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Mating system analysis in a natural population of *Acacia nilotica* subspecies *leiocarpa*

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Abstract The mating-system was investigated in a natural population of the tetraploid taxon *Acacia nilotica* ssp. *leiocarpa* using open-pollinated seeds from 15 families. Six-day-old germinated seeds were used for starch-gel electrophoresis. Three enzyme systems (ADH, EST-1, and 6PGD) were scored. Isozyme banding patterns and segregation of isozyme variants within families suggest that the species is an autotetraploid displaying tetrasomic inheritance. Estimates of single-locus (t_s=0.358) and multilocus (t_m=0.384) population outcrossing rates were homogeneous, and indicate substantial selfing in the population. Heterogeneity of outcrossing estimates among loci and families were marked, suggesting departure from the assumptions of the mixed-mating model. Implications of the result for the utilization of germplasm in tree-improvement programmes are noted.

Key words *Acacia nilotica* · Mating-system · Isozyme Autotetraploid · Inbreeding

Introduction

In tree-breeding programmes, economic and logistic considerations often preclude the use of controlled crosses for estimating genetic parameters. Instead the families derived from open-pollinated seeds are commonly used to quantify genetic variances, heritabilities, and potential genetic gain for characters of interest (Namkoong 1966). The assumption is made that families result from random mating, and that inbreeding does not occur at significant levels. Departure from random mating, especially inbreeding, can bias the estimates of genetic parameters. The situation may be further complicated by the occurrence of correlated mating and biparental inbreeding (Shaw and Allard 1982; Ritland 1989; Muona et al. 1991). Thus, a detailed knowledge of the reproductive pattern, which ultimately determines the genetic structure of the progeny generation, is of paramount importance in formulating an efficient tree-breeding programme that makes use of open-pollinated seed collections.

Traditionally, mating-systems have been studied using observations on floral morphology and the behaviour of pollinators, and from the results of controlled crosses. However, information derived from such studies is far from complete and cannot be used for quantitative estimates of mating-system parameters. The use of electrophoretically-detectable markers, and the development of appropriate genetic models have made possible detailed quantitative studies of plant mating systems (Brown et al. 1985). Allozyme markers are particularly useful for such studies since populations are often polymorphic for many enzyme loci, permitting each individual to be scored for several markers. Moreover, allozymes generally show co-dominant inheritance, so that all genotypic classes can be identified without progeny testing.

The past two decades have witnessed a tremendous exploitation of allozyme data for the analysis of plant mating systems. The vast majority of species studied have been diploids, in which the genetic interpretation of isozyme banding pattern is relatively straightforward. In contrast, the quantitative study of mating systems in polyploids has received far less attention (Murawski et al. 1994). This can be attributed to the complexity of the allozyme banding pattern that can be produced in polyploids, difficulties with inferring the inheritance of this variation, and the lack of suitable statistical models for the analysis of mating systems in species that do not show simple disomic inheritance (Quiros 1982; Barrett and Shore 1987; Ness et al. 1989). However, recent interest in the study of polyploids

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and the development of an appropriate methodology for the analysis of mating systems in polyploids (Barrett and Shore 1987; Ritland 1990) has opened the way for quantitative studies of the mating system in previously-intractable, though economically-important, species.

One genus in which these advances are of great potential benefit is *Acacia*. Acacias possess a range of attributes that suit them for roles in both agroforestry and environmental protection (National Academy of Sciences 1979). Consequently a large number of *Acacia* species are now included in international germplasm collections with the intention of laying the foundations for future genetic improvement programmes. Although a number of detailed studies of reproductive biology have been carried out in diploid Australian species, very little is known about the mating systems of their (often polyploid) counterparts in Africa and Asia (Moran et al. 1989; Muona et al. 1991). In the present paper we report a mating-system analysis in *Acacia nilotica*, a naturally-occurring tetraploid species from Africa, using electrophoretic marker loci. *A. nilotica* is the subject of an ongoing germplasm collection and assessment programme conducted by the Oxford Forestry Institute.

