# Hierarchical Patterns of Correlated Mating in *Acacia melanoxylon*

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ABSTRACT

Pollen of acacias is transported by insects as polyads, composite pollen grains. The polyad has enough pollen grains to fertilize all ovules within a flower and hence all seed within a pod may be full sibs. Isozyme markers were used to test this hypothesis in two populations of *Acacia melanoxylon* R.Br. The proportions of fruit pods with multiple paternity detected in two populations were 0.08 and 0.15. The proportions of fullsib pairs within pods estimated by the sibling pair method were 1 and 0.63 for the two populations. Comparison of the diploid paternal genotypes of pods of single paternity showed that the probability of a common pollen source for a pair of pods was high within globular clusters (0.35) or within inflorescences (0.46) but declined to 0.10 or 0.25 within the tree at random. Thus the reproductive system acted to reinforce a hierarchy of paternal correlation within each tree.

BASIC models of plant mating systems assume that successive outcrosses in a progeny array are due to independent samples from the pollen pool. Thus outcrossed progeny of a maternal plant are not likely to be related through the paternal parent. However, correlated matings can give rise to paternally related offspring of a plant (RITLAND 1989). The type and spatial pattern of such correlated paternity in the fruit crop within and between individuals in a plant population depends primarily on the reproductive structures and mating system of the particular species. Procedures for estimating correlated paternity at various hierarchical levels need to be developed. The statistical procedures will depend in part on what components of the mating system or reproductive structures come into play. For instance, acacias appear to be primarily outcrossing (MORAN, MUONA and BELL 1989b) and hence selfing is not likely to be a significant contributing factor to correlated paternity. On the other hand, for animal pollinated plants such as acacias insect vectors may deposit several pollen grains from the most recently visited parent plant, which could result in full sib progenies within a fruit.

SCHOEN and CLEGG (1984, 1986) developed a model for estimating outcrossing rates in cases where the outcross progeny are expected to be full sibs, and applied it to *Ipomea purpurea*. There was some correlation of paternity within offspring (SCHOEN 1985), but the correlation was far from complete. BROWN, GRANT and PULLEN (1987) found some correlation of matings in *Glycine argyrea* with their paired fruit analysis. RITLAND (1989) has developed an alternative model for estimating the proportion of pairs of seeds from, *e.g.*, the same fruit which are full sibs. The results from *Mimulus guttatus* showed that this proportion was about 0.40 for two seeds from the same capsule. Such correlated paternity influences genetic covariances between relatives (see RITLAND 1989). Relatedness of progeny within fruit may also influence patterns of selection and sibling competition between fertilization and fruit maturation, and of sexual allocation (see BELL 1985).

One of the more likely conditions for such correlated matings to occur is when pollen is not distributed as single grains (monads), but as composite units (polyads) with several grains, or as pollinia, with very large numbers of grains. Polyads occur in only seven families of angiosperms (KRESS 1981). Within Mimosaceae, the genus Acacia is well known for its production of polyads (*e.g.* KENRICK and KNOX 1982) the most common number being 16 (see Figure 1). Furthermore, as KENRICK and KNOX (1982) have shown there is a correlation across species of Acacia between the number of ovules within a flower and the number of pollen grains per polyad. The pollen grain number usually exceeds the ovule number slightly. This means that one polyad could suffice for fertilizing all ovules within a flower. When a stigma is pollinated, KNOX and KENRICK (1983) observed that in 80% of the cases only one polyad is present. These observations strongly suggest that in most fruit in Acacia, all seeds within a fruit pod are full sibs with only one pollen source.

Nonrandom pollination between fruit pods may also occur. Acacia flowers within a tree form a hierarchical system. Flowers are grouped into clusters or balls, which often form parts of a complex raceme or inflorescence (see NEW 1984). All flowers within a cluster often open simultaneously, which suggests that within a cluster the probability that the same insect pollinates

