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Dispersal limitation of the mangrove *Avicennia marina* at its South African range limit in strong contrast to connectivity in its core East African region

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ABSTRACT: Understanding the genetic composition and dynamics of mangrove species along a latitudinal range could provide insight as to how their biogeographical range evolved. In this study, we investigate the genetic composition of the widespread mangrove species *Avicennia marina* in its core region and southern range limit along the East African coast to test for the effect of geographical location and coastal geomorphology on the genetic diversity and differentiation at the southern range limit. A total of 388 *A. marina* individuals from 6 sites in Kenya and Tanzania (core region) and 6 in South Africa (southern range limit) were sampled and genotyped using 8 microsatellite markers. A high genetic differentiation was found between the core and range-limit populations, with strikingly high within-population inbreeding at the range limit with a consequent high fixation (allele fixation at locus level) and stronger between-population differentiation. Despite the fact that *A. marina* propagules may have the capacity to disperse between populations, the gene flow was found to be moderate to high within the core populations and extremely low within the range limit populations. This study highlights a genetically depauperate situation in peripheral populations, most likely as a consequence of historical sporadic arrival of founders with subsequent inbreeding and dispersal limitation due to the coastal geomorphology in combination with range-edge effects.

KEY WORDS: Connectivity · Biogeographic range limit · Microsatellite marker · Genetic diversity · Mangrove

INTRODUCTION

Human pressure on mangrove systems continues to cause quantitative loss and qualitative degradation worldwide (Valiela et al. 2001, Alongi 2002, FAO 2007, Polidoro et al. 2010). Predicting future range shifts and the potential of species to adapt to new environmental conditions requires insight into the environmental factors that control the presence or absence of a species (Quisthoudt et al. 2012). Such information can be obtained from studying the genetic structure and composition of populations at the limits of a species' tolerance range and comparing them with the structure and composition of core populations in the range optimum (Abeli et al. 2014). However, while the absence of mangrove individuals beyond the biogeographical range may reflect unfavorable climatic conditions, they can also be absent

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from climatically suitable sites within the outer range limits (Quisthoudt et al. 2012), for example, in areas situated beyond the reach of dispersing propagules (i.e. dispersal units). Although mangrove propagules are considered to be good potential dispersers, effective long-distance dispersal seems to be of a sporadic nature (Yamashiro 1961, Komiyama et al. 1992, Clarke 1993, McGuinness 1997, Sousa et al. 2007, De Ryck et al. 2012, Van der Stocken et al. 2013) because the viability and buoyancy of propagules as well as barriers to dispersal may limit effective dispersal to other regions. Ocean currents and coastal geomorphology, for example, have been shown to shape spatial genetic patterns and create geographic disjunctions in mangroves (Pil et al. 2011, Wee et al. 2014).

According to the central–marginal hypothesis (Eckert et al. 2008), genetic diversity is expected to be higher in the range optimum as compared with range-edge populations, although exceptions have been observed (Assis et al. 2013). Eckert et al. (2008) showed that 64.2% of 134 studies on 115 animal and plant species reported an expected decline in genetic diversity based on the central–marginal hypothesis, although in most cases the difference in genetic diversity between central and marginal populations was small. For most studies based on microsatellite markers, the genetic diversity was shown to be higher in central populations in terms of their expected heterozygosity (94%) and allelic richness (93%) (Eckert et al. 2008).

The question of why species have spatially restricted ranges has been and still is difficult to answer (Bridle & Vines 2007). Species are restricted to their range because of poor survival rates when there is occasional transport beyond their limits. Moreover, in the typically fragmented range-edge populations, Allee effects, genetic drift and low rates of mutational input may restrict the availability of locally adapted and beneficial alleles, and hence limit range expansion by preventing adaptation (Holt & Keitt 2000, Bridle & Vines 2007). Even if range-edge populations have a high input of new alleles from core populations, beneficial alleles with small selection coefficients may be swamped by migration and will not contribute to local adaptation, i.e. genetic diversity is lost at loci under selection pressure because gene flow is too high (Slatkin 1987, Lenormand 2002, Bridle & Vines 2007).

