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Vagile but inbred: patterns of inbreeding and the genetic structure within populations of the monsoon rain forest tree *Syzygium nervosum* (Myrtaceae) in northern Australia

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ABSTRACT. Patterns of gene flow within 21 northern Australian populations of *Syzygium nervosum* a dominant, mass-flowering, monsoon rain forest canopy tree were investigated using 10 isozyme loci. *S.nervosum* was found to have relatively high genetic diversity within populations (He = 0.307, AP = 3.7, P = 65) but also to have significantly lower frequencies of heterozygotes than expected (Ho = 0.126) and high allelic fixation (F = 0.512). Heterozygosity and allelic fixation were not correlated with measures of genetic diversity within populations, nor were they correlated with rain forest patch size, plant size or population isolation. Within populations, trees, of the same genotype (at each loci tested) were significantly clumped at short distances (*c.* 20 m), whereas trees of unlike genotypes were negatively associated. *S. nervosum* trees however, were not clonal in origin and had unique multilocus genotypes. The results suggest that the high levels of homozygosity recorded are the result of restricted pollination, primarily among flowers within individual trees or among closely related neighbouring trees, rather than rectricted seed dispersal. High homozygosity, the large fruit crop produced by trees of this species and the lack of association between heterozygosity and plant size, indicate that, *S. nervosum* is self-compatible, its fecundity does not appear to be impaired by inbreeding depression.

KEY WORDS: fragmentation, genetic structure, inbreeding, Myrtaceae, pollination, population size, rain forest, spatial autocorrelation, vagility

INTRODUCTION

Genetic studies in rain forest trees have to date primarily concentrated on investigations of breeding systems (Hall *et al*. 1994, Murawski *et al.* 1994,

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O'Malley *et al*. 1988). These studies have predominately indicated that rain forest trees are largely outcrossed, this state being facilitated by self-incompatibility mechanisms (Bawa 1992). It has been proposed that as populations become smaller and more isolated due to habit fragmentation there will be generally greater amounts of effective inbreeding resulting in higher homozygosity (Nei *et al*. 1975). It is also thought that genetic diversity, population size, dispersability and outcrossing are typically correlated (Brown 1989, Ellstrand & Elam 1993). While many theories of population genetics assume random mating within populations, when within-population spatial genetic structure has been studied in plants it has often revealed non-random genetic distributions and evidence of localised family structures, even in species with well dispersed propagules (Epperson 1989). Thus, particularly in self-compatible species in which pollination is facilitated by insects or vertebrates, animal foraging behaviour may ultimately determine the amount of gene flow among individuals within a population.

The present study was conducted as part of a larger investigation into gene flow and rain forest plant genetic diversity among monsoon rain forest patches in the Northern Territory Australia (Shapcott 1998a). Its aims were to: (1) quantify and compare the amount inbreeding/outcrossing in populations of a common rain forest tree across its geographic distribution in Australia, particularly in relation to patch size and isolation as well as to its population genetic diversity; (2) investigate genetic structure among individuals within populations to examine gene flow within populations. In a related paper Shapcott (1998b) investigated patterns of genetic diversity in *Syzygium nervosum*.

STUDY AREA AND SPECIES

In Northern Australia where a strongly seasonal monsoon climate prevails, rain forest is restricted to small scattered patches within a largely flat, eucalypt-dominated savanna landscape (Russell-Smith 1991). While these patches have been interpreted as the fragments of once widespread rain forest vegetation which retracted during the Pleistocene (Trusswell 1990), recent studies determined that many patches have established within the Holocene (Bowman 1992, Fensham 1996, Russell-Smith & Dunlop 1987). Small population sizes are likely to have been typical for many rain forest plant species during this time period. The density of rain forest patches, hence size of the meta-population, declines along a decreasing precipitation gradient from north to south and from west to east (Bowman *et al*. 1991, Russell-Smith 1991).

The Myrtaceae is most notable in Australia for the genus *Eucalyptus* which often dominates the Australian landscape. The Myrtaceae are also an important component of the rain forest vascular plant flora and the genus *Syzygium* has many species in Australia that are largely associated with rain forest or riparian forest habitats (Hyland & Wiffin 1993). In the Northern Territory there are five species of *Syzygium* associated with rain forest or mesic riparian

habitats and several frequently co-occur (Liddle *et al*. 1995). *Syzygium nervosum* is one of the most common rain forest species and is widespread in rain forests across the Northern Territory (Liddle *et al*. 1995, Russell-Smith 1991). Within Australia it is limited to the Northern Territory (Figure 1), but its distribution extends into SE Asia. It occurs in permanently moist monsoon rain forest, associated with basic to neutral, fine textured soils (Russell-Smith 1991). When present, *S.nervosum* is usually a dominant canopy species maintaining relatively large population sizes and high densities relative to other rain forest species (Bowman & Rainey 1996, Russell-Smith & Lee 1992).

S. nervosum is a mass-flowering tree with cream, staminate, hermaphroditic flowers produced in profusion. Plants as small as 3 m in height have been recorded producing flowers within the dense shade of the forest (A. Shapcott, *pers. obs*.). In a 60-m × 60-m quadrat well within the rain forest, over 80% of all *S. nervosum* individuals (< 3 m height, n = 100) produced flowers synchronously during one flowering season (A. Shapcott, unpub. data). Flowers appear with the onset of high humidity, stormy conditions associated with the onset of the annual summer wet season (October to November), and fruit production is completed by Febuary (C. Bach, *pers. comm*.). *S. nervosum* produces masses of purple to black drupes (*c.* 1 cm diameter). The flowers attract many nectivorous organisms including: insects such as bees, flying foxes (*Pteropus alecto,* C. Palmer, *pers. comm*.), and a variety of birds (O. Price, *pers. comm*.). Foraging studies by O. Price (birds) and C. Palmer (flying foxes, *Pteropus alecto*) have confirmed that *S. nervosum* fruit is a highly sought-after food resource for these mobile frugivores.

