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Tina L. Maguire

Peter Saenger
Southern Cross University

Peter R. Baverstock
Southern Cross University

Robert J. Henry
Southern Cross University

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Microsatellite analysis of genetic structure in the mangrove species Avicennia marina (Forsk.) Vierh. (Avicenniaceae)

TL Maguire[1,2], P Saenger[1], P Baverstock[3], R Henry[2]

[1]Centre for Coastal Management, Southern Cross University, PO Box 157, Lismore, NSW, 2480, Australia
[2]Centre for Plant Conservation Genetics, Southern Cross University, PO Box 157, Lismore, NSW, 2480, Australia
[3]Graduate Research College, Southern Cross University, PO Box 157, Lismore, NSW, 2480, Australia

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Corresponding author: Prof. Peter Saenger
Ph: +61-02-6620 3631
Fax: +61-02-6621 2669
email: psaenger@scu.edu.au

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Abstract

The level of genetic variation throughout the entire worldwide range of the mangrove species Avicennia marina (Forsk.) Vierh. was examined using microsatellite markers. Three microsatellite loci detected high levels of allelic diversity (70 alleles total), essential for an accurate estimation of population genetic parameters. The informativeness of the microsatellite loci tended to increase with the increasing average number of repeats. The levels of heterozygosity detected for each population, over all loci, ranged from 0.0 to 0.8, with an average of 0.407, indicating that some populations had little or no genetic variation, while others had a large amount of genetic variation. Populations at the extremes of the distribution range showed reduced levels of heterozygosity, and significant levels of inbreeding. This is not unexpected as these populations may be subject to founder effects and environmental constraints. The presence of genetic structure was tested in A. marina populations using three models: (1) a single panmictic model, (2) the discrete subpopulation model, and (3) the isolation by distance model. The discrete subpopulations model was supported by the overall measures of population differentiation based on the infinite alleles model (F statistics), and the stepwise mutation model (R statistics). In addition, an analysis of molecular variance (AMOVA), using both theoretical models, found that most of the variation was between populations (41 - 71%), and within individuals in the total population (31 - 49%). There was little variation among individuals within populations (0 - 10%). There was no significant isolation by distance. The high levels of genetic differentiation observed among populations of A. marina may be due to environmental and ecological factors, particularly past sea level and climatic changes.

Introduction

Mangrove forests are a feature of the intertidal zone of the tropical and subtropical coastlines of the world. Mangroves comprise some 80 higher plant species in several taxonomic groups. About half of these species occur in Australia, which is believed to be near one centre of mangrove evolution (Saenger 1998). Mangrove forests all over the world are heavily exploited for wood and fishpond operations, as well as other activities. The exploitation of mangroves has resulted in the loss of genetic diversity in mangrove ecosystems, as well as the loss of valuable wood resources. To overcome these losses mangrove reserves, afforestation schemes, and enrichment plantings have been established. Conservation and sustainable management of mangroves is, thus, a major priority in coastal areas of many countries.

Population genetic studies of mangroves, therefore, are aimed at providing the information needed for afforestation, domestication and breeding programs, and for the conservation of genetic resources. A major requirement for natural mangrove populations is the development of efficient strategies for the in situ conservation of genetic resources; in contrast, the goals for breeding programs are the maximum capture of desirable genetic diversity during domestication, and the production of high quality genetic seed orchards. To date, the extent and patterns of genetic diversity in natural mangrove populations is largely unknown.

Avicennia marina (Forsk.) Vierh., is an important mangrove species because it can grow and reproduce across a wide range of climatic, saline, and tidal conditions. It is the most wide-ranging of all the mangrove tree species. A. marina is found in the Indo-West Pacific and ranges in latitude from 25(N to 38(S. The phenologic structure of A. marina stands vary from gnarled shrubbery on dry coastlines and coral atolls, to closed estuarine forests up to 40 m tall within larger systems of wet coastal tropical regions (Duke et al. 1998). The widespread occurrence of A. marina and the large number of propagules produced suggest that this species disperses widely and has little genetic structure throughout its range (Duke et al. 1998). However, field experiments suggest otherwise with propagule dispersal limited by ocean currents, the patchy presence of suitable environmental conditions, and the limited longevity of propagules during dispersal (Clarke 1992). Observations of natural plant populations at different sampling scales have provided contrasting results on spatial differentiation. Most species show little genetic structuring over short distances, but some species show strong genetic structuring (Knowles et al 1992, Shapcott 1995). However, the majority of forest trees show weak genetic structure (Rossetto et al 1999, Leonardi and Menozzi 1996, Leonardi et al 1996).