Various studies have focused on the genetic composition of *Avicennia* species using highly polymorphic markers, mostly in the Eastern Indian Ocean and Atlantic-East Pacific regions (Maguire et al. 2002, Giang et al. 2003, Kahrood et al. 2008, Salas-Leiva et al. 2009, Cerón-Souza et al. 2012, Sandoval-Castro et al. 2014, Mori et al. 2015a,b). A few studies have focused on the genetic composition of the 'central versus marginal' mangrove populations of *Avicennia marina*, *Excoecaria agallocha* and *Rhizophora stylosa* (Maguire et al. 2000b, Arnaud-Haond et al. 2006, Zhang et al. 2008, Islam et al. 2014, 2015). However, along the less investigated eastern and southeastern coast of Africa, the coastline morphology in peripheral mangrove habitats is characterized by deep inlets formed by estuaries that are often seasonally closed by sandbars (Rajkaran et al. 2009). This makes the range limit in South Africa an interesting but challenging case study as only a coupled comparison of core versus range-limit and open-bay versus inland, river-linked populations is possible.

In this study, we investigate the genetic diversity and structure of core and range limit *Avicennia marina* (Forssk.) Vierh. populations along the (south) eastern coast of Africa. We hypothesize an overall deviation from a single panmictic model and that the core region will be genetically differentiated from the range-limit region because of dispersal limitation, ocean current barriers and range-limit effects.

MATERIALS AND METHODS

Sampling design and study area

A total of 388 samples of *Avicennia marina* were collected in 12 locations (see Table 2), of which 6 were in Kenya and Tanzania (core region [C] populations) and 6 in South Africa (southern range limit [SRL] populations) along approximately 1000 and 700 km of coastline, respectively (Fig. 1). The C populations were all located in open (permanently accessible) bays, while the SRL populations were all inland, river-linked populations, intermittently locked from the open ocean. The natural setting did not allow a subset of permanently open bays at the SRL region nor vice versa in the C region, so the coastal geomorphology is a confounding factor that cannot be separated from geographic location. All sites constituted natural forests, except for the range-edge mangroves of Nahoon, which originates from an afforestation event with propagules from the Mgeni River mangroves in Durban (G. Naidoo pers. comm., Hoppe-Speer et al. 2014). We wanted to include the southernmost, natural *A. marina* popula-

