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Genetic structure at range edge: low diversity and high inbreeding in Southeast Asian mangrove (*Avicennia marina*) populations

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Abstract

Understanding the genetic composition and mating systems of edge populations provides important insights into the environmental and demographic factors shaping species' distribution ranges. We analysed samples of the mangrove *Avicennia marina* from Vietnam, northern Philippines and Australia, with microsatellite markers. We compared genetic diversity and structure in edge (Southeast Asia, and Southern Australia) and core (North and Eastern Australia) populations, and also compared our results with previously published data from core and southern edge populations. Comparisons highlighted significantly reduced gene diversity and higher genetic structure in both margins compared to core populations, which can be attributed to very low effective population size, pollinator scarcity and high environmental pressure at distribution margins. The estimated level of inbreeding was significantly higher in northeastern populations compared to core and southern populations. This suggests that despite the high genetic load usually associated with inbreeding, inbreeding or even selfing may be advantageous in margin habitats due to the possible advantages of reproductive assurance, or local adaptation. The very high level of genetic structure and inbreeding show that populations of *A. marina* are functioning as independent evolutionary units more than as components of a metapopulation system connected by gene flow. The combinations of those characteristics make these peripheral populations likely to develop local adaptations and therefore to be of particular interest for conservation strategies as well as for adaptation to possible future environmental changes.

Keywords: *Avicennia marina*, biogeographic limits, genetic diversity, mangrove, mating system, species' distribution margins

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Introduction

The demography of populations at the limits of species' ranges is often influenced by extreme and/or unstable environmental conditions (Brown 1984; Brown *et al*. 1996; Holt & Keitt 2000), leading to habitat fragmentation and low population density (Brown *et al*. 1996; Pulliam 2000). Yet, the resulting consequences for genetic structure and evolution of peripheral populations remain controversial. Genetic isolation and reduced population size (Lawton 1993) are expected to lead to a reduction in gene diversity (Hoffmann & Blows 1994) through increased genetic drift, and thereby to a reduced likelihood to adapt to the extreme conditions often encountered at range edges (Bradshaw 1991). Yet, the migratory fluxes from core populations, which partially compensate for the low density and low reproductive success at range edges, may maintain, in some cases, relatively high genetic diversity in peripheral populations (Kirkpatrick & Ravigne 2002). Moreover, the

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unstable and fluctuating environmental pressures at the species' range limits may even increase genetic diversity in peripheral populations by favouring distinct genotypes and flexibility, thereby balancing the effect of selection (Brussart 1984; Safriel *et al*. 1994; Lesica & Allendorf 1995).

Whereas isolation at the edges of a species' distribution is thought to favour genetic structure (Schaal & Leverich 1996), it has also been suggested that the reduced density of peripheral populations may be a characteristic of a 'source-sink'-like system (Brown *et al*. 1996; Pulliam 2000), with migration from core populations preventing the divergence and adaptation of peripheral populations, thereby restricting local adaptation and range expansion (Peck *et al*. 1998; Kirkpatrick, Ravigne 2002). However, it has been argued that peripheral populations are potentially important for conservation, since they may preserve rare alleles and gene combinations important for adaptation to extreme environmental conditions (Lesica *et al.* 1995; Hampe & Petit 2005). Depending on the strength of drift, the dispersal potential, and the stability of local adaptations, different patterns of genetic diversity and structure can be observed in peripheral, compared to core populations, with a considerable influence on distribution range stability or expansion potential. So far, most comparisons of central and edge populations tend to support two of the previously listed hypothesis: lower genetic diversity and higher divergence are observed in peripheral relative to core populations (El Mousadik & Petit 1996; Durka 1999; Bouzat & Johnson 2004; Faugeron *et al*. 2004; Krauss *et al*. 2004), suggesting a strong role of drift and possible environmental selection as the predominant forces shaping the genetic composition of peripheral populations.

Environmental conditions at range limits are also expected to influence reproductive systems, which in turn will influence the genetic composition of natural populations. Isolation and low density are thought to increase genetic drift, and have been shown to correlate with inbreeding (Wright 1946). They are thought to have a particular impact in species with mixed reproductive systems such as parthenogenetic, clonal, or self-fertilizing species (Peck *et al*. 1998; Jensen *et al*. 2002). As an example, geographical and genetic isolation are thought to out-compete the advantages of sex in marginal populations, and to induce a shift towards asexual reproduction in clonal plants (Eckert 2002; Billingham *et al*. 2003). Among possible explanations for the maintenance of selfing despite its negative effects on fitness, the 'reproductive insurance hypothesis' has been proposed, and supported by several empirical studies, suggesting that selfing can be favoured in situations of low density and habitat fragmentation because it ensures reproductive success for isolated individuals (Jain 1976; Kalisz *et al*. 1999; Rajora *et al*. 2002). More frequent selfing will also contribute to reducing effective population size, thereby favouring local inbreeding and genetic drift (Loveless & Hamrick 1984).