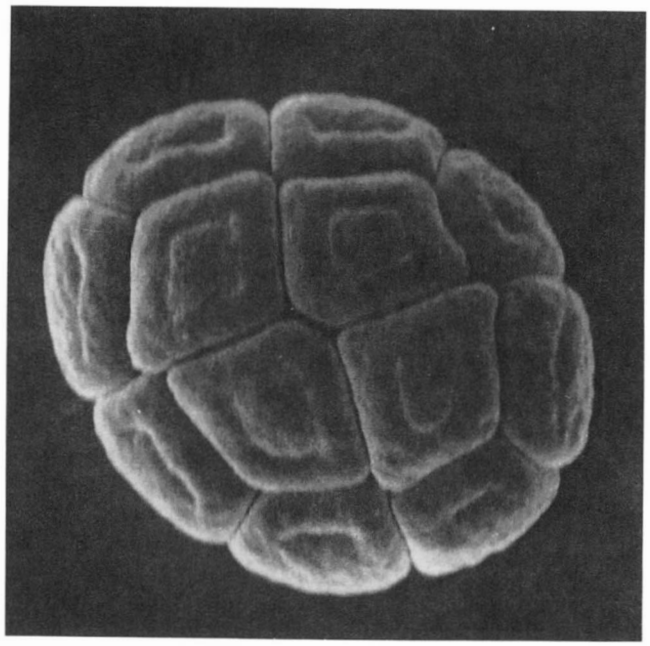

FIGURE 1.—Polyad with 16 pollen grains of *Acacia melanoxylon* (17,000×). (Photograph by MIKE MONCUR, Division of Forestry and Forest Products, CSIRO).

several flowers with polyads from the same tree may be high. For the same reason, the same paternal tree may also have a higher than random probability of donating pollen to flowers on different clusters on the same inflorescence.

The flowering biology of Acacia species suggests a hierarchical spatial pattern of mating. There is a progressively higher likelihood that two seeds share a pollen source as they are sampled from smaller units of the inflorescences. Genetic markers in *Acacia melanoxylon* R.Br. were used first to test the hypothesis that all the seeds within a pod had a single male parent. The extent of paternal correlation was then assessed at different hierarchical levels within the tree.

## MATERIALS AND METHODS

**The species:** *Acacia melanoxylon* R.Br. flowers in New South Wales in the spring (August-October) and the pods develop to maturity in March-April. On the average the flowers have 14 ovules (KENRICK and KNOX 1982), and the polyad consists of 16 grains which derive from two rounds of mitoses preceding meiosis in the sporogenous cell. The fruit pod contains a maximum of 14 seeds, but the average is much less, around six (KENRICK and KNOX 1982). A globose cluster may contain 30–50 flowers, but only a few develop into pods. Single pods from clusters are common and the highest number observed from a single cluster was eight. Each inflorescence consists of a few clusters.

**Populations:** Two populations were chosen, one (Tallaganda) for studying the pollination patterns within pods and between trees, the other (Moss Vale) for more detailed investigations within trees.

*Tallaganda*: The population was situated in Tallaganda shire, New South Wales, Australia (lat. 35° 25′ S, long. 149° 30′ E, alt. 1000 m). *A. melanoxylon* grew in mixed eucalypt species forest on a hillside about 180 m long and 60 m wide around which there was a zone, in which no *A. melanoxylon* occurred. There were altogether 149 stems of *A. melanoxylon* in the area. In March 1986 10–15 pods had been collected randomly around the crown from fifteen of the trees. In all, 129 pods were analyzed, with a mean of 7.3 seeds per pod. Leaves were collected from all trees in the stand in January 1987 for genotyping.

*Moss Vale*: This was a smaller roadside population near Moss Vale, New South Wales (lat. 34° 35′ S, long. 150° 30′ E, alt. 700 m). Eleven trees were sampled with pods collected at two opposite sides of the tree. Each side was represented by a branch containing at least two inflorescences, with several clusters per inflorescence and at least two pods per cluster. In all, 67 pods were analyzed from the six most homozygous maternal trees, with an average of 5.7 seeds per pod.

*Electrophoresis*: Seeds were extracted from the pods and scarified to promote germination (DORAN and GUNN 1987). Extracts were made from 5-day-old seedlings and assayed for enzyme variation using standard methods of starch gel electrophoresis (MORAN and BELL 1983) and assays for: aspartate aminotransferase (AAT, EC 2.6.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), leucine aminopeptidase (LAP, EC 3.4.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), phosphoglucose isomerase (GPI, EC 5.3.1.9) and peroxidase (PER, EC 1.11.1.7) (MORAN, MUONA and BELL 1989b). For the leaf samples homogenates were made by grinding in liquid nitrogen, and extracting in a buffer slightly modified from that of CHELIAK and PITEL (1985).