METHODS

Field methods

Syzygium nervosum plants were sampled from 21 sites across its geographic range within the Northern Territory of Australia (Figure 1). Vegetatively, *S. nervosum* can be distinguished from its most similar congeners occurring in the region by a combination of bark type and leaf venation, *S. nervosum* having a distinctly looped marginal vein. The species also tend to partition themselves within the habitat. Site selection was made in a stratified manner using the existing known distribution of *S. nervosum*. Five geographic regions were identified which would both cover the Australian geographic distribution of the species, and sample a range of patch densities within the distribution of *S. nervosum*. Within each region, sites were then selected that sampled a range of rain forest patch sizes (small to large), and degree of patch isolation. Sites were primarily selected from those previously surveyed by Russell-Smith (1991) so that other site information could be utilized. Sites were located in the field using maps, aerial photographs, local knowledge and a geographical positioning system (GPS).

At each site, samples of leaf material were taken from at least 30 plants (or

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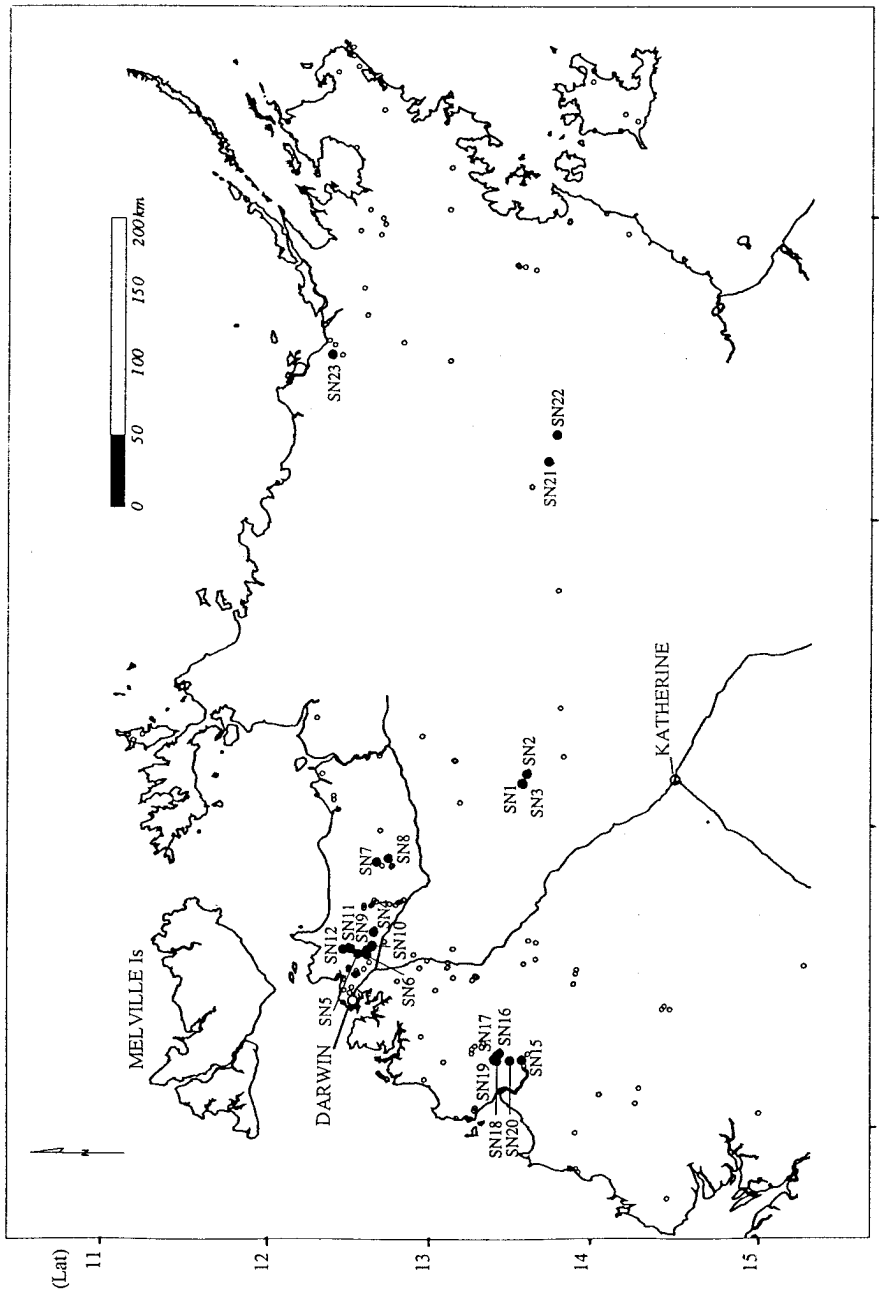

Figure 1. The known distribution of *Syzygium nervosum* in Australia with the study sites indicated. ● Sample site location, ○ Northern Territory records of *S. nervosum* taken from species lists from over 1500 rainforest sites.

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if less, as many as could be collected) across the distribution of the species within the rain forest patch. Samples were taken from all size classes (seedlings to adults). The sampled plants were mapped relative to one another in the field by recording the compass bearing and distance to the next sampled plant. These positions were then converted to coordinates (X,Y) for future reference. The height and diameter (if > 1 cm) of each sample plant was also recorded and the presence or absence of flowers or fruit was noted. At one site Fogg Dam (SN4), in addition to a general site sampling, the relative location of all *S. nervosum* plants (including seedlings) were mapped using X and Y co-ordinates within three contiguous 15-m × 30-m plots. Together the three plots filled three quarters of a 30-m × 60-m area, time prohibiting further mapping. Height, diameter and reproductive status was recorded for each plant and samples were taken from more than 80% of plants within the three plots. Data from these plots were used for more detailed within site population genetic structure analyses. All samples were individually labelled, kept cool and then stored in a refrigerator (4 °C) in the laboratory until used. All material was processed fresh within 2 wk. Voucher specimens are lodged in the Darwin Herbarium (DNA).