Genetic variation in mangrove species has been reported using allozymes (Duke et al 1998, Ballment et al 1988, McMillan 1986), random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers (Balakrishma 1995, Parani et al 1997). In the study by Duke et al., (1998) A. marina populations were divided into two main

groups. However, none of these groups showed fixed gene differences, and shifts in gene frequencies between the three described morphological varieties were no greater than those between populations, within a species. Using allozyme markers, A. marina was reported to have high levels of genetic diversity, with low levels of inbreeding. In addition, it was suggested that the lack of genetic divergence of A. marina into three groups, corresponding to the three morphological varieties, reflected a more recent origin (Duke et al 1998) in contrast to an ancient origin in the late Cretaceous, as has been suggested previously (Duke 1995). Like allozymes microsatellites, a new class of genetic marker, have discrete and co-dominant alleles; but microsatellites have more alleles per locus, and therefore provide a more refined analysis. In a study by Chase et al., (1996) four polymorphic microsatellite loci provided greater resolution and sensitivity for the estimation of population genetic parameters than the combined effect of all allozyme markers previously detected in several detailed studies. The effective number of alleles and expected heterozygosity (gene diversity) of the microsatellites were generally more than double that of allozymes. Recently, microsatellite markers were developed for the mangrove species A. marina (Maguire et al., 1999).

Three models of population structure can be tested in plant species: (1) a single panmictic model is relevant when random mating occurs (Richardson et al 1986). This is the simplest model and requires the entire species population to consist of a single undifferentiated unit, (2) the discrete subpopulation model consists of a series of subpopulations within which mating occurs at random, but the subpopulations are separated from one another by environmental or other barriers that allow limited gene flow between them (Richardson et al 1986), and (3) the isolation by distance model assumes that mobile propagules with a continuous geographical distribution in an undifferentiated environment distribute themselves at various distances from their origin. There are no discrete boundaries between different parts of the range and allele frequencies change gradually without any discontinuities (Richardson et al 1986). These models are simple cases, but more complex field situations may exist. For example, the result is a mixed model of subpopulations, separated by barriers to movement, with significant isolation by distance occurring inside each subpopulation. The aim of this study was to evaluate how much genetic diversity exists throughout the entire range of A. marina, and which model best describes how the genetic variation is distributed within and among populations. This information is essential for conservation and breeding strategies, and for the choice of propagules for rehabilitation or afforestation programs.

Materials and methods

Plant material and DNA isolation

A total of 200 individuals, representing 14 natural populations, were sampled over the entire worldwide range of A. marina (Fig 1). The sampling strategy incorporated a set of samples at the global scale, and a set of

samples at the continental scale within Australia. Leaf material from each population was collected at the locations shown in Table 1. From each of the worldwide locations 10 trees were sampled at random, while from each of the Australian populations 20 trees were sampled at random. Leaf material was stored at -80(C for later analysis. Total genomic DNA was isolated from leaf tissue using a modified CTAB method (Maguire et al. 1994).

Microsatellite analysis

Microsatellite loci were isolated and described previously in Maguire et al. (1999). Primer sequences for the three microsatellite loci used in this study are presented in Table 2. The PCR amplification conditions were: 1X reaction buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3, Boehringer Mannheim), 1.5 mM MgCl2, 200 uM each dNTP, 0.2 uM each primer, 1 unit Taq polymerase (Boehringer Mannheim), and 10 - 50 ng genomic DNA, in a total volume of 25 uL. After an initial heating step at 94(C for 3 min, samples were incubated for 30 cycles of 90(C for 30 sec, 60(C - 55(C for 30 sec, and 72(C for 1 min. The reactions were completed by incubating at 72(C for 5 min and held at 4(C. Cycling conditions were performed using a Perkin Elmer Cetus 9700 Thermal Cycler. The PCR products were fluorescently labelled (FAM, TET, HEX), then denatured for 3 min at 95(C, and separated by capillary electrophoresis on a PE Applied Biosystems 310 Genetic Analyser. GeneScan(and Genotyper(software (PE Applied Biosystems) were used for gel analysis.