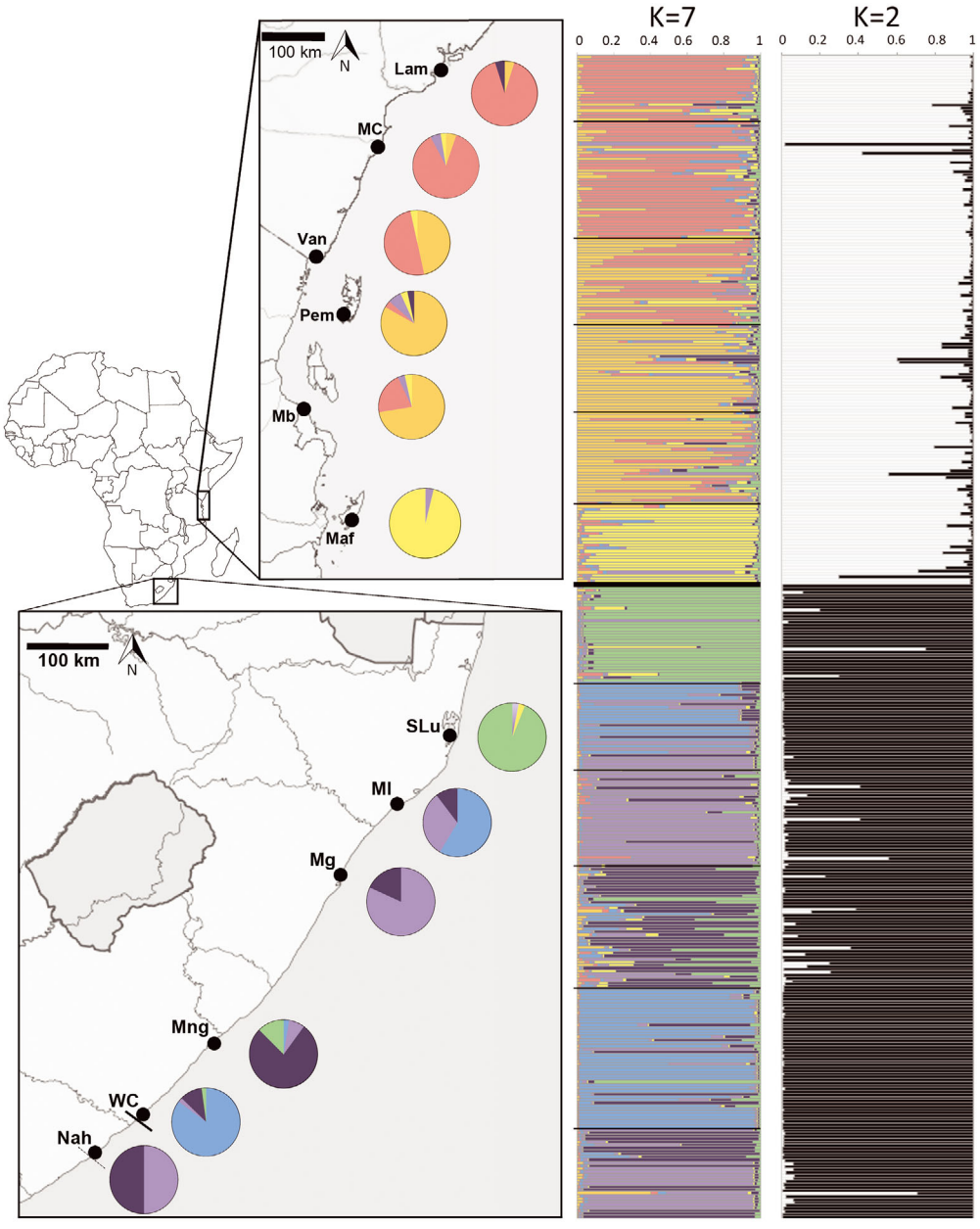

Fig. 1. Illustration of the 12 sample sites along the East African coast in Kenya/Tanzania (upper inset, core mangrove region) and South Africa (lower inset, southern periphery of *Avicennia marina*'s range). The natural range limit of *A. marina* (solid black line) is found at Wavecrest, although there is a more southern mangrove plantation in Nahoon (black dashed line or southernmost mangrove limit). The 12 sites are: Lamu Island (Lam), Mida Creek (MC), Vanga (Van), Pemba (Pem) Mbweni, Dar es Salaam (Mb), Mafia Island (Maf), St Lucia (SLu), Mlalazi (Ml), Mgeni, Durban (Mg), Mngazana (Mng), Wavecrest (WC) and Nahoon (Nah). Bar charts (right; percentage of attribution to different clusters per individual) ($K = 7$ and 2) and pie charts (percentage of individuals attributed to each of 7 clusters) summarizing the genetic structure inferred by STRUCTURE analysis are given

tion of South Africa, i.e. Kobonqaba in our sample design. However, on our fieldtrip in 2010, a die-back of approximately 90% of the *A. marina* trees was observed in Kobonqaba (Adams et al. 2010). Therefore, we opted to take the healthy and large estuary at Wavecrest approximately 5 km north of Kobonqaba as our southernmost natural sampling site.