Laboratory methods

Genetic variation was investigated using isozyme analysis following standard methods (e.g. Conkle *et al*. 1982). The extraction buffer and method was the same as was used by Shapcott (1994) and is similar to that described by Hamrick & Loveless (1986) for use with rain forest plants. Samples were run on 12.5% starch gels at 50 mA for 5 h under constant current conditions. Six standard gel buffer systems were tested and the best resolution of bands was obtained using a lithium hydroxide buffer system (Brewbaker *et al*. 1968). Thirty enzyme stains were screened to find those which gave consistently clear bands. This resulted in the following 10 enzyme stains being used for the analysis: phosphose glucose isomerase (*Pgi*) (EC 5.1.3.1.9, Gurries & Ledig 1978); esterase (Est) (EC 3.1.1.2, Scandalios 1969); acid phosphatase (*Ap*) (EC 3.1.3.2, Adams & Joly 1980); peroxidase (*Per*) (EC 1.11.1.7, Shaw & Prasad 1970); glutamate dehydrogenase (*Gdh*) (EC 1.4.1.2, Shaw & Prasad 1970); isocitric dehydrogenase (*Idh*) (EC 1.1.1.42, Shaw & Prasad 1970); malate dehydrogenase (*Mdh*) (EC 1.1.1.37, Brown *et al*. 1978); 6-phosphogluconic acid (6Pg) (EC 1.1.1.44, Brown *et al*. 1978); aspartate amino transferase (*Aat*) (EC 2.6.1.1, Brown *et al*. 1978); menadione reductase (*Mr*) (EC 1.6.99.2, Burdon *et al*. 1980).

Samples of each of the most similar congeneric species were also run to detect species differences and provide standards with which to confirm species identity. This showed that the population of *S. nervosum* recorded on Melville Island was a miss-identification.

Statistical methods

The bands from each enzyme system were assigned to loci and genotypes based on theoretical expectations and the observed banding patterns (Gottlieb

1981). The BIOSYS-1 package (Swofford & Selander 1981) was used to analyze the results. Allelic frequencies at each site were calculated to determine the following measures of genetic diversity: the mean number of alleles per locus (A); the number of alleles per polymorphic loci (AP); the mean percentage of polymorphic loci (P); the observed mean heterozygosity (Ho): and the expected mean heterozygosity based on Hardy-Weinberg expectations (He), using Levene's (1949) correction for small sample sizes. The alleles which were found in less than 11 out of 21 sites were called uncommon for the purposes of this study. The number of uncommon alleles (U) present in each population was determined. Each population was tested for conformance of genotypic frequencies to those under Hardy-Weinberg expectations using a chi-squared goodness-of-fit test. Heterozygosity was compared for significant differences among sites and among regions using analysis of variance. The degree of past inbreeding in populations was assessed by calculation of genotypic fixation indices (F) at each polymorphic locus (Wright 1965). The values of F range from −1 complete heterosis, 0 Hardy-Weinberg equilibrium, to +1 complete allelic fixation. The partitioning of fixation indices was assessed, among sites, among sites within regions, and among regions using hierarchical F-statistics (Wright 1965).

Spearman's rank correlation tests were used to investigate whether variation in inbreeding (Ho, F) could be explained by geographic or other variables. Using this method the mean observed frequency of heterozygotes (Ho) and the mean fixation index (F), were tested for correlations with: rain forest patch size; the average distance from each site to the three nearest known populations of *S. nervosum*; the mean number of alleles per locus (A); the number of uncommon alleles (U, alleles found in less than 11/21 populations) present; and the expected heterozygosity (He). The mean heterozygosity (Ho) and fixation (F) values for each region were also compared with average regional patch density, average regional patch size and regional genetic diversity measures using analysis of variance. Rain forest patch size, distance to nearest neighbours and patch density data were obtained or calculated from the dataset of J. Russell-Smith and GIS records of the Parks and Wildlife Commission of the Northern Territory. Patterns of fixation index (F) and heterozygosity (Ho) distribution were also investigated at the landscape scale by plotting their geographic distribution using an ARC/INFO GIS system.

During the analysis it became apparent that inbreeding patterns may be better explained by investigating within-site genetic population structure. First, the entire pooled data set was used to investigate the size distribution of genotypes for each variable enzyme locus to see whether there were general trends apparent among size classes. These were plotted on histograms. Second, one site, Fogg dam (SN4), was investigated in detail. This site has been the subject of detailed research by many workers and the vegetation of the entire patch has been mapped by Bowman (e.g. Bowman & McDonough 1991, Bowman & Rainey 1996). Site analyses included: compilation of multilocus

genotypes for each sample to determine the number genetically unique individuals; genotypic size distributions at several variable loci; genotypes at individual loci were mapped for each sampled tree using its location co-ordinates and spatial autocorrelation of genotypes (at variable loci) within the site.

The coordinates *S. nervosum* plants sampled within the Fogg Dam site (SN4) formed an irregular lattice of sample points. In spatial autocorrelation the distribution of points is given, it is the random or non-random identity (e.g. genotype) of defined 'neighbours' which is tested. Spatial autocorrelation analysis requires the construction of a connection matrix defining trees which are considered neighbours or 'joined' (Sokal & Oden 1978). A variety of connection matrixes were constructed defining trees as neighbours within a 5, 10, 15, 20 30 or 40-m radius. At each of the four most variable loci investigated (*Pgi*, *Idh*, *Aat*, *Est*) pairs of trees with each genotype combination (e.g. AA-AA, AA-BB, etc) were assessed for the number of times they co-occurred within a distance class. This number was compared to the number of joins expected and the variance of each type of join, if tree genotypes were randomly distributed (assuming sampling without replacement) (Sokal & Oden 1978). If there were significant excesses of 'joins' those genotype pairs would be said to be positively autocorrelated. A deficiency of 'joins' would indicate negative autocorrelation (Epperson 1989).

In the case of nominal data such as these, the significance was assessed by the estimation of the standard normal deviate (SND), which was compared to a t-distribution (Sokal & Oden 1978). This method has been shown to be very powerful statistically (Epperson & Li 1997). Corrections for small sample sizes and degrees of freedom are given in Sokal & Oden 1978). Fortin *et al*. (1989) demonstrated that the minimum sample size required for this type of analysis was n = 25: the sample size used in this analysis was n = 180. The observed and expected join counts and the variances were calculated using a FORTRAN program (Zaluki *et al.* 1987) based on the formulae given in Sokal & Oden (1978).