The combination of the particular demographic and environmental conditions characterizing range boundaries makes populations occurring there more sensitive to anthropogenic disturbances. Mangrove ecosystems, which rank amongst the most valuable in the world (Costanza *et al*. 1997), are experiencing steep worldwide decline due to a combination of anthropogenic stresses (Valiela *et al*. 2001). Mangroves are of high importance in Southeast Asian regions (Mumby *et al*. 2004), where they have experienced particularly severe losses (Valiela *et al*. 2001). The hermaphroditic species *Avicennia marina* has the broadest distribution of all mangrove tree species, ranging from coastal East Africa to the Western Pacific, and from northern New Zealand to Japan. *A. marina* is thought to have its centre of distribution in the southern Indo-Pacific and its origin in Australia, where the earliest fossil pollen records were found (Ricklefs & Latham 1993). This broad distribution has been linked to its high fecundity and its ability to grow and reproduce across a broad range of climatic, salinity and tidal conditions (Duke *et al*. 1998). For those widespread species, a very common pattern of distribution is a decline in abundance from the core to the edge of distribution (Brown 1984; Brussart 1984; Brown *et al*. 1995), leading to lower effective population size and lower genetic diversity. A decrease in allelic diversity at the range edges of *A. marina* has been suggested by previous studies (Maguire *et al*. 2000b; Giang *et al*. 2003). Nevertheless, the careful comparative analysis of the genetic composition or mating system of populations sampled near its northern limit in Asia, has been hampered by an overall low level of diversity observed with allozymes (Duke *et al*. 1998) and monomorphism of the three microsatellites used on a Japanese population sample (Maguire *et al*. 2000b). Analyses performed with plastid DNA showed very low diversity and apparently high structure in Vietnam, no data for those markers are available in the centre of distribution (Kado *et al*. 2004).

The aim of the present work is to test the hypothesis of lower genetic diversity and higher genetic structure towards the range edges of *A. marina*, and to examine the influence of the distribution limit on the genetic composition of populations. We do so, on the basis of a comparative analysis of the genetic composition of peripheral and core populations. Genetic analyses of nine populations located near the northern range edge of the species, extending from northern Vietnam to northern Philippines, two populations from the core region in northern and eastern Australia, and one from the southern range edge in southern Australia, were conducted using seven microsatellite markers. We also compared the results obtained with published reports for populations from the centre of the distribution (northern Australia and Papua New Guinea) and from the southern limit (southern Australia), performed using three of these microsatellite markers (Maguire *et al*. 2000b).

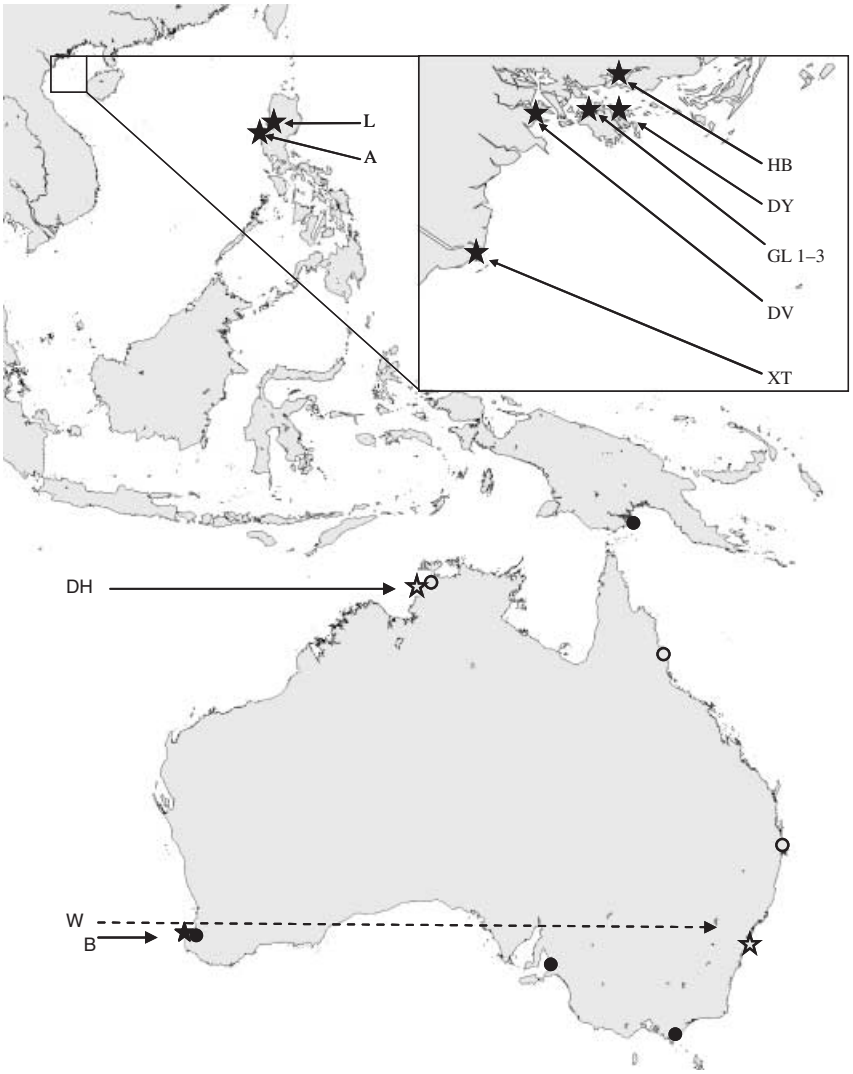

Fig. 1 Location of sites sampled in Australian core populations (grey stars) and in peripheral populations near the Southern limit in southern Australia and near the Northern limit in Vietnam and the Philippines (black stars). The samples from the previous study (Maguire *et al*. 2000b) are also indicated for core and peripheral populations (respectively grey and black circles).

