trees received pollen from many individuals. There was some heterogeneity in allele frequencies among the six pollen pools, but a very low proportion of the total genetic diversity at polymorphic loci (4.7%) was found among the six pollen pools. These results are similar to a previous observation of 2.5% genetic diversity partitioned among populations within Athens, Georgia (Godt and Hamrick, 1997), but lower than the observation of 17.4% genetic diversity partitioned among maternal trees distributed across Athens, Georgia (Irwin et al., 2003). Significant heterogeneity among pollen pools of maternal trees within populations and regions is common in trees (Gibson and Hamrick, 1991; Muona et al., 1991; Godt and Hamrick, 1997) and is usually attributed to spatial or phenological variation among trees. Inflorescences sampled a large proportion (92.0%) of the total genetic diversity, indicating that within individual tree crowns, inflorescences generally sampled from a diverse pollen pool.

While the number of maternal trees included in our sample is limited, apparently there are some differences in within-crown patterns of paternity between population and isolated trees. There were no significant differences between population and isolated trees for any of the genotypic diversity measures, but there was much higher variability in most of these measures among the isolated trees. Excluding JML (discussed later), there was a trend for isolated tree crowns to experience fewer distinguishable pollen donor genotypes, a lower proportion of unique genotypes, and a lower effective number of pollen donor genotypes. There was a trend for more redundancy of common pollen donors within inflorescences in isolated trees (Table 1). The empirically observed proportion of singlets tended to be lower, and thus common pollen donors within inflorescences higher, in isolated than in population trees (Fig. 2). If isolated trees experience decreased visitation and increased within-plant foraging by animal pollinators, fruits are likely to be sired by fewer pollen donors overall and by common pollen donors more often within inflorescences. Measurements of fruit set and germination rates for an expanded sample of trees coupled with observations of visitation rates and foraging behavior within crowns could address this possibility.

Isolation and density have been shown to affect the reproductive biology of other angiosperm tree species with low densities or fragmented populations. Selfing rates were higher in pasture individuals of several tropical tree species (Aldrich and Hamrick, 1998; Dick, 2001; Dick et al., 2003). Foraging time within crowns increased and fruit set decreased in isolated individuals of *Shorea siamensis* Miq. (Ghazoul et al., 1998; Ghazoul and McLeish, 2001). Solitary or asynchronously flowering individuals of *Pachira quinata* (Jacq.) Alverson (isolated in space or time) had lower outcrossing rates and higher correlations of paternity among progeny, indicating progeny were sired by fewer pollen donors (Fuchs et al., 2003). Contrasting results were observed for isolated individuals of the mimosaceous, tropical tree *Enterolobium cyclocarpum*. Isolated pasture trees had a lower correlation of paternity than population trees, indicating that fruits on isolated trees were sired by relatively more pollen donors than population trees, but seedlings from isolated trees were less vigorous (Rocha and Aguilar, 2001). If pollen limitation reduces selective fruit abortion, fruits pollinated by fathers that would normally be aborted might be allowed to mature but

may have low germination or poor performance. Nonrandom seed abortion has been observed in several leguminous tropical tree species and has been attributed to selective abortion based on identity of paternity (Bawa and Webb, 1984).

Isolated tree JML is a major contributor to variability in genotypic diversity among isolated trees. The small sample size from this tree (15 fruits) and occurrence of only one duplicated pollen donor skewed the genotypic diversity higher, made the distribution of genotypes more even, and increased the effective number of genotypes per sample for JML relative to the other isolated trees. JML was the most isolated tree (≈600 m) and consistently had lower fruit set, seed viability, and germination over multiple years, and died 2 years later (J. L. Hamrick and E. A. Pardini, personal observation). Low fruit set and seed germination may have been due to limited resource availability, low pollinator visitation combined with greater within-plant pollinator movement, lack of selective abortion, and/or senescence.