The genetics of the loci were inferred from the large single tree progeny arrays and by comparison of enzyme systems with other acacias (MORAN, MUONA and BELL 1989a,b). Banding patterns that did not display regular Mendelian segregation were excluded from the analysis. The designation of alleles and loci followed MORAN, MUONA and BELL (1989b). The adult samples of Tallaganda for the loci *Aat2*, *Adh* and *Gdh* are from only the 15 trees where large progenies were analyzed.

## DATA ANALYSIS

**Genetic variability:** Allelic and genotypic frequencies were estimated separately for the Tallaganda adults and seeds. For the adult trees all stems in the stand were included, but some neighbouring stems were clearly members of the same clone, as they shared one multilocus genotype. In the Tallaganda population there was little replication of the diploid genotype among the trees (except for clonal groups).

**Mating system analysis by the sibling pair method:** The sibling pair method of RITLAND (1989) was used to estimate mating system parameters. A pair of sibs can either be both selfed, one outcrossed and one selfed, or both can be outcrossed. If both are outcrossed, they can have the same pollen parent or two different pollen parents. The model estimates the inbreeding coefficients for parents ($F$) and progeny, and the correlation of paternal outcrossed gametes ($f$). The proportion of full sib progeny among the pairs, or correlation of paternal genotypes, is then estimated as $r_p = 2f/(1 + F)$. All single locus estimates of $F$ were small and thus the correlation is determined

by $f$. Two random seeds per fruit pod in both populations were examined for the proportion of fullsibs among pairs of sibs from within fruit pods. The method can be readily extended to examining correlated paternity at other hierarchical levels by sampling the pairs of sibs not within pods but between pods within, *e.g.*, clusters. Each sampling scheme yielded a set of parameter estimates for the model. Results from different loci were combined as minimum-variance estimates over loci. Variance estimates were obtained from 100 bootstraps. The resampling units were pods, clusters, inflorescences, branches or trees for the different levels of estimation. The estimations were made with a computer program provided by KERMIT RITLAND. We obtained estimates of outcrossing rates from this model and also based on the simple mixed mating model, using the method of RITLAND and JAIN (1981). For this analysis, all progeny of a tree were combined which eliminates most of the correlation between progeny.

**Measuring the number of distinct pollen sources per pod with the pollen array method:** The second method for estimating mating parameters followed the general approach for studying correlated matings of BROWN, GRANT and PULLEN (1987). We used information from the total progeny array from each pod. The maternal genotypes of all trees from which pods were collected were determined either directly from leaf samples or the large progeny arrays. The multilocus genotype of the fertilizing pollen grain for each seed was inferred for those loci where the maternal parent was homozygous. For maternal heterozygous loci the pollen genotype was ambiguous, if it did not differ from the two maternal alleles. Then the array of the pollen genotypes of a fruit pod was screened to detect multiple paternity. The following criteria were used.

1. More than two alleles in the pollen pool of a single pod. This event is unambiguous proof of multiple pollen sources but not all such pods will provide this evidence.

2. Multilocus segregation. If one pod contains paternal contributions with two kinds of alleles at several loci, this can be due either to segregation in a heterozygote, or to two (or more) fathers (see BROWN, GRANT and PULLEN 1987). We assume that the loci are not linked. Acacias have $2n = 26$ chromosomes, so close linkage is not likely. Segregation in a multiple heterozygote should thus give rise to all different gametic types in equal proportions, *e.g.*, with two heterozygous loci, to four gametic types. In contrast, two different fathers each homozygous for those loci, would give rise to only two gametic types. All pods were tested for this kind of evidence. The number of loci that the pods segregated for ranged up to eight. If two loci segregated, the pair of loci were examined but if more loci segregated, all possible triplets of loci were screened. The expected frequencies of gametic types for a double (or triple) heterozygote were computed, given independent segregation. The purpose of this test was to check for segregation between loci, not for single locus ratios. Hence single locus allelic frequencies within the fruit pod were used. If the probability of the array given a single father was lower than 0.01, then multiple paternity was inferred.

3. More than eight paternal copies of an allele of a kind at a segregating locus. If there is just one polyad per stigma, there should not be more than eight copies of one allele from a heterozygous pollen source. If more than eight alleles of one kind were found, there must have been two polyads per stigma. However, they could have been from the same pollen source.