Data analysis

Genetic diversity

Genetic diversity was calculated per population and overall, as the average number of alleles per locus (A), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and the inbreeding coefficient (Fis) using GDA, version 1.0, (Lewis and Zaykin 1999). The estimates of He were unbiased for sample size (Weir 1996). F-statistics (Fis, Fit and Fst) were estimated for each locus. The probability of the F-statistics being greater than zero was determined by bootstrap analysis using 1000 replicates, with a 95% confidence interval, using GDA, version 1.0. The outcrossing rate was calculated as:

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(Weir 1996; p 263). Hardy-Weinberg, linkage disequilibrium, and tests of significance for allele frequencies between populations, were assessed using the exact tests in the program GENEPOP, version 3.1b (Raymond and Roussett 1995), where significance levels were determined using the Markov chain method. Multilocus estimates of the effective number of migrants (Nm) between populations were calculated using the private allele method of Slatkin (1985), and were corrected for sample size as given in Barton and Slatkin (1986). For all Markov chain tests, the default parameters in GENEPOP were used, with 50 batches of 1000 iterations each. Sequential

Bonferroni adjustments were used to judge significance levels for all tests with an initial (level of 0.05 (Rice 1989).

Genetic structure

Assessment of genetic structure was performed using two models: the infinite alleles model (Kimura and Crow 1964), and the stepwise mutation model (Ohta and Kimura 1973, Kimura and Ohta 1978). Values of Fst (Weir and Cockerham 1984), Rst (Slatkin 1995) and hierarchical analysis of molecular variance (AMOVA) (Michalakis and Excoffier 1996) were calculated using the program ARLEQUIN, version 1.1 (Schneider et al 1997), where significance levels for the overall values were determined after 1023 permutations, and for the population pairwise values after 992 permutations. Isolation by distance was tested by using the log10 values of the population pairwise estimates of the overall number of migrants per generation (Nm) and regressing against the log10 of the population pairwise geographical values (Slatkin 1993), where:

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where Xst is either Fst or Rst. Prior to calculating Nm, all negative Xst values were changed to 0.0001. The significance of the regression was determined after 10,000 permutations of a Mantel procedure to assess the correlation between log10Nm and log10distance. Calculation of the Xst values, the regression and the Mantel tests were performed using the program GENEPOP, version 3.1b (Raymond and Rousset 1995).

Results

Genetic diversity

The 200 individuals, representing 14 natural populations, of A. marina produced a total of 70 alleles with 3 microsatellite loci. Allele sizes varied from 3 to 21 repeats for the locus M3, from 10 to 52 repeats for the locus M40, and from 5 to 37 repeats for the locus M47; which corresponds to the di-nucleotide repeat sequences (TG)15, (AG)32, and (CA)13 respectively. Locus M3 had 16 alleles, locus M40 had 34 alleles, and locus M47 had 20 alleles. The average number of alleles per locus per population was 3.143 for locus M3, 7.857 for locus M40 and 3.214 for locus M47 (Table 2). The observed heterozygosity (Ho) for each locus ranged from 0.332 to 0.582 (Table 2); with the longer di-nucleotide repeat giving greater values of heterozygosity. For each locus there were 7, 8 and 10 private alleles detected for the loci M3, M40 and M47 respectively. The frequencies of the private alleles detected ranged from 0.025 to 1.0 (Table 3). Interestingly, 8 of the private alleles occurred in the population UAE, and 6 occurred in the population NT.

The total number of alleles per population for all loci ranged from 3 to 37. The average number of alleles per population ranged from 1.0 to 12.333 (Table 4). For each population, over all loci, the expected heterozygosity (He)

was generally higher than the observed heterozygosity (Ho), leading to positive inbreeding coefficients (Fis). For each population the observed heterozygosity ranged from 0.0 to 0.8, with an average heterozygosity of 0.407 (Table 4). The levels of inbreeding for each population ranged from negative 0.125 (heterozygote excess) to 0.623, with an average inbreeding coefficient of 0.181 (low level of inbreeding) (Table 4). In contrast, the outcrossing rate (1-Fis/1+Fis) ranged from 0.232 to 1.286 (complete outcrossing), with an average of 0.693 (high outcrossing) (Table 4). Overall, A. marina populations were generally outcrossing with low levels of inbreeding. However, those populations at the extremes of the range, such as NSW, WA, VIC, STHAF and JAPAN (Figure 1), show reduced levels of heterozygosity, and significant levels of inbreeding (Table 4). Over all populations and loci, F statistic analysis showed significant positive levels of Fis, Fit and Fst (0.169, 0.509, and 0.410 respectively); with all estimates falling within the bootstrap confidence intervals, at the 5% significance level (Table 5).