Materials and methods

Plant material and DNA isolation

A total of 303 individuals were sampled in 12 locations (Table 2, Fig. 1) distributed in three regions: the Northern limit (seven populations from northern Vietnam, two from northern Philippines), the Southern limit (one population from southern Australia) and the core region (one population from northern and one from eastern Australia). We shall refer to populations or samples from both limits (Northern and Southern limits) as 'peripheral', as opposed to those from the centre of distribution, which we will refer to as 'core' populations. Samples were collected within areas of about 1 km^2. Samples from southern Australia were collected in the Reserve of Bunbury, 700 km south of the nearest stands of *Avicennia marina* (Semeniuk *et al*. 2000). Leaves for DNA extraction (2–3 per tree) were collected and stored in bags with silica gel. Total genomic DNA was isolated using the CTAB (cetyltrimethyl ammonium bromide) method (Doyle & Doyle 1987).

Microsatellite analysis

The microsatellite loci used in this study were described previously in Maguire *et al*. (2000a; Table 1), but we used slightly modified primers shorter than the ones proposed in that study (most primers composed of 20–22 bp instead of 25–30 bp). This slight modification was performed in order to minimize the risks of primer mismatch, that are more likely to arise when working on large geographical scales, and that can result in the occurrence of null alleles. Loci Am3, Am32, Am40, Am47, Am49, Am81 and Am98 were analysed with primers fluorescently labelled as to enable the use of an automated sequencer. Amplifications were performed by polymerase chain reaction (PCR), in 10-μL volumes containing 10–50 ng of template DNA, 1× reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4;

Locus	Motif	T°	Primer sequence
Am 3	$(TG)_{15}$	50	F: GGTTCCTGCAAGTATGTCAACACCCTC^{FAM}
			R: ACCTCGATTCCTCCCCGAATGC
Am 32	$(AC)_{14}$	55	F: AACTTTGCTTCAGAGTCTCG^{FAM}
			R: AATGGAGCCTCATTCCTCCG
Am 40	$(AG)_{32}$	50	F: TGACGGCAATCTTATGATCC^{HEX}
			R: ATAAAATAAATCTCCCTCCC
Am 47	$(CA)_{13}$	55	F: CCAAGGGAAATCAACATGCC^{FAM}
			R: CGACCAATAGATCATCCTGG
Am 49	$(TG)_{16}$	55	F: ACGACAGACTAGAAACCACC^{HEX}
			R: TGGATAAAGGCAACTCCGAC
Am 81	$(CA)_9(CT)_{16}$	55	F: ATCGGATGTTGCTACTCCTG^{NED}
			R: CAAAGCCCCAAAAATAATCC
Am 98	$(CGT)_8$	50	F: CTCGTTACGATGGATGACTTC^{FAM}
			R: TGCGGTAAAATGAGACGTGC

Table 1 Modified primer sequences used in this study for the microsatellite loci described for *Avicennia marina* by Maguire *et al*. (2000a). The motif, forward (F) and reverse (R) primers used for amplification, and the fluorescent (FAM, HEX or NED) labelling are indicated

Invitrogen), 0.5 U of *Taq* polymerase (Invitrogen), 0.5 μL of 1% W-1 solution (Invitrogen), 2 mM of $MgCl_2$, 1 μM of each primer, 60 μM of dNTP mix. Reactions were performed in a Gene Amp PCR system 9700 (PE Applied Biosystems) with the following programme: 5 min denaturation at 95 °C, then 30 cycles of 45 s at each temperature of 95 °C, 50 °C to 55 °C (Table 1), 72 °C, and a final extension of 7 min at 72 °C. The PCR products obtained were denatured for 4 min at 95 °C and separated by vertical electrophoresis on a PE Applied Biosystems 377 Genetic Analyser. GENSCAN™ software (PE Applied Biosystems) was used for gel analysis.

Data analysis

The mean number of alleles per locus (allelic diversity), the expected (H_E) and observed (H_O) proportion of heterozygotes, and the inbreeding coefficient (F_{IS}) were estimated using GENETIX, 4.0 (Belkhir *et al*. 1996–2001). Significance levels were estimated by bootstrapping (1000 replicates). Microsatellite genotypes were tested for linkage disequilibrium according to Black & Krafsur (1985), and a permutation approach (1000 permutations) was used to estimate significance levels.