At each site, 2 to 4 leaves per tree were collected along a transect parallel to the shore or river and stored in bags with dry silica gel for transport. To

Table 1. The 8 selected microsatellite markers used in this study: Am3 and 81, described by Maguire et al. (2000a), and Avma 1, 2, 8, 10 and 17, described by Geng et al. (2007). A modified version of Am81 was used as suggested by Arnaud-Haond et al. (2006). Locus name, repeat motif and forward/reverse primer sequence with indication of fluorescence marker (PET, VIC, 6-FAM and NED) are given

Locus	Repeat motif	Primer sequence (5′–3′)	Size range (bp)
Am3	$(TG)_{15}$	F: GGTTCCTGCAAGTATGTCAACACCCTC R: ACCTCGATTCCTCCCCGAATGC^{PET}	179–191
Am81	$(CA)_9(CT)_{16}$	F: ATCGGATGTTGCTACTCCTG^{VIC} R: CAAAGCCCCAAAAATAATCC	132–155
Avma1	$(TC)_6(AC)_8A_2(AC)_2$	F: TCTCTCTCTCTCACACACACAC R: CCAAAGAGTCACAGCAGAGGCACT^{VIC}	126–130
Avma2	$(TC)_6(AC)_9AT(AC)_3$	F: TCTCTCTCTCTCACACACACAC R: CTGTGTTGAGGTGGTTGATGAGAT^{VIC}	86–98
Avma8	$(TC)_6(AC)_7(AG)_2$	F: TCTCTCTCTCTCTCACACAC R: GGGCGAGGAGATGGGAAATTAG^{6-FAM}	175–181
Avma10	$(TC)_6(AC)_{10}$	F: TCTCTCTCTCTCTCACACAC R: CACCATTATATCTAGTGGCTTGTG^{NED}	68–80
Avma16	$(TC)_6(AC)_6$	F: TCTCTCTCTCTCACACACACAC R: TTTTAGGGTTGTGGACGTGGAAGG^{NED}	86–90
Avma17	$(TC)_6(AC)_{10}$	F: TCTCTCTCTCTCTCACACAC R: GCACTACCTGTTGATAGAGC^{PET}	70–76

maximize the probability of capturing genotypic diversity, we adjusted the transect length according to the size of the studied mangrove (ranging from 10 to 100 m between samples). Total genomic DNA extractions were performed on 20–30 mg leaf material using the E.Z.N.A. SP Plant DNA Mini Kit (Omega bio-tek, Norcross) or the NucleoSpin 96 Plant II (Macherey-Nagel) for subsequent microsatellite analysis.

Microsatellite analysis

Out of 16 microsatellite primer pairs developed by Maguire et al. (2000a) and Geng et al. (2007), 8 were selected based on their amplification performance and the polymorphism of East African populations (Table 1). The PCR mix to amplify the multiplex of the 8 fluorescence-labeled (VIC, PET, 6-FAM and NED, see Table 1) microsatellite primers (Life Technologies) was made based on the Qiagen Multiplex PCR Kit (Qiagen) and consisted of 25 µl reactions containing 5 µl H_2O, 12.5 µl QIAGEN Multiplex PCR Master Mix, 2.5 µl primer mix and 5 µl DNA. The primer mix consisted of 2 µM of each forward and reverse primer. PCRs were carried out in a BIO-RAD T100 thermal cycler (Bio-Rad Laboratories,), starting with an initial activation step of 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 57°C and 90 s at 72°C, and ended with a final extension for 10 min at 68°C. Fragment analysis was performed at Macrogen, after which allele sizes were assessed with the software Genemarker 2.4.0 (Softgenetics).