The variation in autocorrelation, and hence genetic relationships, between trees that occurs with increasing distance between trees, was analysed by assessing autocorrelation with systematically increasing distances and constructing correlograms. These were used to investigate patterns in relationships between trees with different distances between them. A correlogram is a plot of the standard normal deviate of each pair type at increasing distance class or annuli (Legendre & Fortin 1989). Increments of 10 m were used in this analysis. Correlograms were plotted for each site for the association of each genotype. The point where a correllogram becomes non-significant and crosses the X-axis (SND = 0) is thought to represent the patch size (Epperson 1989). In this study, correllogram patterns at annulus distances of 40 m were unlikely to be reliable due to edge effects reducing the number neighbouring trees in the outer annulus (Epperson 1989).

RESULTS

Genetic diversity

Syzygium nervosum populations contained considerable genetic diversity, for example the mean expected heterozygosity in populations (He) was 0.307 (Table 1). Seven out of the ten loci studied were polymorphic for the species and this high level of polymorphism was found across populations with a mean percentage polymorphic loci per population (P) of 65 (Table 1). The mean number of alleles per locus (A) in populations was 2.2 and 2.9 for the species. The mean number of alleles per polymorphic loci (AP) was 3.7. However, there was not a great amount of genetic differentiation among the populations studied (Fst = 0.118). No patterns in the distribution of allelic variation or genetic diversity were found among populations across the geographic distribution of the species in the Northern Territory. The distribution and amount of genetic diversity in *S. nervosum* is discussed in more detail in Shapcott (1998b).

Inbreeding in populations

Generally, the observed heterozygosity was significantly less than under Hardy-Weinberg expectations ($P < 0.001$), leading to high fixation indices in the variable loci and indicating effective inbreeding within populations (Table 1). Analysis of variance analysis (ANOVA) indicated that there was significant variation ($P < 0.001$) in mean heterozygote frequency among the *S. nervosum* populations, but there was no significant variation among regions (Table 1). Fixation index (F), however, did not differ significantly among populations at different sites or among regions (ANOVA , $P > 0.05$). The highest mean frequency of heterozygotes (Ho = 0.201) was recorded at Glasswater (SN16) in the Litchfield region and the lowest (Ho = 0.064) at Bukbukluk (SN1) in the south of Kakadu. The highest fixation was also recorded at this latter site (F = 0.708; Table 1). Measures of inbreeding (Ho, F) were not correlated ($P > 0.05$) with measures of genetic diversity (alleles/locus A, number of uncommon alleles U, expected heterozygosity He) nor with patch size and population isolation (Table 2). Such results suggested that within-population dynamics were controlling gene flow among individuals and hence levels of inbreeding/outcrossing.

It is generally thought that heterozygotes should be favoured within populations as they mask deleterious alleles and are associated with hybrid vigour (Ennos 1989). Evidence of selection favouring heterozygotes would expected to be seen in the relative contribution of heterozygotes within each size class (Ennos 1989). To see whether there were any overall trends supporting this view, size class distributions of heterozygotes were investigated (Figure 2). Heterozygote frequencies were pooled among all 21 populations for each of the seven variable enzyme loci, and the percentage of heterozygotes in each size class was then plotted (Figure 2). No clear trends in heterozygote frequency

Table 1. Summary of inbreeding measures across *Syzygium nervosum* populations and summarized by region. Total sample size is 948.

Region	Site	Population	Mean sample size (n)	% loci polymorphic (P)	Mean observed heterozygosity (Ho)	Mean expected heterozygosity (He)	Mean fixation (F)
Kakadu	SN1	Bukbukluk	51	70	0.064	0.240	0.708
	SN2	South Kakadu	26	60	0.119	0.295	0.476
	SN3	Bukbukluk East	29	50	0.066	0.196	0.403
		Mean	35	60	0.083	0.244	0.529
Gunn Point	SN4	Fogg Dam	170	70	0.117	0.312	0.632
	SN5	Koolpinya 8	62	70	0.174	0.383	0.475
	SN6	Koolpinya 9	56	70	0.107	0.354	0.619
	SN9	Black Jungle 13	40	60	0.134	0.295	0.479
	SN10	Black Jungle 18	52	60	0.124	0.294	0.490
	SN11	Bankers	33	70	0.144	0.343	0.527
	SN12	Mallacca	30	70	0.127	0.371	0.570
		Mean	63	67	0.132	0.336	0.542
Wildman	SN7	Point Stuart	47	70	0.158	0.361	0.483
	SN8	Wildman	25	70	0.159	0.323	0.457
		Mean	36	70	0.159	0.342	0.470
Litchfield	SN15	Taro	27	60	0.105	0.283	0.549
	SN16	Glasswater	29	60	0.201	0.328	0.320
	SN17	Pajawarr	39	70	0.111	0.134	0.566
	SN18	Overflow	31	70	0.103	0.322	0.614
	SN19	Old Mill	33	70	0.086	0.293	0.640
	SN20	Canteen	29	60	0.141	0.303	0.374
		Mean	31	65	0.125	0.307	0.510
Arnhemland	SN21	Weemol	60	60	0.120	0.313	0.558
	SN22	Midnight	28	70	0.132	0.274	0.546
	SN23	Powerhouse	18	60	0.161	0.252	0.268
		Mean	35	63	0.138	0.279	0.457
Overall mean			43	65	0.126	0.307	0.512

Table 2. Summary of Spearman's rank correlation (r_s) analysis across 21 *Syzygium nervosum* sites of inbreeding variables (mean fixation (F) and observed heterozygosity (Ho)) with genetic diversity and patch size and isolation measures. Genetic diversity measures; (A) mean number of alleles per locus (10 loci); (U) number of uncommon alleles (alleles present in less than 11/21 sites) and (He) the mean expected heterozygosity. Patch size and isolation measures; rainforest patch area, the average distance from each site to the nearest known sites where *S. nervosum* is present (three nearest neighbours). For each pairwise correlation between measures (r_s) the probability for each comparison (P) is given.