Measures of allelic richness were included to investigate differences in the number of alleles among populations. In order to test for the difference in genetic diversity among peripheral populations, two series of comparisons were performed. The first one including all loci, in order to compare the allelic richness in the populations from Northern edge (in Southeast Asia) and core (northern and eastern Australia) region analyzed in the present study. The second series of comparisons, based on a subset of three microsatellites (Am3, Am40, Am47) loci, included the populations analysed in our study from the Northern limit (in Southeast Asia) and core regions (in northern and eastern Australia), compared with the populations at the Southern limit (three populations from southern Australia) which had been analysed with these three markers in a previous study (Maguire *et al*. 2000b). Two one-tailed *t*-tests were performed in order to test the null hypothesis H_O: $\hat{A}$ peripheral populations = $\hat{A}_{\text{core populations}}$, with the alternative hypothesis H1: $\hat{A}_{\text{peripheral populations}} < \hat{A}_{\text{core populations}}$, where $\hat{A}$ is the allelic richness. Differences in the level of diversity ($\hat{A}$) between samples collected in both sets of peripheral populations: near the Southern (Maguire *et al*. 2000b) and near the Northeastern limit (present study) were also tested, by applying, in the absence of an a priori hypothesis, a two-tailed *t*-test.

When comparing diversity levels among samples with different number of individuals, a resampling approach should be used to obtain comparison based on homogeneous subsamples, with the minimum sample size available in the data set (Leberg 2002). Therefore we performed resampling (bootstrap, 1000 replicates) of 17 individuals (the smallest sample size analysed in this study, Lucero, Philippines) in all population samples. These repeated random subsampling analyses for allelic richness were performed with a routine written in Delphi (Borland) for the purpose (Arnaud-Haond, available upon request).

To test for the influence of range-edge on Hardy–Weinberg equilibrium (HWE), the same series of *t*-tests were also performed in order to test for the significance of differences in the mean levels of inbreeding in core and peripheral populations, with the same two groups of peripheral populations: the populations sampled near the Northern limit analysed in our study, and the Southern limit Australian populations analysed by Maguire *et al*. (2000a). Here also, comparison of Northern and core populations were performed on the basis of the seven markers analysed in the present study, and the comparisons with published data on populations from Southern limit (Maguire *et al*. 2000b) with the subset of three microsatellite markers used in that study. Finally,

differences in inbreeding estimated in samples collected near Southern and Northeastern limits were also tested with a two-tailed *t*-test.

In order to test for a reduction in effective population size linked to bottleneck or founder events in the peripheral populations, heterozygosity tests were used to compare the estimates of expected heterozygosity based on allele frequencies (H_E) and on the number of alleles and sample size (H_{EQ}). Indeed, when a population experiences a bottleneck, the number of alleles decreases faster than heterozygosity, resulting in an apparent excess of heterozygosity ($H_E > H_{EQ}$) that can be used as an indicator of a recent bottleneck event (Cornuet & Luikart 1996). Wilcoxon tests were used (with 1000 iterations) with estimates of H_{EQ} calculated under the two extreme mutation models: stepwise-mutation model (SMM) and infinite allele model (IAM), using BOTTLENECK 1.2.02 (Cornuet & Luikart 1996).

The *F* estimator of genetic structure θ (Weir & Cockerham 1984) was calculated for each locus and over all loci. The probability of the *F*-statistics being greater than zero was determined by a permutation analysis (10 000 replicates) with GENETIX software (Belkhir *et al*. 1996–2001). A Mantel test was performed to test for the existence of a relationship between genetic distance, expressed as $F_{ST}/(1 - F_{ST})$ and log-transformed geographical distances, as suggested by Rousset (1997) in a two dimension space.

Results

Genetic variability, linkage disequilibrium and bottleneck tests

From the 303 individuals assayed, a total of 118 alleles were detected with the seven polymorphic microsatellite loci. Locus Am3 was monomorphic for all populations from the Northern limit, whereas all other loci were polymorphic in all three regions (Northern and Southern limit as well as core region). Significant linkage disequilibrium was detected only in the population samples from the Northern limit: between Am 98 and Am 47 in the sample from Lucero (Philippines), between Am 98 and Am 40 in the sample from Arnedo (Philippines) and Gia Luan 1 (Halong Bay, Vietnam). Significant values were also observed among Am 32, Am 49 and Am 81 in samples from Gia Luan 3 (Halong Bay, Vietnam) and Xan Thuy (Vietnam).

The total number of alleles per population for all loci ranged from 12 (Xan Thuy) to 25 (Gia Luan 3) in peripheral populations and 57 (Wollongong) to 67 (Darwin Harbor) in core populations. The standardized (on the basis of the smallest sample size $N = 17$) number of alleles per locus per population varied from 1.26 (Xan Thuy) to 3.05 (Arnedo) in peripheral populations, and reached 8.24 (Darwin Harbor) in the core region (Table 2). The unbiased heterozygosity (H_E) varied between 0.15 and 0.37 in peripheral populations, and 0.54 and 0.79 in core populations, and the observed heterozygosity (H_O) ranged from 0.02 to 0.30 in peripheral and 0.52 to 0.75 in core populations (Table 2).

The values of F_{IS} per locus per population ranged from –0.09 to 1.00 (see online Supplementary material). Over all loci, the observed heterozygosity (H_O) showed a significant ($P < 0.01$) tendency to be lower than expected in all populations from the Northern limit (Southeast Asia), resulting in highly significant ($P < 0.001$) positive values of F_{IS}, ranging from 0.35 in Arnedo to 0.90 in Xan Thuy (Table 2; Supplementary material). The population from the Southern limit in Australia exhibited a limited, but significant ($P < 0.05$) F_{IS} value of 0.13, and the core population from Darwin Harbor a much smaller but significant ($P < 0.05$) F_{IS} of 0.05.