No significant linkage disequilibrium was detected between different genotypes at each of the different microsatellite loci. Hardy Weinberg Equilibrium (HWE) departures were measured using F statistics, and their departures from zero were tested. The null hypothesis tested was the ìrandom union of gametesî. Out of the 14 populations, 5 populations had significant departures from HWE; which correspond to those populations with significant levels of inbreeding (Fis) (Table 4). In each case, departures from HWE were due to homozygote excess.

Genetic structure

In order to test which model best describes how the genetic variation is distributed within and among populations the following hypotheses concerning the allelic and genotypic distributions across all populations and between population pairs were tested. For each locus, a probability test was performed on contingency tables and an estimate of P was calculated. The first two null hypotheses tested were ìthe allelic distribution is identical across populations, and between population pairsî. P values were highly significant ($P < 0.001$), therefore indicating the presence of genetic structure. There were a few exceptions which were not significant at the 0.05 level. For the locus M3: populations NSW and NEWCAL, WA and PNG. For the locus M40: populations SA and JAPAN, NT and INDIA. For the locus M47: populations NSW and JAPAN, NSW and NEWCAL, VIC and JAPAN, VIC and NZ, VIC and NEWCAL. The second two null hypotheses tested were ìthe genotypic distribution is identical across populations, and between population pairsî. P values were highly significant ($P < 0.001$), therefore indicating the presence of genetic structure. Again, there were a few exceptions which were not significant at the 0.05 level. For the locus M3: populations PNG and WA, NEWCAL and NSW. For the locus M40: populations PNG and NT, JAPAN and SA, INDIA and NT. For the locus M47: populations JAPAN and NSW, JAPAN and VIC, NZ and NSW, NZ and VIC, NEWCAL and NSW, NEWCAL and VIC. Those population pairs which were not significantly different, for allelic or genotypic distributions, were the

same in both cases; except for two which bordered the rejection zone at $P < 0.05$.

In order to measure the degree of population differentiation two theoretical models were used, the infinite alleles model (Fst) and the stepwise mutation model (Rst). The overall values of genetic differentiation for each model produced different values and each was significantly different than zero ($P < 0.001$): Fst = 0.410, Rst = 0.710. The overall values of Fis and Ris were 0.169 and -0.079 respectively, indicating little to no inbreeding. The overall values of Fit and Rit were 0.509 and 0.685 respectively. For the population pairwise comparisons the values of Fst were generally lower than Rst (Tables 6 and 7).

For both Fst and Rst, the majority of all pairwise comparisons were significantly greater than zero at the 0.05 level. Those comparisons which were not significant were between populations: VIC and NZ for Fst; SA and PNG, NSW and VIC, NSW and NZ, NT and PNG, for Rst. Using Wrights (1978) interpretive guidelines, for Fst there were 5 population pairs in the range of 0.05 to 0.15, and 1 population pair in the range of 0 to 0.05 (Table 6), indicating little and moderate genetic differentiation. For Rst there were 3 population pairs in the range 0.05 to 0.15, and 3 population pairs in the range 0 to 0.05 (Table 7), indicating little and moderate genetic differentiation. These populations also had estimates of the number of migrants (Nm) above 1, indicating some gene flow. Whereas most of the pairwise comparisons had estimates less than 1, indicating very low levels of gene flow. For Fst those populations with moderate differentiation were: NSW and VIC, NSW and NZ, NSW and NEWCAL, NT and QLD, NT and UAE. Estimates of the number of migrants were 1.375, 2.241, 2.652, 1.933, and 1.417 respectively. For Fst those populations with little differentiation were: VIC and NZ. The estimate of the number of migrants was 4.822. For Rst those populations with moderate differentiation were: SA and QLD, NT and PNG, JAPAN and INDIA. Estimates of the number of migrants were 1.437, 2.964 and 1.629 respectively. For Rst those populations with little differentiation were: SA and PNG, NSW and VIC, NSW and NZ. Estimates of number of migrants were 12.165, 5.664, and 23.175 respectively. Overall populations and loci, the number of migrants using the private alleles method according to Barton and Slatkin (1986) was 0.209; which is less than 1, indicating very low levels of gene flow. It should be noted however that these values of Nm represent a historical average of the number of migrants per generation.