**Examining the power of multiple paternity tests:** The power of this test depends critically on the amount of genetic variability in the population. Loci with more than two alleles are especially useful, and homozygous mother trees provide more accurate information than heterozygous ones. It is difficult to gauge the power of the test exactly, because so many factors affect it. Therefore we used Monte Carlo simulations with an artificial data set, where the pods always had two pollen parents in our initial simulations. The probability of detecting multiple paternity was computed by submitting these simulated pods to the multiple paternity tests. For simulating the mating pattern, two paternal trees were chosen at random from the 102 trees with different multilocus genotypes in the population. Both trees contributed equal numbers of pollen grains to the pod in the initial simulations. The necessary number of pollen grains from each of these were formed assuming 1:1 segregation at heterozygous loci and independent segregation between loci. A maternal tree was then chosen from among the original 15 trees from which seeds had been collected. Egg genotypes were formed also assuming normal segregation and independence. Egg cells and pollen grains were joined to form seeds, the number of which varied between three and 14 per pod. From these pods, the genotypes of pollen were inferred with the same method used for the actual pods. The pollen genotype arrays were subjected to our multiple paternity tests. For each pod size, 1000 simulated pods were tested to assess the power of the estimation procedure for our data set.

The simulations were then extended to study the influence of number of potential pollen parents in the population, partial selfing, and fertility selection after pollination. Two pollen parents were chosen for each pod. With partial selfing, the maternal tree was also chosen as a paternal tree with a specified probability, and the other paternal tree was chosen at random. Both trees still contributed about equal numbers of

TABLE 1

**Allelic frequencies, sample sizes (2N) and observed heterozygosities ($H_{obs}$) in the two populations**

| Locus | LS | Allele 1 | 2 | 3 | 4 | 2N | $H_{obs}$ |
|---|---|---|---|---|---|---|---|
| *Aat-1* | MS | 1.00 | | | | 754 | 0.00 |
| | TS | 0.99 | 0.01 | | | 1680 | 0.02 |
| | TA | 0.99 | 0.01 | | | 298 | 0.02 |
| *Aat-2* | MS | 1.00 | | | | 754 | 0.00 |
| | TS | 0.97 | 0.03 | | | 1676 | 0.06 |
| | *TA | 0.97 | 0.03 | | | 30 | 0.07 |
| *Aat-3* | MS | 0.02 | 0.03 | 0.95 | | 752 | 0.09 |
| | TS | 0.03 | 0.33 | 0.64 | | 1758 | 0.50 |
| | TA | | 0.14 | 0.86 | | 282 | 0.16 |
| *Adh* | MS | 0.79 | 0.21 | | | 750 | 0.25 |
| | TS | 0.91 | 0.09 | | | 1674 | 0.16 |
| | *TA | 0.93 | 0.07 | | | 30 | 0.13 |
| *Gdh* | TS | 0.17 | 0.83 | | | 1800 | 0.30 |
| | *TA | 0.20 | 0.80 | | | 30 | 0.27 |
| *Gpi-2* | MS | 0.221 | 0.79 | | | 754 | 0.34 |
| | TS | 0.43 | 0.57 | | | 1860 | 0.53 |
| | TA | 0.40 | 0.60 | | | 298 | 0.49 |
| *Idh* | MS | 0.11 | 0.71 | 0.18 | | 740 | 0.49 |
| | TS | 0.03 | 0.80 | 0.17 | | 1118 | 0.32 |
| | TA | 0.02 | 0.86 | 0.12 | | 294 | 0.24 |
| *Lap* | MS | | 0.27 | 0.41 | 0.32 | 688 | 0.79 |
| | TS | 0.01 | 0.38 | 0.52 | 0.09 | 1736 | 0.56 |
| | TA | 0.01 | 0.23 | 0.66 | 0.10 | 298 | 0.42 |
| *Mdh-2* | MS | 0.46 | 0.54 | | | 750 | 0.33 |
| | TS | 0.43 | 0.56 | 0.01 | | 1694 | 0.59 |
| | TA | 0.51 | 0.49 | | | 298 | 0.58 |
| *Mdh-3* | MS | 0.09 | 0.91 | | | 754 | 0.18 |
| | TS | 0.06 | 0.93 | 0.01 | | 1730 | 0.12 |
| | TA | 0.14 | 0.85 | 0.01 | | 298 | 0.30 |
| *Pgd-1* | MS | 0.16 | 0.84 | | | 628 | 0.28 |
| *Pgd-2* | MS | 0.08 | 0.91 | 0.01 | | 750 | 0.17 |
| | TS | 0.17 | 0.82 | 0.01 | | 1708 | 0.29 |
| | TA | 0.24 | 0.76 | | | 298 | 0.47 |