Comparison of the Northern limit and core populations showed a significantly higher allelic richness ($P < 0.01$) and lower departure from HWE ($P = 0.01$) in the core than in the Northern range edge populations (Table 3, Fig. 2). Indeed, the number of alleles per locus ranged between 1.26 and 3.05 for populations from the Northern limit (and 2.91 in the southern Australian populations analysed therein), as opposed to 6.55–8.24 for core populations. Based on the three microsatellite loci those samples had been analysed with (Maguire *et al*. 2000b), some discrepancies are also observed in the number of alleles per locus, heterozygosity and inbreeding coefficient (Table 2, Table 3; Fig. 2). With these three loci, the number of alleles per locus ranged between 1.63 and 2.92 for populations from the Northern range edge and 2.33–4.00 in populations from the Southern limit populations, as opposed to 7.01–10.36 for samples from core populations. Mean allelic diversities for these three regions were thus most reduced in the north of the distribution (Southeast Asia), significantly lower than in the Southern limit (southern Australia) ($P < 0.001$), whereas the comparison between southern vs. core populations was inconclusive (i.e. $P = 0.10$).

In the present study, the estimates of the inbreeding coefficient (F_{IS}, Table 2; Table 3; Fig. 2) were higher in the Northern limit (0.37–0.90) than in core (0.03–0.06, $P = 0.01$), or the Southern limit (0.13) populations. On the basis of a subset of three microsatellites (from Maguire *et al*. 2000a), the comparison of estimates based on our samples from the Northern limit and core region with the data previously published for the Southern distribution limit (Maguire *et al*. 2000b), shows significantly higher F_{IS} values in Northern than in Southern limit ($P = 0.00$). Conversely, no significant difference was observed ($P = 0.63$) between core and Southern limit populations.

A significant probability of occurrence of a recent reduction in effective population size ($P > 0.95$) was detected in five of the nine population from the Northern limit (Hoang Bo, Gia Luan 2 and 3, Xan Thuy and Arnedo) and

Table 2 Sampling localities in the peripheral populations from Northern (N) or Southern (S) limits, or in the core (C) regions, sample size (*N*), average number of alleles standardized (Â) for the smallest sample size analysed (17 individuals, 1000 permutations) analysed in the present study, and number of alleles (A) in the previous study, expected heterozygosity (H_E), observed heterozygosity (H_O), and heterozygote deficiency (F_{IS}) for all loci in the Southeast Asian populations, and for three microsatellite loci (Am3, Am40 and Am47) in the Southeast Asian and core (Australia and Papua New Guinea) *Avicennia marina* populations. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

					All markers				Three microsatellites				
Region		Population	Code	*N*	Â	H_E	H_O	F_{IS}	Â/A	H_E	H_O	F_{IS}	Studies
Vietnam	N	Hoang Bo	HB	19	1.65	0.20	0.13	0.37***	2.90	0.29	0.23	0.21	Present study
	N	Dai Yen	DY	19	2.52	0.33	0.20	0.41***	2.60	0.35	0.29	0.18	Present study
	N	Gia Luan 1	GL-1	33	1.75	0.20	0.10	0.53***	2.26	0.35	0.13	0.63***	Present study
	N	Gia Luan 2	GL-2	34	2.10	0.20	0.07	0.67***	1.67	0.22	0.14	0.39**	Present study
	N	Gia Luan 3	GL-3	30	2.95	0.37	0.13	0.66***	2.63	0.35	0.22	0.39***	Present study
	N	Din Vu	DV	19	1.84	0.21	0.10	0.56***	2.63	0.39	0.14	0.64***	Present study
	N	Xuan Thuy	XT	19	1.26	0.15	0.02	0.90***	1.63	0.09	0.04	0.63***	Present study
Philippines	N	Lucero	L	17	2.29	0.36	0.20	0.47***	2.00	0.20	0.16	0.23	Present study
	N	Arnedo	A	19	3.05	0.34	0.23	0.35***	2.92	0.26	0.21	0.21*	Present study
Northern Australia	C	Northern Territory	DH	30	8.24	0.79	0.75	0.06*	10.36	0.88	0.80	0.08*	Present study
Eastern Australia	C	New South Wales	W	34	6.55	0.54	0.52	0.03	7.01	0.64	0.63	0.02	Present study
Southern Australia	S	Bunburry Reserve	B	30	2.91	0.35	0.30	0.13***	3.34	0.42	0.37	0.11*	Present study
Papua New Guinea	C	Umuda island		10	—	—	—	—	4.67	0.48	0.47	0.03	Maguire *et al*. 2000a
Northern Australia	C	Northern Territory		20	—	—	—	—	12.33	0.85	0.80	0.06	Maguire *et al*. 2000a
	C	Queensland		20	—	—	—	—	7.33	0.71	0.65	0.08	Maguire *et al*. 2000a
Eastern Australia	C	New South Wales		20	—	—	—	—	5.33	0.61	0.42	0.32***	Maguire *et al*. 2000a
Southern Australia	S	Bunburry Reserve		20	—	—	—	—	2.33	0.27	0.18	0.34*	Maguire *et al*. 2000a
	S	St Kilda Reserve		20	—	—	—	—	3.67	0.46	0.50	−0.08	Maguire *et al*. 2000a
	S	Victoria, Port Albert		20	—	—	—	—	4.00	0.26	0.18	0.30**	Maguire *et al*. 2000a