	Mean fixation (F)		Heterozygosity (Ho)	
Site variables	r_s	P	r_s	P
Expected heterozygosity (He)	0.021	0.926	0.466	(0.033)
Alleles/locus (A)	0.110	0.663	0.099	0.697
Uncommon alleles (U)	0.161	0.524	–0.062	0.806
Rainforest patch area (m^2)	0.071	0.778	–0.062	0.807
Average distance to three nearest neighbours (m)	–0.126	0.586	–0.049	0.832

with size class were apparent across the loci (Figure 2) therefore no further analysis was undertaken on the data.

Within-population genetic structure

Population genetic structure at Fogg dam (SN4) was studied in detail (sample size n = 180). The comparison of multilocus (10 loci) genotypes for each plant revealed that all plants sampled represented a genetically unique individual, i.e. no two plants sampled had identical genotypes. Histogram plots indicating the relative contribution of heterozygotes to each height class size found no apparent trends that related the frequency of heterozygotes with the size of plant (Figure 3).

Spatial autocorrelation analysis at the four most variable loci indicated that plants with the same genotypes (at each loci) were locally clustered. When between neighbour plant distances were up to 10 or 20 m the SND values were consistently significantly positive for all enzyme loci (e.g. Table 3) but at distances greater than 20 m values became non-significant and then negative. That is, the near neighbours for any plant within a 20-m radius were more likely to be of the same genotype for any particular enzyme locus but outside of 20 m genotypes are randomly distributed. The patch size within species populations is generally defined as the point where 'like' join and SND values become non-significant to zero. In the Fogg Dam site (SN4) the average patch size at each enzyme locus was *c*. 25 m.

The analysis also indicated that trees that were homozygous but of differing genotypes (e.g. *Idh* AA and *Idh* BB) tended to be spatially separated from each other as evidenced by significant negative autocorrelations among unlike homozygote pairs at all distances measured (e.g. Table 3). This spatial segregation of homozygous genotypes at each locus was apparent in the *S. nervosum* genotype distribution maps at this site (e.g. Figure 4). The maps also indicate, however, that trees with dissimilar genotypes were often in close enough proximity to one another such that outcrossing should not be inhibited by distance (Figure 4).

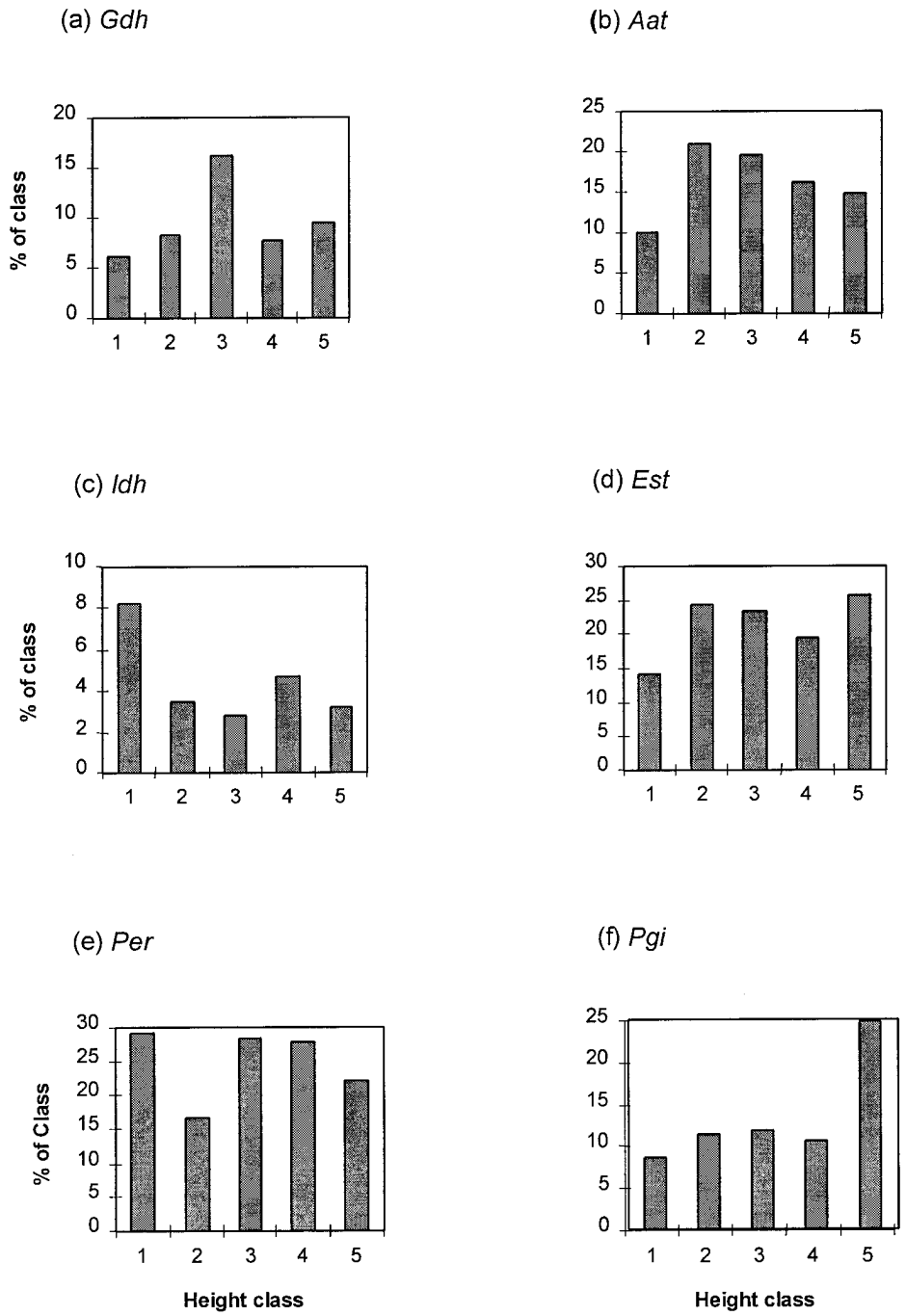

Figure 2. Height distribution of *Syzygium nervosum* heterozygotes at six enzyme loci; (a) *Gdh*, (b) *Aat*, (c) *Idh*, (d) *Est*, (e) *Per* and (f) *Pgi*. The data from all 21 sites were pooled and the percentage of the plants in each height class which were heterozygotes is plotted for each locus (n = 948). Height classes: 1 = 0–1; 2 = 1.1–3; 3 = 3.1–7, 4 = 7.1–15; 5 = 15.1–30 m.