For life stages (LS) T, Tallaganda, New South Wales; M, Mossvale, New South Wales; S, seeds; A, adult trees. Adult samples for those loci which could not be scored from leaf samples, and where the adult sample is based on the 15 maternal trees are denoted with *A.

pollen grains to the seeds of a pod, so that within a pod half the seeds were selfs and half outcrossed. For examining the influence of fertility selection, two paternal trees were chosen at random, and then the number of seeds within a pod that each fertilized, was allowed to vary from 3 to 14.

**Estimation of correlated paternity at different hierarchical levels with the pollen array method:** The frequency with which two pods had the same pollen parents was determined by examining whether the same pollen parent could have produced the array of pollen grain genotypes within the two pods compared. If the probability that the two pods had the same pollen source was low ($P < 0.05$), it was concluded that two different trees were pollen sources of the pods. For this test, all pods were excluded that demonstrably had two pollen parents. Multiply pollinated pods included in the data set will cause some error in these deductions, but the error should be of the same magnitude at different levels of our comparisons. Two different pollen sources are not always detected. However, the expectation is that there will be successively higher probabilities of identity of pollen sources for the pairs of fruit pods that are sampled from smaller units of the tree. At all levels of comparison the power of the test should be similar.

TABLE 2

**Frequency of multiple paternity in the two populations based on the "pollen array" method**

| Population | N of pods | Multiple | Proportion | Corrected | SD |
|---|---|---|---|---|---|
| Tallaganda | 129 | 19 | 0.15 | 0.33 | 0.07 |
| Moss Vale | 67 | 5 | 0.08 | 0.17 | 0.07 |

SD is the standard deviation within pods.

## RESULTS

**Genetic variability and outcrossing rates:** Table 1 presents the estimates of allelic frequencies for the two populations. In the Tallaganda population the allelic frequencies in adults and seeds were similar at most loci. The RITLAND (1989) sibling pair method estimated outcrossing for Tallaganda as complete (1.0), between 0.86 and 0.96 (SD = 0.07 and SD = 0.04, respectively), depending on the sampling scheme, for Mossvale. Outcrossing rates estimated by the method of RITLAND and JAIN (1981) were 0.86 (SD = 0.01) for Tallaganda and 0.88 (SD = 0.03) for Moss Vale.

**Number of pollen parents per pod:** Among the 129 pods examined in the Tallaganda population, seven were found to have more than one pollen parent

TABLE 3

**Power of the tests to detect multiple paternity in pods from the Tallaganda population**

| N of seeds | N of all | Mend | Seg | Joint |
|---|---|---|---|---|
| 3 | 7 | 0 | 0 | 7 |
| 4 | 12 | 0 | 3 | 15 |
| 5 | 17 | 0 | 15 | 31 |
| 6 | 21 | 0 | 9 | 30 |
| 7 | 22 | 0 | 24 | 46 |
| 8 | 25 | 0 | 23 | 48 |
| 9 | 26 | 0 | 23 | 49 |
| 10 | 27 | 27 | 32 | 71 |
| 11 | 27 | 50 | 37 | 85 |
| 12 | 31 | 59 | 34 | 93 |
| 13 | 27 | 67 | 36 | 95 |
| 14 | 30 | 68 | 37 | 98 |

One thousand pods were simulated. The columns show the percentages of detection of multiple paternity with one of our three tests. N of all = 3 alleles in pollen array, Mend = more than 8 alleles of a kind at a segregating locus, Seg = unlikely segregation for pair or triplet of loci. Joint = combined percentage of detection. If "N of all" was positive, other tests were not made. See text for details.