Table 3 Comparisons (based on *t*-test) of allelic richness (Â) and F_{IS} estimates using all seven loci for northern limit (Southeast Asia) vs. core (northern and eastern Australia) populations comparisons, and using a subset of three loci for comparisons using results published (Maguire *et al*. 2000b) for the Southern distribution limit. Significant results at the 5% level are indicated in bold

Data set	Test	Â or F_{IS}	Mean ± SE (peripheral vs. core)	*P* value
7 loci	Northern limit (SE Asia) vs. core (N and E Australia)	Â	**2.16 ± 0.20 < 7.40 ± 0.84**	**0.000**
		F_{IS}	**0.51 ± 0.07 > 0.04 ± 0.01**	**0.013**
3 loci	Southern limit (S Australia) vs. core (N and E Australia)	Â	3.74 ± 0.14 ≅ 8.69 ± 1.68	0.104 (w)*
		F_{IS}	0.12 ± 0.11 ≅ 0.04 ± 0.01	0.630
	Southern limit (S Australia) vs. northern limit (SE Asia)	Â	**3.74 ± 0.14 > 2.36 ± 0.17**	**0.000**
		F_{IS}	**0.51 ± 0.07 > 0.12 ± 0.11**	**0.001**

*w indicates Welch's correction was applied whenever the variances where found to differ significantly among compared groups.
<, > or ≅ means significantly smaller, larger, or not significantly different, respectively.

in the population from the Southern limit (Bunbury) under the SMM model. Under the IAM, none of the values were significant.

Genetic structure

The values of pairwise F_{ST} varied from 0.01 to 0.67 (data not shown), revealing a high genetic structure between populations. Among 36 pairwise comparisons, all but two values of F_{ST} were significantly different from zero ($P < 0.05$). Therefore, apart from very small geographical scale, all Vietnamese population pairs (distances of 20 to about 180 km) were significantly different, with F_{ST} values ranging between 0.06 and 0.20, without any significant relationship at the Mantel test ($P = 0.20$) between geographical and genetic distances (Fig. 3). F_{ST} values among Philippine and Vietnamese populations (about 1500 km apart) located near the Northern limit ranged between 0.40 and 0.66, and were therefore comparable with the differentiation estimates (0.32−0.66) between Southeast Asian and Australian samples

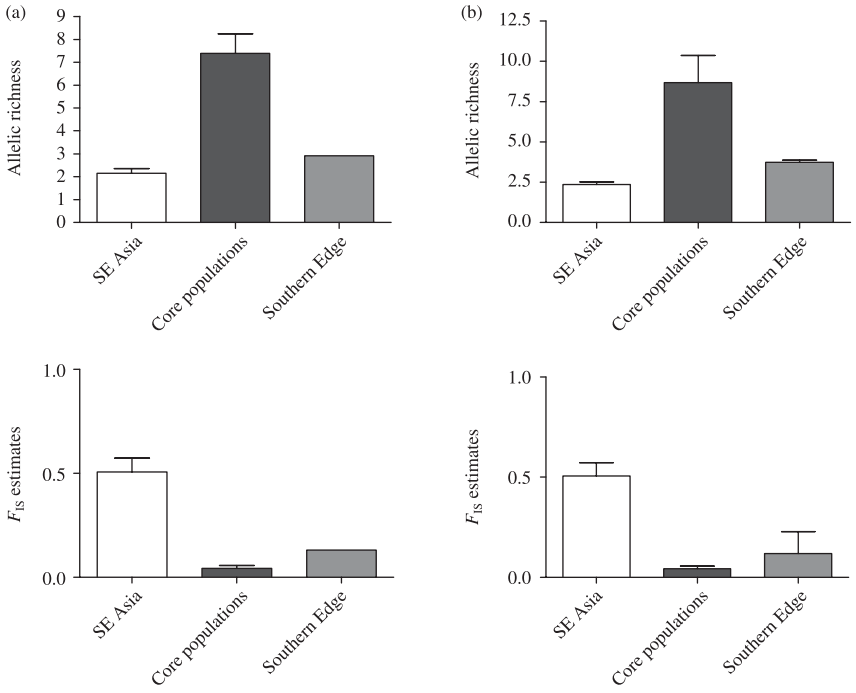

Fig. 2 Average estimates (± SE) of allelic diversity (Â) and inbreeding coefficient (F_{IS}) in samples from core populations (northern and eastern Australia) and range-edge populations sampled near Northeastern (Vietnam and the Philippines) and Southern limits (southern Australia). The comparison is illustrated on the basis of (a) the data at seven microsatellite loci analysed in the present study, and (b) the comparison of a subset of three microsatellites in the same populations from Southeast Asia and northern and eastern Australia with previously published data (Maguire *et al*. 2000b) from three populations sampled at the Southern distribution limit.