Population structure was also tested at different hierarchical levels using both Fst and Rst (Table 8). Most of the variation was found to be between populations (41 - 71%) and within individuals in the total population (31 - 49%). There was little variation among individuals within populations (0 ñ 10%) .This corresponds to the estimates of both Fst and Rst (0.410 and 0.709 respectively), indicating great genetic differentiation among populations.

The isolation by distance model analyses resulted in non-significance, therefore, the null hypothesis of ìindependence between two variablesî was retained. This means there was no statistically significant correlation between geographical distance and migration.

Discussion

Genetic diversity

The aim of this study was to evaluate how much genetic diversity exists throughout the entire range of A. marina, and which model best explains how the genetic variation is distributed within and among populations. Three microsatellite loci detected a total of 70 alleles, with the average number of alleles per locus per population ranging from 3.143 to 7.857. This is much higher than previously described levels of polymorphism using allozymes (Duke et al 1998); where a total of 26 alleles were detected using 11 allozyme loci, with the average number of alleles per locus ranging from 1.0 to 1.9. The informativeness of the microsatellite loci tended to increase with the increasing average number of repeats, as reported by Weber (1990). The longest repeat (AG)32 detected 34 alleles, whereas the smaller repeats (TG)15 and (CA)13 detected 16 and 20 alleles respectively.

The levels of heterozygosity (Ho) detected for each population, over all loci (Table 4), indicate that some populations had little or no genetic variation, while others had a large amount of genetic variation. In addition, these estimates of heterozygosity were much higher than those previously reported using allozymes; where estimates of Ho ranged from 0.0 to 0.217 (Duke et al 1998). Overall, A. marina populations were found to be generally outcrossing with little or no inbreeding, which corresponds to previous data using allozymes (Duke et al 1998). However, those populations at the extremes of the range, such as NSW, WA, VIC, STHAF and JAPAN (Figure 1), show reduced levels of heterozygosity, and significant levels of inbreeding (Table 4). This is not unexpected as these populations may have been subjected to repeated bottlenecks or founder effects in earlier times, due to episodes of glaciation and transgressions (Saenger 1998). For example, the low level of genetic variation found in populations of Acacia mangim, a coastal tree species, was thought to be due to a bottleneck occurring during the Pleistocene glaciations (Moran et al 1989). A. mangium typically occurs on coastal tropical lowlands in northern Australia, Indonesia and Papua New Guinea, and during the interglacial periods in the Quaternary these environments were largely underwater and the species was restricted to small refuge populations. Similar contractions and expansion of mangroves have been associated with glacial and interglacial periods in West Africa (Saenger and Bellan 1995).

Environmental constraints such as temperature, humidity, fresh water supply, as well as ecological factors such as the mating system, population size, suitable habitat for seedling establishment and isolation by environmental barriers may also have a significant effect on the levels of heterozygosity, inbreeding and population structure. Recently, it has been

reported that populations of A. marina which are under pressure due to the constant use of foliage for fodder and grazing, as well as environmental pollution, show reduced levels of polymorphism (Parani et al 1997).

Genetic structure

Three models of population structure were tested. The single panmictic model was suggested previously by Duke et al (1998), because A. marina has a widespread occurrence and large propagule production. The probability values of allelic and genotypic distributions across all populations and between population pairs were highly significant, providing strong evidence for genetic structure. This was also supported by observations that A. marina propagule dispersal is limited by ocean currents, the presence of suitable environmental conditions, and longevity of propagules during dispersal (Clarke 1992). Pattern analysis of A. marina also showed small scale patterns corresponding to reproductive origin, that is fruits that fall from trees tend to be trapped in the heterogeneous habitat produced by the lower part of the trees, whereas on a larger scale the pattern corresponds to environmental heterogeneity (Saifullah et al 1994).

The discrete subpopulation model was then tested and the values of Fst and Rst were 0.410 and 0.710 respectively, indicating very great genetic differentiation using Wrights (1978) interpretive guidelines. Some population pairs however, showed little and moderate levels of genetic differentiation. Those populations also had estimates of the number of migrants (Nm) greater than 1, indicating some gene flow; whereas most population comparisons had very low levels of gene flow. Hierarchical analysis of molecular variance (AMOVA) also showed that most of the variation was partitioned among populations and within individuals in the total population. There was little variation among individuals within populations. From these analyses the discrete subpopulations model is likely for A. marina.