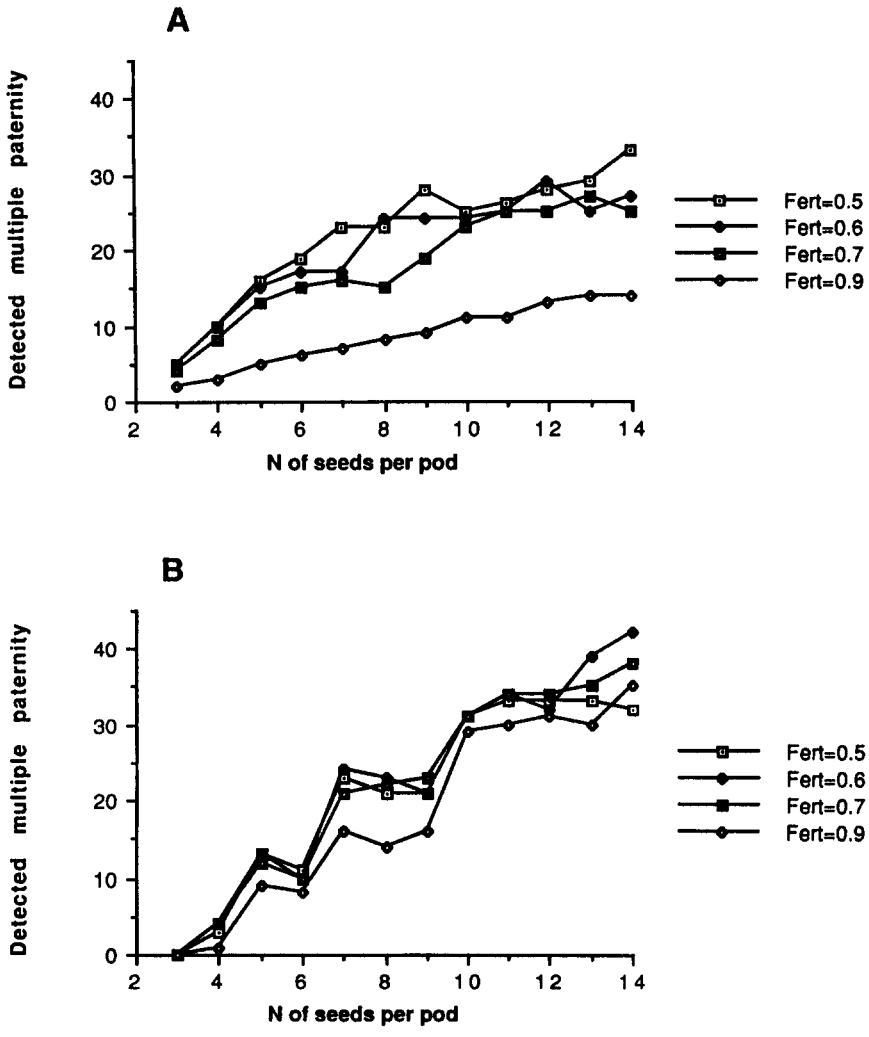


FIGURE 2.—Influence of fertility selection on the power of the multiple paternity tests. A, The number of alleles test; B, segregation test. See text for details.

by the number-of-alleles test. The test for more than eight alleles of a kind at a segregating locus was positive in three cases. The diagnosis of two pollen parents was more likely than one heterozygous pollen parent in nine cases. Altogether 19 pods were considered to be pollinated by two trees (Table 2). In the Moss Vale population, a similar analysis revealed three cases of multiple paternity based on the number-of-alleles test. Segregation tests gave two more positive results among the 67 pods examined (Table 2). These estimates have to be corrected upward, because not all multiply pollinated pods are detected in our analysis.

Table 3 shows the results of simulations on the power of tests. If the number-of-alleles test was positive, further tests were not applied. For large pod sizes, the criterion of more than eight alleles of a kind in a segregating pod and the segregation test were efficient. The final column gives the proportion of pods where at least one test indicated multiple paternity. With average pod sizes of about six to eight seeds, 30–50% of multiple paternity is detected. The probability of detection of multiple paternity for our data set obtained by weighting by the actual pod size distribution, was 0.45.