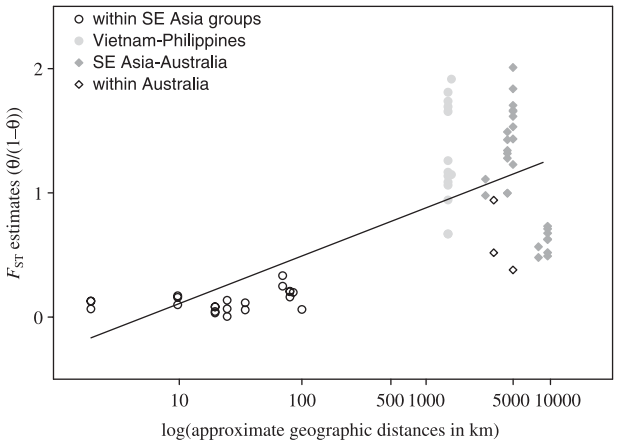

Fig. 3 Estimates of genetic differentiation [$F_{ST}/(1 - F_{ST})$] among populations, plotted against log-transformed geographic distance, as used to perform the Mantel test. Detail is given of the pairwise distances corresponding to pairs of samples compared within Vietnam and within the Philippines in Southeast Asia (white circles), within Australia (white rhombs), and among groups (grey rhombs).

(5000–10 000 km). Consequently, most population pairs (3 and 23, respectively) showed F_{ST} greater than 0.15 and 0.25 and the estimated number of migrants was in most cases $Nm < 1$, indicative of very low levels of gene flow if populations were at migration–drift equilibrium. At a larger scale, a significant result was observed with the Mantel test, showing a relationship between genetic and geographical distance ($P = 0.01$ and $r^2 = 0.50$; Fig. 3). However, it is clear that this relationship does not reflect a gradual increase in genetic distance with increasing geographical distance (Fig. 3), but is mostly driven by the difference in genetic differentiation estimates at very small scale (within Asiatic regions) vs. very large scale (among regions). Indeed, no such relationship is observed at small scale (among Vietnamese populations), and there is a clear absence of a relationship when comparing very large-scale pairwise differentiation, with Australian populations being much less genetically differentiated than Vietnamese–Philippines or Southeast Asia–Australia populations at similar geographical scales (Fig. 3).

Discussion

The results obtained in this study show that populations of *Avicennia marina* near the Northern limit of distribution are characterized by low genetic diversity at microsatellite loci, high levels of inbreeding and high genetic divergence. The number and frequency distribution of alleles revealed low levels of heterozygosity and allelic richness for both distributional limits, lower in both Northern (Southeast Asia) and Southern (southern Australia) limits compared with core populations from northern and eastern Australia. A significant impoverishment in allelic diversity was detected towards the Northern range edge (Southeast Asia) of *A. marina* distribution (Fig. 2). These patterns confirm the previously reported general tendency for lower allelic diversity in populations at distributional limits, particularly observed with the monomorphism of a Japanese sample (Maguire *et al*. 2000b) and with the low diversity reported

in Vietnam (Giang *et al*. 2003). The low level of diversity observed in samples from populations located near the Northern limit in Vietnam and the Philippines (Southeast Asia: 1.26–3.05), is intermediate between that observed in samples from populations from the core region (northern and eastern Australia: 6.55–8.24), and population from the extreme northeastern range in Japan (1). This pattern is in agreement both with the theoretical predictions based on the assumption that drift or stable directional selection are major determinants of gene diversity (Brussart 1984; Hoffmann *et al.* 1994), and with some empirical observations reported so far. Theory predicts that demographic instability inducing low effective population size, repeated bottlenecks or founder events, should lead to genetic impoverishment and higher genetic structure through the effect of accentuated drift. Empirically, this pattern has indeed been recently reported, although not always tested for significance, in most studies comparing central and peripheral populations in algae (e.g. Faugeron *et al*. 2004), seagrasses (e.g. Alberto *et al*. 2001, 2006; Billingham *et al*. 2003), terrestrial plants and trees (e.g. Chang *et al*. 1994; Durka 1999; Lammi *et al*. 1999; Lonn & Prentice 2002; Gapare & Aitken 2005), as well as animals (e.g. Brussart 1984; Wang *et al*. 2002), where reduced gene diversity, often combined with enhanced genetic structure, have been observed among peripheral populations. Occurrence of repeated bottlenecks or founder events at the margin of distribution is, for *A. marina*, additionally supported by the significant probabilities of bottlenecks in five upon nine tested populations from the Northern range limit.