The isolation by distance model was rejected, therefore, the discrete subpopulations model was retained. Further support for this model, with high levels of genetic differentiation among populations, has been reported in the literature. Using allozymes, the value of Fst was reported to be 0.384 overall populations of A. marina, indicating great genetic differentiation among populations (Duke et al 1998). Using RAPDs, low levels of polymorphism were reported at the intrapopulation level of A. marina (27%), but higher levels of polymorphism were reported at the interpopulation level (77% and 66% for RAPD and RFLP markers respectively), indicating greater genetic variability among populations rather than within populations (Parani et al 1997).

For a wide range of plant species the mating system also affects the patterns of genetic variation both within and among populations. Most forest trees have been found to have mating systems with high levels of outcrossing (Muona 1990), although some species, such as the eucalypts, have mixed mating systems (Sampson et al 1989). There have been limited studies on

the floral biology, pollination and breeding systems of mangroves. However, work by Primack and Tomlinson (1980) suggest that there is a trend towards outcrossing. In Avicennia species there is evidence for protandry and limited self compatibility based on controlled pollinations, observation of the fruiting of isolated individuals, or of heavy fruit set in natural populations (Tomlinson 1986). Theoretical arguments would suggest that mangroves, if they are primarily colonising species, would retain the need for self fertility if they are to establish populations in isolated localities (Primack and Tomlinson 1980). However, more direct confirmation of this hypothesis is needed.

Differences in breeding systems may result in differences in population genetic structure. Hamrick and Godt (1990) generalised that in outcrossing species, the majority of the total genetic diversity (Ht) resides within populations (Hs), while in selfing species more is proportioned among populations (Fst). In a study by Awadalla and Ritland (1997) they compared two monkey-flower species with contrasting mating systems using microsatellite markers. They found that mean Fst values were greater among populations of inbreeding species than outbreeding species, and that selfing populations were characterised by relatively high Ht values and low Hs values. Thus, selfing populations maintain as much diversity as outcrossing populations, but more is distributed among populations. Colonisation events were thought to be important in patterning this variation as colonisation, which is frequently associated with selfing populations (Schoen and Brown 1991), increases the among population component of genetic diversity, but decreases within population heterozygosity and numbers of alleles (Waples 1989). The observed frequency distribution of alleles and the large difference in the variance of allele sizes within populations, relative to among populations, is indicative of past colonisation events. Because of the dynamism of mangrove habitats, colonisation is a frequent occurrence for mangrove species. For example, floating propagules or seedlings can establish at a distance founding new colonies, or populations experience sequential periods of significant contraction or expansion with resultant bottleneck or founder effects due to past sea-level and climatic changes (Saenger 1998). For example, eight cycles of wet and arid climatic phases associated with sea-level changes are known from the East African coast in the past 3 million years (Rossi 1981).

Other factors can also affect the patterns of genetic variability both within and among populations. Given that A. marina has buoyant water-borne propagules, their dispersal can be constrained by land masses blocking current flow, and by large bodies of water. Such limitations usually confine a species to a particular region, depending on its dispersive range, and its ability to become established in a new location. Thus, present A. marina distributions as discrete subpopulations may have resulted from environmental constraints, imposed ecological conditions, the past changes in these conditions, and the limited dispersive range of the species.

Conclusions

The potential loss of genetic diversity in mangrove forests due to disturbances, such as wood harvesting and shrimp pond operations, is reason for concern and the conservation of genetic diversity may be one of the most important issues facing the future of mangrove forestry practices. Knowledge of how the genetic variation is partitioned within and among populations is a pre-requisite for decision making in the management of breeding and natural populations (Williams and Hamrick 1996). In addition, the general patterns of genetic diversity can provide valuable information; such as the populations at the extremes of the range tend to be genetically depauperate. For the establishment of a breeding program, or seed orchard, populations can be identified which have high levels of genetic diversity so as much variation as possible can be captured in the base populations. This will ensure that sufficient genetic variation exists for the selection of superior individuals or populations to advance the genetic improvement program.

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Author Information Box

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Figure 1
The entire worldwide distribution of Avicennia marina. Stars represent sampling locations (Table 1), the dotted line represents the distribution limits of Avicennia marina.

Table 1
The 14 Avicennia marina populations tested (name and code given), latitude and longitude of the location, and the collectorís name.