Our analysis estimated the effective number of pollen donor genotypes (N_{eg}), which is often interpreted as a measure of the effective number of fathers, as is a similar parameter, N_{ep}. Our estimates of N_{eg} for *A. julibrissin* were higher (mean = 22.3) than values of N_{ep} obtained by MLTR mating system analysis (2.1–3.2; J. L. Hamrick, unpublished data) and TwoGener analysis (2.05 in one year to 2.87 over several years; Irwin et al., 2003). In reviewing differences between two general approaches to measuring pollen flow, Smouse and Sork (2004) distinguished between results from TwoGener studies, which suggest smaller effective numbers of pollen donors, and results obtained by parentage analyses, which typically suggest many pollen donors with uneven contributions. Our results were obtained using a kind of paternity analysis, which may be why they indicated many pollen donors with uneven contributions. Furthermore, our results suggest that within-plant variation in allele frequencies may be higher than previously thought. Nonrandom, within-plant mating patterns could represent potentially significant biological noise that is not accounted for in some estimates of the effective number of fathers. Significant variation at the within-plant level should decrease the proportion of total variation attributable to among-plant differentiation, thus leading to higher estimates of the effective number of fathers using methods based on differentiation among maternal pollen pools.

Overall, our results show that *A. julibrissin* individuals receive very diverse pollen from many sources. Heterogeneity among pollen donors and the effect of flower position on the chance of sharing common pollen donors suggests that mating events within plants are correlated. Because correlated mating within plants may influence estimates of pollen dispersal (Irwin et al., 2003; Hardy et al., 2004), it should be considered when developing sampling schemes for genetic analyses. Genetic structuring within individual seed pools and clumping of full-sibling progeny dispersed in singly sired fruits may transmit preexisting genetic structure to the next generation. Routine long-distance pollination, self-incompatibility, and the combination of high genetic diversity of seed pools and exceptionally high seed set in this species probably prevent detrimental effects of inbreeding, but footprints of strong family structure may be evident in colonizing populations. Results of our study suggest subtle differences in the amount of pollinator visitation and within-plant movement experienced by isolated and population trees. However, there are no major qualitative differences in the way these trees receive pollen; isolated trees receive diverse pollen from many sources, suggesting pollen routinely moves long distances in this species. As a consequence, spatially distant trees are not effectively isolated, rather they remain integrated parts of the breeding population.

LITERATURE CITED

ALDRICH, P. R., AND J. L. HAMRICK. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* 281: 103–105.

APSIT, V. J., J. L. HAMRICK, AND J. D. NASON. 2001. Breeding population size of a fragmented population of a Costa Rican dry forest tree species. *Journal of Heredity* 92: 415–420.

BOSHIER, D. H., M. R. CHASE, AND K. S. BAWA. 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 2. Mating system. *American Journal of Botany* 82: 476–483.

BROWER, J. E., AND J. H. ZAR. 1977. Species diversity, field and laboratory methods for general ecology, 2nd ed., 153–160. Wm. C. Brown Publishers, Dubuque, Iowa, USA.

CHAISURISRI, K., J. B. MITTON, AND Y. A. EL-KASSABY. 1994. Variation in the mating system of Sitka spruce (*Picea sitchensis*): evidence for partial assortative mating. *American Journal of Botany* 81: 1410–1415.

CHELIAK, W. M., AND J. A. PITEL. 1984. Electrophoretic identification of clones in trembling aspen. *Canadian Journal of Forest Research* 14: 740–743.

DICK, C. W. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society of London Series, B, Biological Sciences* 268: 2391–2396.

DICK, C. W., G. ETCHELECU, AND F. AUSTERLITZ. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12: 753–764.

DYER, R. J. 2005. GENER: a server-based analysis of pollen pool structure. *Molecular Ecology Notes* 5: 971–973.

DYER, R. J., AND V. L. SORK. 2001. Pollen pool heterogeneity in shortleaf pine, *Pinus echinata* Mill. *Molecular Ecology* 10: 859–866.

EL-KASSABY, Y. A., J. PARKINSON, AND J. B. DEVITT. 1986. The effect of crown segment on the mating system in a Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) seed orchard. *Silvae Genetica* 35: 45–54.

ELIAS, T. S. 1980. The complete trees of North America. Gramercy Publishing, New York, New York, USA.

ELLSTRAND, N. C., AND M. L. ROOSE. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* 74: 123–131.

EXCOFFIER, L. 2001. Analysis of population subdivision. *In* D. J. Balding, M. Bishop, and C. Cannings [eds.], Handbook of statistical genetics, 271–307. John Wiley, Chichester, UK.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

FAGER, E. W. 1972. Diversity: a sampling study. *American Naturalist* 106: 293–310.

FUCHS, E. J., J. A. LOBO, AND M. QUESADA. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conservation Biology* 17: 149–157.

GHAZOUL, J., K. A. LISTON, AND T. J. B. BOYLE. 1998. Disturbance-induced density-dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. *Journal of Ecology* 86: 462–473.