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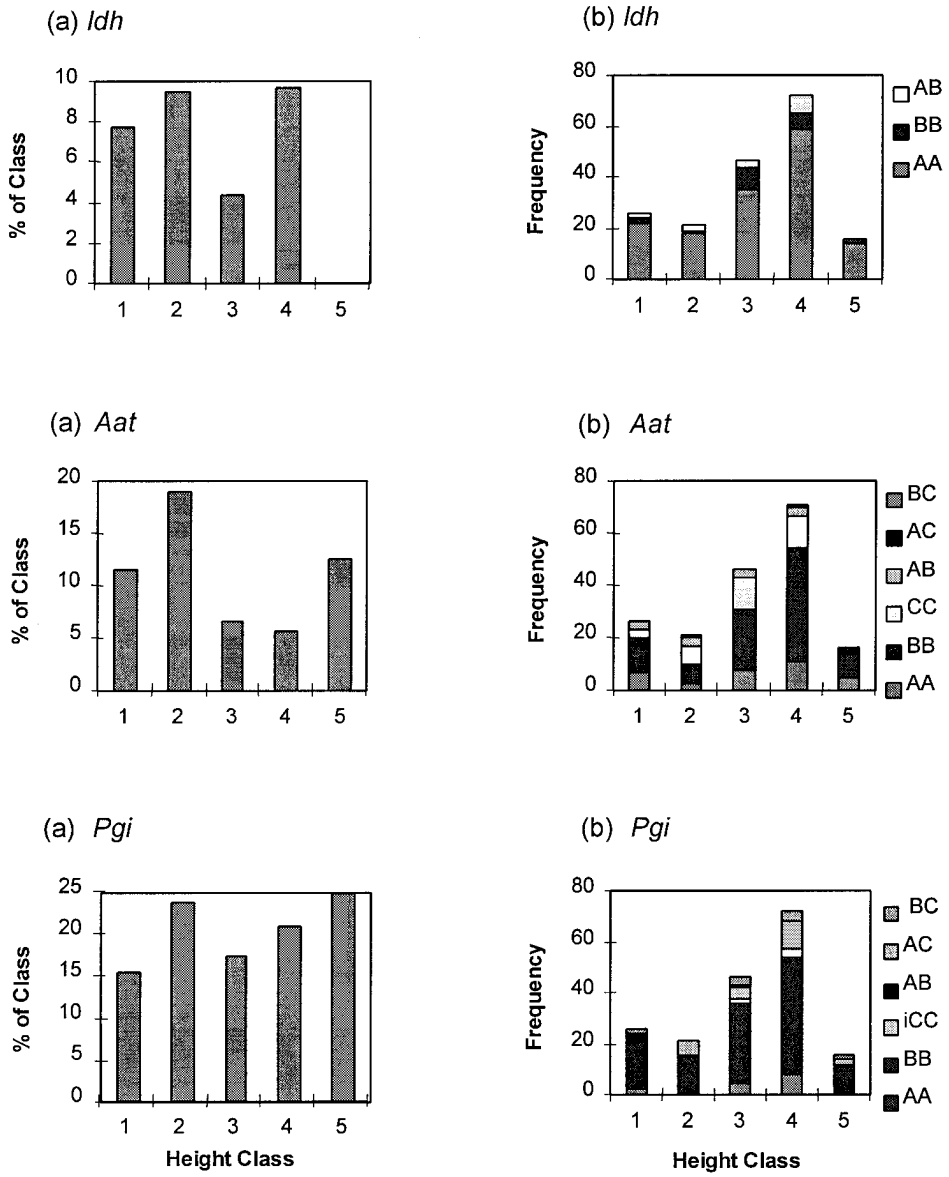

Figure 3. Size distribution of genotypes and heterozygotes across five height classes for each variable locus at Fogg Dam (SN4). The three loci which had the greatest variability and numbers of heterozygous genotypes, *Idh*, *Aat* and *Pgi*, are illustrated. (a) The percentage of heterozygotes in each height class at each locus. (b) The frequency of genotypes within each size class (n = 180). Height classes are as in Figure 2.

DISCUSSION

Comparison of inbreeding with other species

Despite considerable diversity within populations of *Syzygium nervosum* (P 65, AP 3.7) the observed heterozygosity (Ho = 0.126) occurred at less than half the frequency expected (He = 0.307) and the mean fixation index (F = 0.512) was

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Table 3. Summary of spatial autocorrelation analysis at four enzyme loci, between pairs of *Syzygium nervosum* plants at Fogg Dam (SN4). Plants within a 20-m radius were considered neighbours, n = 180. The percentages of all like pairs (e.g. AA-AA), all unlike pairs (e.g. AA-AB) and all unlike homozygote pairs (AA-BB) with SND (significant normal deviate) values which were significant ($P < 0.05$) are shown. Whether the SND is significantly positive (+) or negative (–) is indicated.