Population•Code•Latitude•Longitude•Collector••Australian populations••••••Northern Territory,
Darwin Harbour•NT•12:27:00S•130:50:00E•Darwin Botanic Gardens••Queensland,
Moody Creek Cairns•QLD•16:55:00S•145:46:00E•Department of Primary Industries••New South Wales,
Ballina Reserve•NSW•28:48:00S•153:34:00E•T. Maguire••Western Australia,
Bunbury Reserve•WA•33:20:29S•115:38:26E•Department of Conservation and Land Management••South Australia,
St Kilda Reserve•SA•34:56:00S•138:36:00E•E. Maguire••Victoria,
Port Albert
•VIC•38:39:00S•146:39:00E•R. Harrison••World-Wide populations••••••Malaysia, Batu Maung
SE Penang Island•MALAY•05:17:34N•100:16:98E•M. Haywood••Papua New Guinea,
Umuda Island Fly River•PNG•08:30:00S•143:41:00E•M. Rau••India,
Dona Paula Goa•INDIA•15:27:00N•73:48:00E•A. Untawale••New Caledonia,
Noumea•NEWCAL•22:16:00S•166:27:00E•B. Richer de Forges••United Arab Emirates,
Abu Dhabi•UAE•24:28:00N•54:22:00E•T. Youssef••Japan,
Iromote Island Okinawa•JAPAN•26:20:15N•127:48:09E•S. Baba••South Africa,
Durban, Beechwood•STHAF•29:48:00S•31:03:00E•T. Steinke••New Zealand,
Auckland•NZ•38:20:00S•176:00:00E•K. Parnell••

Table 2
Descriptive statistics for the three microsatellite loci studied overall populations. A, total number of alleles; Ave, average number of alleles per locus, per population; He, expected proportion of heterozygotes; and Ho, observed proportion of heterozygotes.

Locus•Repeat unit•Primer sequence
(5í-
3í)•A•Ave•He•Ho••M3•(TG)15•GGTTCCTGCAAGTATGTCAACACCCTC•16•3.
143•0.405•0.332••••ACCTCGATTCCTCCCCGAATGC••••••M40•(AG)32•CCC
ATAGATGACGGCAATCTTATGATCC•34•7.857•0.706•0.582••••ACCATCCAA
AATAAAATAAATCTCCCTCCC••••••M47•(CA)13•TGACACCAAGGGAAATCAA
CATGCC•20•3.214•0.370•0.307••••GAACCTAGCGACCAATAGATCATCCTG
G••••••

Table 3
Private alleles for each locus and the population of Avicennia marina in which it occurs.

Locus•Allele•Frequency•Found in••M3•21•0.350•UAE••M3•20•0.200•UAE••M3•10•0.850•INDIA••M3•9•1.000•JAPAN••M3•7•0.025•QLD••M3•11•0.050•NT••M3•3•0.050•WA••M40•38•0.350•NEWCAL••M40•35•0.100•NEWCAL••M40•34•0.025•VIC••M40•11•0.100•QLD••M40•13•0.225•QLD••M40•41•0.025•NT••M40•47•0.025•NT••M40•52•0.025•NT••M47•37•0.050•UAE••M47•22•0.200•UAE••M47•34•0.050•UAE••M47•20•0.050•UAE••M47•19•0.100•UAE••M47•16•0.200•UAE••M47•25•0.200•STHAF••M47•26•0.650•STHAF••M47•13•0.125•NT••M47•15•0.025•NT••

Table 4
Descriptive statistics over all loci for each population of Avicennia marina. N, number of individuals; A, number of alleles; Ave, average number of alleles per locus; He, expected proportion of heterozygotes; Ho, observed proportion of heterozygotes; Fis, the inbreeding coefficient; and the outcrossing rate. Levels of significance for Fis > 0 are: *P < 0.05; **P < 0.01; ***P < 0.001.

Population•N•A•Ave•He•Ho•Fis•Outcrossing rate••SA•20•11•3.667•0.464•0.500•-0.080•1.173••NSW•20•16•5.333•0.605•0.417•0.317***•0.519••WA•20•7•2.333•0.274•0.183•0.337*•0.496••NT•20•37•12.333•0.853•0.800•0.063•0.881••QLD•20•22•7.333•0.708•0.650•0.084•0.845••VIC•20•12•4.000•0.261•0.183•0.303**•0.535••STHAF•10•12•4.000•0.598•0.233•0.623***•0.232••PNG•10•14•4.667•0.482•0.467•0.034•0.933••JAPAN•10•3•1.000•0.000•0.000•0.000• N/A••NZ•10•8•2.667•0.268•0.300•-0.125•1.286••INDIA•10•14•4.667•0.616•0.567•0.084•0.845••NEWCAL•10•11•3.667•0.447•0.367•0.189•0.683••UAE•10•20•6.667•0.816•0.733•0.106•0.808••MALAY•10•12•4.000•0.521•0.300•0.438*•0.391••Mean•14.286•14.214•4.738•0.494•0.407•0.181***•0.693••

Table 5
F-Statistic analysis estimates the parameters Fis, Fit and Fst for each locus, and over all loci over all populations. F-statistic bootstrap analysis obtains bootstrap confidence intervals for the F statistics, using 1000 replicates and a 95% confidence interval (CI).