Materials and methods

Study organism

A. nilotica is a naturally-occurring polyploid species that is widely distributed in Africa, Arabia and the Indo-Pakistan subcontinent where it is managed as a multipurpose tree (Ali and Quaiser 1980). The species is very variable, and presently nine subspecies are recognised. Most of these subspecies are tetraploid (2n=4x=52), though higher-ploidy levels (2n=8x=104, and 2n=8x=208) have been recorded in the subspecies *nilotica* and *tomentosa* (Nongonierma 1976). This study involves the subspecies *leiocarpa* whose distribution is restricted to Arabia and East Africa, where it occurs in Kenya, Somalia, Ethiopia and Tanzania.

A. nilotica produces showy, bright-yellow flowers that are pollinated by bees of the families Megachilida and Anthophoridae (Tybirk 1989). Pollen is dispersed as polyads, which generally contain 16 pollen grains. This offers the potential for full-sib mating within pods. Another important aspect of the reproductive biology is that the species is andromonoecious, with on average, only about 30% of flowers per tree, being hermaphrodite. The remaining fraction of flowers produce abundant pollen but have no female function. Flowering may occur a number of times each year, depending upon water availability. Although profuse flowering occurs, most flowers abort and pod set is low. The degree of self-incompatibility within the species is not known, though self-incompatibility is found in varying degrees within other members of the genus (Kenrick and Knox 1989).

Seed and seed collection site

Open-pollinated seeds from 25 individual trees of *A. nilotica* ssp. *leiocarpa* were obtained from the collection maintained by the Oxford Forestry Institute. Seeds had been collected by the Institute during January/February 1990 from the Sabaki area near Malindi in the Coast Province of Kenya (3°11′S, 40°07′E and altitude 20 m). Here the population of trees is spread over an extensive area measuring 7 km×4 km. In some places the trees are found as scattered individuals that have been deliberately retained in farmers fields, while elsewhere it occurs as thickets in overgrazed areas and near cattle trails. The seed analysed from each tree comprised the contents of a number of pods that had been bulked.

Electrophoresis

Electrophoretic analysis was carried out using open-pollinated seeds from 15 randomly-chosen trees. Approximately 40 seeds from each family were analysed, making a total of 580 seeds assayed in the study. Large numbers of seed were assayed per tree in order to allow the maternal genotype of each tree to be inferred from the progeny genotype array with reasonable precision. Seeds were clipped to allow them to imbibe water, and were subsequently germinated on Petri dishes for a period of 1 week. Seed tissues were ground over ice in extraction buffer and absorbed onto filter-paper wicks. Samples were subjected to electrophoresis on 11% horizontal starch gels for 5 h. Three enzyme systems, alcohol dehydrogenase (ADH), esterase (EST), and six-phosphogluconate dehydrogenase (6PGD), were assayed. The buffers used were 0.0045 M DL-histidine HCl, 0.0037 M NaOH, pH 6.5 (gel), 0.041 M sodium citrate, 0.0003 M citric acid, pH 6.5 (electrode) for EST, and 0.0052 M DL-histidine HCl, 0.042 M Tris, 0.0029 M $LiOH.H_2O$, 0.022 M H_3BO_3, pH 8.2 (gel), 0.029 M $LiOH.H_2O$, 0.192 M H_3BO_3, pH 8.1 (electrode) for ADH and 6PGDH.

For each of the enzymes, one clearly-staining, polymorphic system was scored. The three loci were designated Adh, Est-1, and 6Pgd. As well as recording the position of bands on the electrophoresis gel, the relative staining intensities of the enzyme bands were noted. This was essential for assigning putative genotypes to the progeny that had been scored in this tetraploid species.

Mating-system analysis

Mating-system analysis was carried out on the assumption that the tetraploid *A. nilotica* displays tetrasomic inheritance i.e., there is no preferential pairing among the four homologous chromosomes at meiosis. Such a mode of inheritance is expected if *A. nilotica* has an autotetraploid, rather than an allotetraploid, origin. The assumption of tetrasomic inheritance is consistent with the lack of fixed heterozygotes and the variation in gene dosage levels, detected at isozyme loci in the study. It should be noted, however, that confirmation of this mode of inheritance can only be derived from formal genetic analysis which was not performed in this investigation.