Despite the widespread occurrence of *A. marina* and the large number of buoyant propagules produced, migration from core populations is not expected to counterbalance the effects of drift and possible local adaptation on genetic variability. Dispersal in *A. marina* is actually expected to be extremely limited by the low buoyancy of propagules and high predation during the initial stages of seed development (Clarke 1992, 1993; Minchinton & Dalby-Ball 2001; Clarke & Kerrigan 2002), supported by the observation of significant levels of genetic structure among Australian and Indo-Pacific populations, even at small or intermediate geographical scales (Parani *et al*. 1997; Duke *et al*. 1998; Maguire *et al*. 2000b). Studies of its reproductive phenology in core populations have shown that *A. marina* is hermaphroditic, but selfing is thought to be limited both by protandrous development of flowers and early mortality of fruits (Clarke & Myerscough 1991). In agreement with those biological features, genetic studies using allozymes (McMillan 1986; Ballment *et al*. 1988; Duke *et al*. 1998) and microsatellites (Maguire *et al*. 2000a, b) have shown that core populations sampled around Australia exhibit significant levels of genetic variability and overall HWE, as well as significant genetic structuring. Our results provide evidence for a greater restriction to gene flow between peripheral populations (Fig. 3) of Vietnam and the Philippines (F_{ST} ranging between 0.40 and 0.67 for about 1500 km distance), compared to values among samples from Australia (0.27–0.48 for about 1000 to more than 5000 km). High genetic structure is particularly clear at lower scale in populations near the Northern limit, with F_{ST} among Vietnamese and among Philippine populations ranging between 0.01_{NS} and 0.25 at a scale of about 10–100 km. Both reduced diversity and increased structure suggest that drift due to very low effective population sizes, plays a major role in shaping the genetic composition of peripheral populations of *A. marina*. Yet, strong and stable directional selection may also contribute to reduce the level of genetic diversity in peripheral populations, since fewer niches and extreme environmental conditions at the margins may favour fewer specialized genotypes (da Cunha & Dobzhansky 1954). Moreover, small and inbred populations may not be able to afford 'heterotic buffering' (Brussart 1984), and selection may then favour homozygotes vs. slightly detrimental heterozygotes.

Accentuated drift and possible selection for homozygotes in range-edge populations may also account for the high levels of inbreeding observed near the Northern limit in Vietnamese and Philippine populations, when compared with those reported in core populations (Table 3). A Wahlund effect, if each site sampled in Asia had an admixture of individuals originating from distinct gene pools, may account partially for the observed departure to HWE. Yet, given the limitations to propagule dispersal (Clarke 1992, 1993; Minchinton & Dalby-Ball 2001; Clarke & Kerrigan 2002), this effect alone would generate F_{IS} values comparable to the F_{ST} values estimated at local scale, and could therefore not explain the much higher values obtained here. Among additional factors that could generate such high F_{IS}, both strong selective pressure favouring homozygotes and accentuated drift due to low density can result in high levels of inbreeding. Levels of inbreeding observed in most Asiatic populations strongly suggest the occurrence of selfing in those populations. Although studies of *A. marina* phenology suggested that self-fertilization is scarce and outcrossing is favoured both by protandrous development and pollinator behaviour (Clarke *et al.* 1991), these observations performed on Australian populations may not apply at the distribution limit. In fact, selfing can circumvent the Allee effect (Brown *et al*. 1996; Groom 1998) induced by the combination of low plant density and pollinator scarcity (Lloyd 1979; Affre & Thompson 1999). Moreover, marginal habitat conditions could also lead to selection of individuals able to complete their reproduction earlier, thereby favouring selfing (Masuyama 1996; Runnions & Geber 2000). Although the general advantage of reproductive assurance has been questioned by the observation of high genetic load in selfed vs. outcrossed progeny (Byers & Waller 1999; Herlihy &

Eckert 2002), range edges may represent particular cases where selfing may remain advantageous due to the combined effect of reproductive assurance, short life cycle and possible strong adaptive selection favouring homozygotes.

Occurrence of a recent bottleneck or founder event at the Southern limit of the distribution was supported by a significant bottleneck test for Bunbury. Allelic diversity was lower, though the difference was not significant, in Southern limit than in core samples, and was still significantly higher than that for populations near the Northern limit. Moreover, levels of F_{IS} at the Southern limit were not highly distinct from those reported for core populations, but significantly lower than those estimated here for Northern limit populations. Several factors may account for the apparently more accentuated range-edge effect in Northeastern than in Southern populations. Allelic diversity difference might be due to historical factors such as different bottleneck strength or later recolonization during glacial episodes (Saenger 1998), whereas the radically distinct level of inbreeding implies that present factors are acting differently in Northeastern and Southern populations. Among the possible (nonmutually exclusive) hypotheses are: (i) lower effective population sizes, (ii) lower pollinator density, and/or (iii) stronger environmental constraints at the Northern range, whereas the Southern range may be mostly defined by the absence of habitat/land further south.

Conclusion

The results obtained in this study show that demographic and environmental conditions near the Northeastern range-edge limit of *Avicennia marina* favour inbreeding and possibly selfing, and that consequently accentuating drift, possibly combined with local selection, leads to lower gene diversity and high genetic structure. The Asiatic populations of *A. marina* therefore seem to behave as independent evolutionary units more than as components of metapopulations connected by gene flow (Barrett 2003). The combinations of those characteristics make these peripheral populations likely to develop local adaptations to extreme environmental conditions, and therefore are likely to be of interest for conservation strategies and breeding or genetic improvement programs, as well as of potential importance for possible environmental changes (Lesica *et al.* 1995; Hampe & Petit 2005).

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Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2997/MEC2997sm.htm

Table S1 Descriptive statistical data for each population at each of the seven polymorphic microsatellite loci. *N*, total number of individuals per population; H_{nb}, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, heterozygote deficiency, in bold when significant after a 1000 permutation test; allele frequencies.

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