The power of these tests depends critically on the level of polymorphism. Loci with more than two alleles are especially useful. Our additional simulations showed that the tests distinguished equally well between pollen parents whether the number of pollen sources was very low (15) or high (100). Partial selfing had virtually no influence on the power of the tests (data not shown). The tests were as efficient in distinguishing between polyads from two random outcrossing sources as between polyads from the maternal and a random tree. Fertility selection after pollination, however, had a significant effect (Figure 2). When the proportion of seeds within a pod due to the two pollen sources was not equal, the power of the number of alleles test decreased. The segregation test was influenced to a lesser degree. With large pod sizes, the "more than eight copies of a kind" test was more powerful than without fertility selection. However, larger pods were infrequent in our data set. As no

TABLE 4

**Estimates of correlation of outcrosses, *i.e.*, proportion of full sibs among compared pairs of seeds at different hierarchical levels within a tree**

| Comparison | Tallaganda | Moss Vale |
|---|---|---|
| Within pods | 1.02 (0.07) | 0.63 (0.19) |
| Within cluster | | 0.58 (0.20) |
| Within inflorescence | | 0.43 (0.19) |
| Within branches | | 0.10 (0.14) |
| Within trees | 0.05 (0.05) | 0.03 (0.12) |

Standard deviations are given in parentheses.

information on the occurrence of fertility selection was available, we assume no fertility selection, and use the above detection probability of 0.45.

This resulted in a final estimate of 0.33 multiple paternity for Tallaganda (Table 2). The stringent number-of-alleles test alone yielded a lower estimate of 0.24. The power of the test was examined only in Tallaganda, for which the genotypes of all trees had been determined. The proper correction factor for Moss Vale is not known, so the same factor was assumed. The corrected estimate of multiple paternity for Moss Vale was 0.17.

The analysis by the Ritland method on correlation of outcross paternity within pods is given in Table 4. These estimates are not directly comparable to the estimates of the number-of-pollen parents-per-pod. In acacias many pods are likely to have one father, some have two, but three are unlikely because of the polyad mechanism. Within pods with only one pollen parent, the correlation of outcross paternity should naturally be one. For pods with two pollen parents, the average correlation of paternal genotype between sibling pairs should be 0.5, assuming equal male fertility. An estimate of 0.75 of pods with a single pollen parent would result in a correlation estimate of 0.87. The Tallaganda correlation estimate of 1.02 was high, as the other method provides definite evidence of multiply-pollinated pods. However, the estimates from the Ritland method have large standard errors as noted by RITLAND (1989).

**Correlated paternity between pods at different hierarchical levels:** The results on correlated paternity at different hierarchical levels are given in Tables 4 and 5. The sibling pair method showed that there was a high probability of shared paternal parentage when comparing seeds within clusters (0.58) or within inflorescences (0.43) (Table 5). However, when any two seeds from a branch or within a tree were considered, the probability was much lower (0.05–0.10). The results of the two methods were similar for inflorescences. For Tallaganda, both methods showed that the probability of shared paternity for two random pods within the tree was 0.05 to 0.10. The "pollen array" method also allowed estimation of probability

TABLE 5

**Comparison of pairs of pods for identity of the inferred diploid paternal genotype**

| Comparison | Same father | Total | Proportion of pairs with same father |
|---|---|---|---|
| Tallaganda | | | |
| At random | 195 | 6328 | 0.03 |
| Between trees | 150 | 5911 | 0.03 |
| Within trees | 45 | 417 | 0.11 |
| Moss Vale | | | |
| At random | 178 | 1770 | 0.10 |
| Between trees | 91 | 1421 | 0.06 |
| Within trees | 87 | 349 | 0.25 |
| Within branch | 61 | 230 | 0.27 |
| Within inflorescence | 21 | 46 | 0.46 |
| Within cluster | 10 | 29 | 0.35 |

of identity of pollen parents in pairs of pods from different trees. This was 0.06 in Moss Vale and 0.03 in Tallaganda (Table 5). The probability is much greater that the errors made in the "pollen array" analysis are in not detecting two different pollen sources rather than claiming two pollen parents when there is one. This last result suggests that the test has reasonable power in distinguishing between different paternal genotypes.

## DISCUSSION

**Outcrossing rate:** This study along with a few earlier ones found that acacias are predominantly outcrossing species. The estimates of outcrossing rate for the two populations were between 0.86 and 1.0. Previous studies of acacias in plantations have suggested that both *A. mearnsii* (MOFFETT 1956) and *A. decurrens* (PHILP and SHERRY 1946) are predominantly outcrossing. Recent studies on two tropical acacias also indicated a predominant outcrossing system for *A. auriculiformis* and *A. crassicarpa* (MORAN, MUONA and BELL 1989b). A strong self-incompatibility system has been demonstrated in some species of Acacia (KENRICK, KAUL and WILLIAMS 1986).