Quantitative analysis of the mating system made use of the MLTET programme (Ritland 1990; Murawski et al. 1994), specifically written for the analysis of mating systems in autotetraploids. Data on putative progeny-genotype arrays from the 15 parent trees were used both to infer maternal genotypes, and to jointly estimate pollen allele frequencies and outcrossing parameters. Estimates of single-locus outcrossing rate (t_s), multilocus outcrossing (t_m), and multilocus family estimates of outcrossing rate (t_{mi}), were determined. The method makes the normal assumptions of the mixed-mating model, but the programme is modified to account for tetrasomic inheritance. Variances of estimates were obtained by conducting 200 bootstraps. Due to the low number of families assayed (15), bootstrap resampling was performed within families.

Results

The three enzyme systems studied each showed a wide variety of banding phenotypes (Fig. 1). These were distinguished not only by the position of bands, but also by the relative staining intensity of the bands. Differences in staining intensity were interpreted as differences in allelic dosage in this tetraploid species, where, within a single individual, a particular allele may be present in from one to

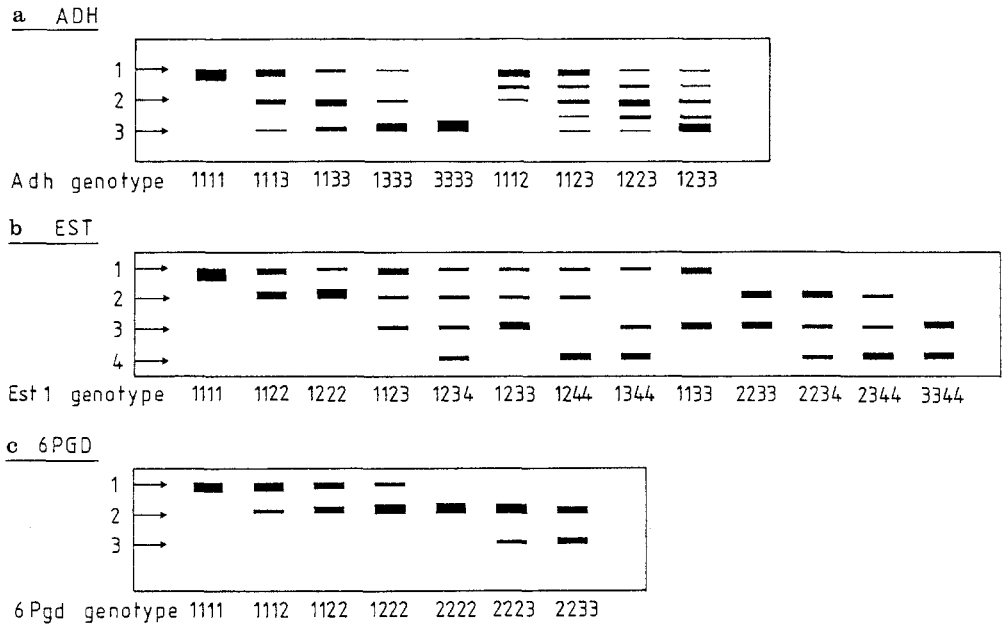

Fig. 1 Observed variation in isozyme banding patterns of *A. nilotica* ssp. *leiocarpa* for alcohol dehydrogenase (ADH), esterase (EST), and 6 phosphogluconate dehydrogenase (6PGD). Also shown are the inferred genotypes at the Adh, Est1 and 6Pgd loci

four copies. Also shown in Fig. 1 are the putative genotypes associated with each banding pattern for Adh (dimeric enzyme with three alleles), Est-1 (monomeric enzyme with four alleles), and 6Pgd (monomeric enzyme with three alleles). All enzyme systems showed extensive segregation for putative heterozygous and homozygous phenotypes, with no evidence of 'fixed' heterozygosity. In addition it was possible to distinguish both 'balanced' and 'unbalanced' heterozygotes, differing in the intensity of their band staining. This electrophoretic evidence suggests, but does not prove, that *A. nilotica* ssp. *leiocarpa* is unlikely to be an allopolyploid, showing digenic-disomic inheritance. In such a case a high proportion of 'fixed' and 'balanced' heterozygotes would be expected. Moreover, the number of alleles per locus would be restricted to two in a recent allopolyploid derived by hybridisation and chromosome doubling. For these reasons, further analyses of the data were carried out on the assumption that *A. nilotica* is an autotetraploid displaying tetrasomic inheritance.