Enzyme loci	% like pairs	% unlike pairs	% unlike homozygote pairs
Pgi	100 +	40–	67–
Idh	100 +	67–	100–
Aat	100 +	40–	100–
Est	83+	47–	100–

significantly greater than zero indicating significant inbreeding/ selfing within the sampled populations. This contrasts with results found for other rain forest species (mean $F = 0.048$), which have indicated them to be predominantly outcrossed plants (Aradhya *et al*. 1991, Eguiarte *et al*. 1992, Hall *et al*. 1994, Loiselle *et al*. 1995, Murawski & Bawa 1994, Murawski *et al*. 1994, Sytsma & Schaal 1985). These data are more consistent, however, with the studies of trees in Australia in temperate, subtropical and monsoon rain forests (Shapcott 1994, 1998a; Shapcott & Playford 1996). Other studied Myrtaceae have varied from heterozygote-deficient species (e.g. rain forest *Austromyrtus* species, Shapcott & Playford 1996) to highly outcrossed *Eucalyptus* species (Moran 1992). Studies of Myrtaceae generally (not including *Syzygium*) indicate that they are predominately outcrossed (Coates & Hnatiuk 1991, Moran 1992). This likely reflects self-incompatibility mechanisms known to be widespread in the Myrtaceae (Beardsell *et al*. 1993). Self-incompatibility mechanisms are also thought to contribute to the predominance of outcrossing observed in many rain forest plants (Bawa 1992)

Population size and inbreeding

As highlighted in the review by Jain (1976) the phenomenon of selfing and hence inbreeding has fascinated many scientists and is one of the most significant differences between plants and animals, in their evolutionary biology and ability to persist in small populations, and thus in issues relating to their conservation. The ability to be self-pollinated enables species to pass through bottlenecks in population size (Jain 1976). However, selfing is also associated with the build-up of deleterious alleles and inbreeding depression (Lynch & Gabriel 1990, Schemske & Lande 1985). It has also often been cited that inbreeding should increase in small populations and this is usually seen as a problem when habitats become fragmented as populations will become less fit due to inbreeding depression (Ellstrand & Elam 1993, Frankham 1996). It is generally considered that higher within-population diversity leads to greater outcrossing rates (Brown 1989). In addition, and especially in rain forest plants, it has been assumed that higher densities of plants enhance the potential for outcrossing among individuals (Bawa 1992, Hamrick & Murawski 1991).

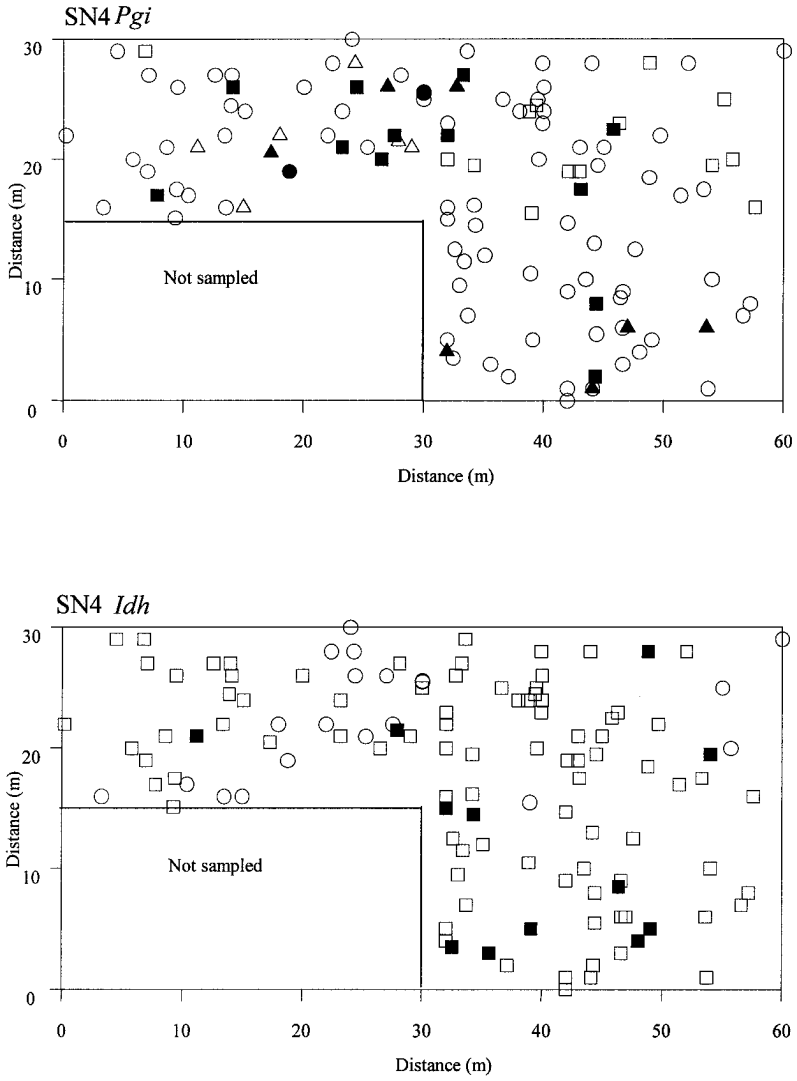

Figure 4. The spatial distribution of *Syzygium nervosum* plants within three contiguous 15 × 30-m plots at Fogg dam (SN4). For each plant sampled, the genotypes at (a) the *Pgi* locus, and (b) the *Idh* locus, are indicated by different symbols. Plant genotype symbols for the *Pgi* and *Idh* loci: □ AA; ○ BB; △ CC; ■ AB; BC; ● AC, respectively.

In this study, genetic diversity in *S. nervosum* was high (Table 1), but not correlated with fixation or heterozygosity (Table 2). As well, *S. nervosum* generally occurs at relatively high densities within patches (Bowman & Rainey 1996), especially when compared to rain forest plants that have been studied by other authors. Therefore, the above assumptions (i.e. of Bawa 1992, Brown 1989, Frankam 1996) have to be questioned. Given that seed dispersal by highly mobile frugivores in this species is considerable (O. Price and C. Palmer; *pers*.

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comm.), and estimates of gene flow among populations are fairly high (Shapcott 1998b), then the lower-than-expected frequencies of heterozygotes observed in this species is perhaps best explained by poor pollen dispersal.

Pollinator movements

Many canopy trees, notably many rain forest Myrtaceae species, produce mass displays of flowers per tree over a short period of time, synchronously among plants (van Schaik *et al*. 1993). This syndrome is particularly prevalent in seasonal rain forest species (van Schaik *et al*. 1993) and has been observed in *S. nervosum* (C. Bach, *pers. comm*; A. Shapcott, *pers. obs*.). It has been postulated that mass synchronous flowering attracts pollinators and satiates predators, and may queue pollinators so that they will seek out other individuals of the same species to obtain superabundant nectar rewards (Augspurger 1980, Lack & Kevan 1984, Stephenson 1982). However, the phenomenon of mass flowering in individual trees has provided a quandary to pollination biologists since this syndrome is expected to reduce pollinator movements and hence reduce outcrossing (Bawa 1992, Stephenson 1982).