Locus•Repeat
unit•Fis•Fit•Fst••M3•(TG)15•0.166•0.575•0.490••M40•(AG)32•0.181•0.385
•0.249••M47•(CA)13•0.150•0.588•0.515••Overall••0.169•0.509•0.410••Boo
tstrap CI••••••Upper••0.181•0.588•0.515••Lower••0.150•0.385•0.249••

Table 6
Matrix of pairwise comparisons of population genetic differentiation calculated using the infinite alleles model (Fst).

•SA•NSW•WA•NT•QLD•VIC•STHAF•PNG•JAPAN•NZ•INDIA•NEW CAL•UAE••••••••••••••••NSW•0.232••••••••••••••WA•0.525•0.550••••••••••••NT•0.243•0.187•0.408••••••••••••QLD•0.310•0.250•0.481•0.115•••••••••••VIC•0.352•0.154•0.723•0.375•0.409••••••••••STHAF•0.481•0.393•0.599•0.256•0.326•0.607•••••••••PNG•0.451•0.443•0.494•0.260•0.288•0.651•0.450••••••••JAPAN•0.493•0.524•0.818•0.409•0.541•0.755•0.701•0.746••••••••NZ•0.330•0.100•0.719•0.328•0.369•0.049•0.567•0.625•0.799••••••INDIA•0.386•0.341•0.571•0.196•0.276•0.554•0.391•0.402•0.623•0.509•••••NEW CAL•0.303•0.086•0.648•0.236•0.328•0.240•0.477•0.535•0.664•0.215•0.414••••UAE•0.363•0.270•0.467•0.150•0.198•0.485•0.267•0.344•0.592•0.429•0.226•0.340•••MALAY•0.473•0.386•0.589•0.264•0.354•0.608•0.396•0.433•0.739•0.571•0.355•0.500•0.325••

Table 7
Matrix of pairwise comparisons of population genetic differentiation calculated using the stepwise mutation model (Rst).

•SA•NSW•WA•NT•QLD•VIC•STHAF•PNG•JAPAN•NZ•INDIA•NEW CAL•UAE••••••••••••••••NSW•0.697••••••••••••••WA•0.228•0.847••••••••••••NT•0.198•0.329•0.277••••••••••••QLD•0.148•0.816•0.221•0.344•••••••••••VIC•0.691•0.042•0.881•0.292•0.827••••••••••STHAF•0.816•0.889•0.961•0.643•0.884•0.932••••••••PNG•0.020•0.726•0.357•0.078•0.271•0.765•0.913••••••••JAPAN•0.469•0.894•0.813•0.311•0.448•0.939•0.987•0.681••••••NZ•0.751•0.011•0.927•0.357•0.858•0.256•0.969•0.850•0.983••••••INDIA•0.281•0.845•0.573•0.255•0.213•0.880•0.927•0.389•0.133•0.919•••••NEW CAL•0.817•0.359•0.937•0.497•0.889•0.628•0.956•0.879•0.971•0.415•0.926••••UAE•0.623•0.830•0.856•0.548•0.723•0.850•0.490•0.709•0.795•0.865•0.722•0.886•••MALAY•0.553•0.440•0.804•0.185•0.737•0.550•0.963•0.661•0.953•0.748•0.849•0.802•0.849••

Table 8
Hierarchical analysis of molecular variance of 14 populations of Avicennia marina. %, percentage molecular variation explained by the hierarchical level; P, level of significance for the distribution of variation for that hierarchical level being different from random.

Source of Variation•d.f.•% Variation•P Value•% Variation•P Value••••Fst•Fst•Rst•Rst••Among populations•13•40.97•<0.001•70.86•<0.001•••••••••Among individuals within populations•186•9.98•<0.001•-2.31•<0.001•••••••••Within individuals•200•49.05•<0.001•31.45•<0.001••