Table 1 shows single-locus outcrossing estimates (t_s) and their standard errors for the Adh, Est-1, and 6Pgd loci, the minimum variance mean single-locus outcrossing rate (t_s), and the multilocus outcrossing estimate (t_m) that uses information from all three loci. Single-locus outcrossing estimates range from 0.18 for Est-1 to 1.02 for 6Pgd, and are significantly heterogeneous. The mean single-locus outcrossing rate, t_s=0.358, and the multilocus outcrossing rate, t_m=0.384, are very similar, both values being significantly less than one, indicating a substantial degree of selfing in the population. The close correspondence between these values suggests that the low values of t are caused by true selfing rather than biparental inbreeding.

Table 2 shows the estimated multilocus family outcrossing rates (t_{mi}) for the 15 trees in this study. These values were calculated on the assumption that pollen-allele frequencies are the same for each maternal parent. The values obtained run from 0.16 to 1.04 and are statistically significantly heterogeneous. Such a result indicates that the assumptions of the mating system model are violated either because there is true variation in outcrossing rate among trees, or because the allele frequencies in outcross pollen received by different trees are significantly different.

Table 1 Single and multilocus estimates of outcrossing rate in *Acacia nilotica* ssp. *leiocarpa*

Locus	Outcrossing rate (t)	SE (t)
Adh	0.327	0.333
Est-1	0.180	0.018
6Pgd	1.017	0.076
Mean single locus	0.358	0.074
Multilocus	0.384	0.080

Table 2 Multilocus outcrossing rates for 15 families of *Acacia nilotica* ssp. *leiocarpa*

Family	Outcrossing rate (t_{mi})	SE (t_{mi})
1	0.16	0.08
2	0.22	0.08
3	0.18	0.12
4	0.39	0.12
5	0.67	0.19
6	0.73	0.18
7	0.17	0.08
8	0.80	0.12
9	0.39	0.13
10	0.90	0.22
11	0.44	0.12
12	0.25	0.12
13	1.04	0.22
14	0.20	0.09
15	0.41	0.19

Discussion

Although polyploids comprise a large fraction of the world's flora, very few quantitative studies of mating systems have been performed on these species in comparison with their diploid counterparts (Barrett and Shore 1987; Murawski et al. 1994). This is largely a reflection of the technical difficulties associated with the analysis of polyploids, difficulties that are well illustrated in the present study. The first problem is in finding suitable genetic markers. Isozyme banding patterns in polyploids are often extremely complex due to the multiplicity of alleles that may be found in a single individual, making genetic interpretation of the variation difficult (Roose and Gottleib 1976; Soltis and Rieseberg 1986; Ness et al. 1989). Moreover, systems must be found in which differences in allelic dosage can be scored. Alleles may be present in from one to four copies, and this number must be known in order to assign a genotype to an individual. Although clear banding patterns were found for other enzyme stains, difficulties with interpretation precluded their use in the present mating-system analysis.

The second major difficulty with analysing polyploids is in knowing with confidence the mode of inheritance of genetic variants. Allopolyploids are often considered to display regular disomic inheritance, while autopolyploids exhibit polysomic inheritance patterns. However, the origin of many polyploids is not known. Moreover, these general rules may not always apply, especially in cases where hybridisation takes place between very-closely related species, or where pairing behaviour of chromosomes is modified subsequent to the formation of the polyploid (Soltis and Rieseberg 1986). The only certain way of finding the mode of inheritance is by conducting formal genetic crosses (Quiros 1982; Soltis and Soltis 1988, 1989). In the present case the polyploid origin of tetraploid *A. nilotica* ssp. *leiocarpa* is not known, and controlled crosses were not available. Analysis has been conducted on the assumption that the population is showing tetrasomic inheritance of isozyme variants. This assumption is based on the argument that if *A. nilotica* ssp. *leiocarpa* was an allotetraploid, formed by hybridisation and chromosome doubling, 'fixed' heterozygosity would be detected, and the frequency of segregating allelic variants would be low. Isozyme analysis, however, revealed no 'fixed' heterozygosity, and at the loci scored either three or four alleles were segregating at substantial frequencies in the population.