**Pollination by polyads:** The "pollen array" results showed that each of the majority of the pods of *A. melanoxylon* has a single pollen parent. The estimates agree reasonably well with the occurrence of multiple polyads on stigmas of *A. retinodes* (KNOX and KENRICK 1983). Clearly the genetic methods used in this study were quite effective in testing whether a single polyad is the sole paternal parent of seeds in a pod.

**Correlated paternity at higher hierarchical levels:** Insect pollination resulted in correlated matings between fruit as well as within fruit. Pods within the same cluster or inflorescence had a high proportion of shared paternity, about 0.40, indicating that a hierarchy of correlated mating paralleled the spatial arrangement of the pair of fruit being compared.

**Polyads and breeding systems:** The polyad mech-

anism gives rise to fruit where offspring have a high degree of relatedness. Several mechanisms have been suggested to account for the evolution of polyads. Polyads have been considered to provide an efficient mechanism for assuring pollination, and in fact species with polyads have low pollen/ovule ratios (CRUDEN 1977). WILLSON (1979) proposed that the evolution of polyads is due to sexual selection. A polyad from one male can fertilize all ovules within a flower and prevent fertilization by other males by blocking the stigmatic surface. KRESS (1981) suggested that kin selection may have given rise to the evolution of polyads, as seeds within an ovary compete for the same resources and higher relatedness may result in reduced competition. UMA SHAANKER, GANESHAIAH and BAWA (1988) proposed that reduced parent-offspring conflict may also have contributed to the evolution of polyads. In *A. melanoxylon*, although most of the flowers are pollinated by a single polyad and the resultant pods consist of offspring which are fullsibs, nevertheless a significant fraction of pods have more than one pollen parent. This departure from fullsib arrays within pods suggests that some form of selection must operate to maintain the polyad system.

The mechanism and distribution of correlated matings differ among species where pollen is spread as single grains, *e.g.*, Ipomea (SCHOEN 1985), Glycine (BROWN, GRANT and PULLEN 1987) and Mimulus (RITLAND 1989). Glycine and Mimulus have significant proportions of selfing, so that the pattern of correlated matings is more complicated than in *A. melanoxylon*, where selfing and correlation of selfing can largely be ignored.

**Estimation methods:** Several methods are available for estimating multiple paternity within sibships. AKIN *et al.* (1984) and WILLIAMS and EVARTS (1989) have developed single locus methods, which would probably be less efficient for this data set than the multilocus approaches. Two different approaches in assessing the correlated matings were employed here. The "pollen array" approach utilizes fully the information in a pod. It will only be useful in populations with high genetic variability. However, it is difficult to estimate the proportion of nondetected multiple paternity and the size of errors this causes in further comparisons. The parametric sibling pair method of RITLAND (1989) could readily be used at different hierarchical levels of comparisons. The pollen array method is useful in that the diploid genotypes of pollen sources may be identified for further studies. Thus, we find that the two approaches complement each other.

**Future studies on fertility selection and migration:** Much recent interest has focused on paternity and migration studies in plant populations (*e.g.*, ELLSTRAND and MARSHALL 1985). It is difficult to identify the paternal genotype with the level of isozyme variability often available, based on the genotype of the pollen grain, except in very small populations (see CHAKRABORTY, MEAGHER and SMOUSE 1988). When a fruit with many progeny is pollinated by a single plant, the diploid genotype can be obtained. In the case of polyads, just 4–5 seeds would suffice for accurate determination, as the sampling is without replacement from a finite pool of 16 gametes. However, fruit with numerous seed are needed to ensure that fruit with multiple paternity can be excluded. The diploid paternal genotypes can be used for efficient study of migration or fertility selection patterns. Genetic markers with more variability than those used in this study would be especially helpful in identifying multiple paternity.

**Implications for plant breeding:** Acacias are becoming important agroforestry species, which are currently entering plant breeding programs (TURNBULL 1987). A part of such programs is determining the mating system and estimating quantitative genetic parameters. Biased estimates of outcrossing rates at or below the individual tree level may result from ignoring the pattern of correlated matings. The correlation structure also influences genetic covariances [see RITLAND (1989) for discussion]. If a pair of progeny are assumed to be half sibs, and they are in fact full sibs, heritabilities would be substantially overestimated. The easiest way to minimize such correlations is to make a bulk seed collection for a tree from as many fruits as possible.

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