GHAZOUL, J., AND M. MCLEISH. 2001. Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecology* 153: 335–345.

GIBSON, J. P. 2001. Ecological and genetic comparison between ray and disc achene pools of the heteromorphic species *Prionopsis ciliata* (Asteraceae). *International Journal of Plant Sciences* 162: 137–145.

GIBSON, J. P., AND J. L. HAMRICK. 1991. Heterogeneity in pollen allele frequencies among cones, whorls, and trees of table mountain pine (*Pinus pungens*). *American Journal of Botany* 78: 1244–1251.

GIBSON, J. P., AND A. D. TOMLINSON. 2002. Genetic diversity and mating system comparisons between ray and disc achene seed pools of the heterocarpic species *Heterotheca subaxillaris* (Asteraceae). *International Journal of Plant Sciences* 163: 1025–1034.

GODT, M. J. W., AND J. L. HAMRICK. 1997. Estimation of mating system parameters of *Albizia julibrissin* (Fabaceae). *Forest Genetics* 4: 217–221.

HARDY, O. J., S. C. GONZALEZ-MARTINEZ, B. COLAS, H. FREVILLE, A. MIGNOT, AND I. OLIVIERI. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics* 168: 1601–1614.

HEDRICK, P. W. 2005. Genetics of populations. Jones and Bartlett Publishers, Boston, Massachusetts, USA.

IRWIN, A. J., J. L. HAMRICK, M. J. W. GODT, AND P. E. SMOUSE. 2003. A multiyear estimate of the effective pollen donor pool for *Albizia julibrissin*. *Heredity* 90: 187–194.

LI, C. C., AND D. G. HOROVITZ. 1953. Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics* 5: 107–117.

MATLAB. 2002. MATLAB 6.5 (Release 13). Math Works, Natick, Massachusetts, USA.

MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.

MUONA, O., G. F. MORAN, AND J. C. BELL. 1991. Hierarchical patterns of correlated mating in *Acacia melanoxylon*. *Genetics* 127: 619–626.

MURAWSKI, D. A., AND J. L. HAMRICK. 1991. The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* 67: 167–174.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.

NEI, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.

OMI, S. K., AND W. T. ADAMS. 1986. Variation in seed set and proportions of outcrossed progeny with clones, crown position, and top pruning in a Douglas fir seed orchard. *Canadian Journal of Forest Research* 16: 502–507.

PAETKAU, D., AND C. STROBECK. 1994. Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* 3: 489–495.

PARKER, K. C., AND J. L. HAMRICK. 1992. Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schottii*. *American Journal of Botany* 79: 86–96.

PARKER, P. G., A. A. SNOW, M. D. SCHUG, G. C. BOOTON, AND P. A. FUERST. 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* 79: 361–382.

PEET, R. K. 1974. The measurement of species diversity. *Annual Review of Ecology and Systematics* 5: 285–307.

PIELOU, E. C. 1969. An introduction to mathematical ecology. Wiley-Interscience, New York, New York, USA.

ROCHA, O. J., AND G. AGUILAR. 2001. Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. *American Journal of Botany* 88: 1607–1614.

SHEN, H. H., D. RUDIN, AND D. LINDGREN. 1981. Study of the pollination pattern in a Scots pine seed orchard by means of isoenzyme analysis. *Silvae Genetica* 30: 7–15.

SMOUSE, P. E., R. J. DYER, R. D. WESTFALL, AND V. L. SORK. 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. *Evolution* 55: 260–271.

SMOUSE, P. E., AND V. L. SORK. 2004. Measuring pollen flow in forest trees: an exposition of alternative approaches. *Forest Ecology and Management* 197: 21–38.

SMYTH, C. A., AND J. L. HAMRICK. 1984. Variation in estimates of outcrossing in musk thistle populations. *Journal of Heredity* 75: 303–307.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27.

SORK, V. L., P. E. SMOUSE, V. J. APSIT, R. J. DYER, AND R. D. WESTFALL. 2005. A two-generation analysis of pollen pool genetic structure in flowering dogwood, *Cornus florida* (Cornaceae), in the Missouri Ozarks. *American Journal of Botany* 92: 262–271.

WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in Papago. *American Journal of Human Genetics* 22: 24–49.

WRIGHT, S. 1922. Coefficients of inbreeding and relationship. *American Naturalist* 56: 330–338.

WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19: 395–420.