The final difficulty with analysing the mating system in polyploids concerns the application of models for outcrossing estimation (Ritland 1990; Murawski et al. 1994). These models require substantially more data than do similar models for diploids. This is especially true where maternal genotypes are unknown, and large numbers of progeny need to be scored to allow these genotypes to be inferred with precision. Moreover, data from heterozygous maternal parents yields relatively-little information on outcrossing rates. This is true for Adh in the *A. nilotica* population studied where the two common alleles are present at roughly equal frequencies. Data from a number of polymorphic loci may, therefore, be essential to obtain outcrossing estimates with low standard errors. Finally the models themselves are necessarily complex, involving far more parameters than diploid models, so reducing the strength of the conclusions that can be drawn from them.

Acknowledging these difficulties and shortcomings, the present study gives a best estimate of the outcrossing rate of *A. nilotica* ssp. *leiocarpa* as t_m=0.384. This is highly significantly less than one, the figure expected under random outcrossing. The multilocus estimate is very little different from the mean single-locus estimate, showing that biparental inbreeding is unlikely to be important in the population (Shaw et al. 1981). These figures suggest that *A. nilotica* ssp. *leiocarpa* is self-compatible, and that approximately 60% of seeds are set through self pollination. These results are comparable to those obtained for another African tetraploid species, *A. tortilis*, with an estimated mean outcrossing rate, over ten populations, of 0.35 (Olng'otie 1991). They differ substantially, however, from estimates for diploid *Acacia* species from Australia which range from 0.84 to 0.99, very close to random mating (Philp and Sherry 1946; Moffett 1956; Moran et al. 1989; Muona et al. 1991).

A number of factors may be important in accounting for these differences. The first is the degree of self-compatibility exhibited by the species. Studies using controlled crosses have shown that there is a complete spectrum of compatibility among *Acacia* species in Australia (Kendrick and Knox 1989). Higher levels of outcrossing may therefore be associated with a greater degree of self-incompatibility. An alternative is that the species are uniformly self-compatible, but differ in the degree of pollen dispersal among individuals. In populations of *A. nilotica* ssp. *leiocarpa*, where the density of trees is low and the time of flowering among individuals may vary, limited pollen dispersal between trees could limit the extent to which outcrossing occurs. Controlled pollination and observations of pollination in the field would be needed to distinguish between these hypotheses.

The possibility of restricted pollen dispersal among trees within the *A. nilotica* population is supported by the fact that single-locus outcrossing rates are significantly heterogeneous over loci. Such a result would be expected if, through restricted pollen dispersal, the allele frequencies in outcrossed pollen received by different trees varied (Ennos and Clegg 1982; Brown et al. 1985). Whatever its cause, this heterogeneity in single-locus outcrossing rates indicates significant departures of the population from the assumptions of the mixed-mating model. Another indication of departures from model assumptions is the significant variation in outcrossing rates found among families. These range from 0.16 to 1.04. Possible reasons for heterogeneity are restricted and non-random outcross pollen dispersal, as well as true differences in outcrossing rate among trees (Brown 1985).

Although there are undoubtedly some difficulties in applying mating-system analysis to this population of *A. nilotica*, there are clear indications of a significant degree of

self fertilisation ocurring in the field. This has important implications for the use of these open-pollinated progenies in the estimation of genetic parameters for practical tree-breeding programmes. The most important of these is that among-family variance for quantitative characters will be greater than expected under random mating in a tetraploid (Bever and Felber 1992). Unless this is accounted for, heritability will be overestimated from the analysis of open-pollinated progeny trials. Future research in such tree-breeding programmes needs to be directed at clarifying such issues as the degree of self-compatibility within populations, confirming the proposed mode of inheritance of genetic variation, and assessing factors controlling the extent of outcrossing in this species. Such information is an essential foundation for future genetic improvement of the species.

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