While many authors have found that mass-flowering species attract many organisms, few of these visitors seem to act effectively as pollinators (Augspurger 1980, Hopper 1980). Hopper (1980) studied the mass-flowering canopy tree *Syzygium tierneyanum* (Myrtaceae) in North Queensland, Australia. He observed 45 insectivorous animals visiting the flowers, though he found that honey-eaters and hawk moths were the most important pollinators (Hopper 1980). Hopper (1980) also found that almost all (99.95%) inter-flower movements by these two pollinators were within the same plant and concluded that the species must be self-compatible. Similarly, the superabundant floral resource of *S. nervosum* is utilised by many species. Flying foxes (*Pteropus alecto*) appear to track the flower resource of *S. nervosum* and to preferentially forage on its flowers (C. Palmer, *pers. comm*.). Flying foxes are known to pollinate many *Syzygium* species (Williams & Adam 1994). While feeding they spend long periods in a single tree, but it might be expected that pollen carried on their fur would be dispersed to many flowers during intertree movements (C. Palmer, *pers. comm*.). *S. nervosum* produces masses of flowers in large tree crowns and it seems likely that pollinators are primarily moving between flowers within single trees with relatively few between tree movements leading to outcrossing compared with the total number of flower pollinations. The high levels of homozygosity recorded in *S. nervosum* and the expected predominance of within-plant pollinations, together with the large fruit crops recorded for this species (C. Bach, *pers. comm*.), suggest that *S. nervosum* is self-compatible and therefore similar to *S. tierneyanaum* from North Queensland (Hopper 1980). The results contrast, however, with those of Lack & Kevan (1984) for the mass-flowering canopy tree *Syzygium syzygoides* in Sulawasi, which was visited sparingly by nectivores and was found to exhibit substantial self-incompatibility.

Spatial genetic structure

Random spatial genetic patterns have been found in some plant species (e.g. Coates 1992, Dewey & Heywood 1988, Epperson & Allard 1989). However, localised genetic structure similar to that found in this study, has been reported for several plant species (e.g. Argyres & Schmitt 1991, Perry & Knowles 1991, Schnabel *et al*. 1991, Schoen & Latta 1989, Shapcott 1995, Wagner *et al*. 1991). This pattern is typically found in populations with relatively greater proportion of homozygotes (Epperson 1989) as was found in *S. nervosum*. The pattern usually represents aggregates of homozygotes with zones of heterozygotes intergrading so that unlike trees (especially unlike homozygotes) are negatively associated (Epperson 1989). Short-distance positive autocorrelation is found where family clusters have developed (Epperson 1989, Legendre & Fortin 1989). Modelling studies have shown that family aggregates develop quickly in populations with limited gene flow, such as self-compatible insect pollinated species or where most seed falls beneath the parent plant (Ennos & Clegg 1982). It has been shown that uncommon long-distance dispersal events have little effect on this family structure development (Epperson 1989, Schnabel *et al*. 1991).

The results of this study are similar to those found for *Acer saccharum* stands of mixed age (Perry & Knowles 1991). They suggested that although *Acer saccharum* seed can be dispersed for great distances, seed dispersal within dense forests is restricted. This may also be the case for *S. nervosum* as most seed falls beneath the parent plant and frugivore dispersers will drop most seed eaten beneath the parent plant (O. Price and C. Palmer; *pers. comm*.). Dispersed seed is likely to contribute only a small proportion of the seed rain (C. Bach, *pers. comm*.). That seed is also most likely to have arisen from flowers that have received pollen from the same parent tree thus reinforcing development of genetic substructuring. The spatial structure of enzyme loci in *S. nervosum* is not due to vegetative spread given that all plants surveyed at Fogg Dam posses unique multilocus genotypes.

Size distribution of heterozygotes, self-compatibility and evidence of inbreeding depression

Several authors have suggested that heterozygotes should be selected for over homozygotes, since heterozygotes have been shown to have more 'vigour' in some species, and higher levels of homozygosity are often thought to lead to inbreeding depression due to the expression of deleterious alleles (Ennos 1989). Several authors (e.g. Brown 1989, Ennos 1989, Farris & Mitton 1984) have shown examples of selection favouring heterozygotes and predicted that this should be evident in the distribution of heterozygotes among plants of differing size classes/generations. Other authors have pointed out that self-compatibility and high homozygosity in populations is advantageous in some circumstances, for example where species characteristically occur in small populations (Jain 1979). Ellstrand & Elam (1993), have also argued that some species/genera are characteristically inbred or outcrossed and it is the change

in conditions which may affect their future viability. In this study there were no clear trends in the proportion of heterozygotes across size classes. Thus in *S. nervosum* there is no clear evidence that positive selection generally favours heterozygotes (Ennos 1989).

It has been predicted that populations going through genetic bottlenecks are likely to be purged of deleterious alleles and therefore do not show evidence of inbreeding depression (Nei *et al*. 1975, Schemske & Lande 1985). *S. nervosum* appears to be an actively colonising species which occurs in small rain forest patches of fluctuating population size (Shapcott 1998b). If this is so, then populations are likely to be frequently passing through bottleneck conditions and hence are likely to be frequently purged of deleterious alleles. Purging of deleterious alleles can also occur in species with a long history of inbreeding. This suggests that *S. nervosum* should not be greatly affected by inbreeding depression even small populations. This appears to be so as *S. nervosum* is one of the most successful species in monsoon rain forests of Northern Australia with massive flower and fruit productions (C. Bach, *pers. comm*.), massive seedling recruitment, and continuous, reverse-J size distributions (Bowman & Rainey 1996). *S. nervosum* therefore, by most measures, shows a high degree of genetic and reproductive fitness (Ennos 1989). The relatively high level of genetic diversity within populations appears to ensure that, even when most pollination is achieved by selfing, there is still a very high level of uniqueness among individuals in a population. These characteristics appear to have enabled this species to survive in the small rain forest patches found in this highly fragmented landscape.

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