Genetic diversity analysis

GenAlEx 6.5 (Peakall & Smouse 2012) was used to calculate parameters of genetic diversity, such as average number of alleles, observed heterozygosity (H_o) and unbiased heterozygosity (uH_e), and FSTAT 2.9.3.2 (Goudet 2001) was used to calculate the allelic richness (A_r). A Mann-Whitney U-test (MWU) was performed to test for difference in allele numbers between C and SRL populations. We used INEst 2.0 (Chybicki & Burczyk 2009) to check for the possibility of null alleles in sample sites with a heterozygote deficit (50 000 burn-in and 500 000 cycles keeping every 50th update), MICRO-CHECKER to detect large allele dropout and Bottleneck 1.2.02 (Cornuet & Luikart 1996) to test for possible recent bottleneck events. For the latter, we ran the 2-phase mutation model (95% single-step mutations, 12% variance, 10 000 iterations) using the mode shift and 1-tailed Wilcoxon signed-rank test to test for significancies (Williamson-Natesan 2005, Peery et al. 2012).

Genetic structure analysis

To investigate the population genetic structure, pairwise F_{ST} values (Weir and Cockerham's θ) as well as the within-population inbreeding coefficient F_{IS} (Weir and Cockerham's f) were calculated along with their significance levels with FSTAT (Weir & Cockerham 1984, Goudet 2001). Principal coordi-

nate analyses (PCoA), an analysis of molecular variance, probability of identity and the standardized genetic differentiation (F'_{ST}) per pair of populations were calculated with GenAlEx. To test for linear association between genetic distance and geographic distance (isolation by distance), Mantel tests were conducted between pairwise F_{ST} values and the shortest coastal distance between population pairs (using Google Earth, www.google.com/earth/index.html). Mantel tests were performed for the whole data set, the C populations and the SRL populations (9999 permutations). The afforested site of Nah was excluded from this analysis because of its artificial origin. Furthermore, a 2-sided comparison (10 000 permutations) of A_r, H_e, H_s (within-population heterozygosity), F_{IS} and F_{ST} was done (FSTAT), again excluding Nah, to check whether these parameters were significantly different between C and SRL populations. Lastly, we ran the program STRUCTURE 2.3.2 (Pritchard et al. 2000), using the admixture model with correlated allele frequencies without prior population information, to infer genetic structure in our different populations. We carried out 20 replicates for $1 \leq K \leq 15$ with 1 000 000 Markov chain Monte Carlo iterations, discarding the first 100 000 as burn-in for all 12 populations together. We used STRUCTURE HARVESTER 0.6.94 (Earl & von Holdt 2012) to implement the Evanno method to infer the optimal K through the ΔK statistic, which is based on the rate of change of log probability of the data between successive K-values (Evanno et al. 2005).

RESULTS

Descriptive population statistics and genetic diversity

A total of 42 alleles were detected for 8 microsatellite loci in 388 individuals. Loci were polymorphic for all populations, with the exception of Avma16, which was only polymorphic in the Maf population (C), and Avma1, Avma17, Am81 and Avma8, which were monomorphic in the Ml, WC and Nah populations. Private alleles were only found in C populations, with a maximum of 4 per population (Table 2). No significant linkage disequilibrium was detected between the different loci ($p < 0.05$, data not shown) and the probability of identity showed that samples could be identified correctly with the 8 selected loci (data not shown). Null alleles may be present in 2 populations (Lam and Pem). No evidence for large allele dropout was detected for any of the loci. Evidence of recent bottleneck events (1-tailed Wilcoxon signed-rank test, $p < 0.01$) was found in 2 C (Mb and Maf) and 3 SRL populations (Ml, Mg and WC).

Total numbers of alleles per population for all loci ranged between 11 and 30, and were significantly higher in C populations than in SRL populations (MWU: $p < 0.01$, $N = 12$), with an allelic richness per population between 2.67 (Mb) and 3.66 (Lam) in the C populations and between 1.33 (Ml) and 2.41 (Mng) in the SRL populations (Table 2). Forty-one (41) and 22 alleles occurred in the C and SRL regions, respectively. Out of these, 21 alleles occurred only in the C

Table 2. Sample locations in Kenya, Tanzania (Core) and South Africa (southern range limit, SRL), with population names and codes, sample size (N), allelic richness (A_r), unbiased expected heterozygosity (uH_e), observed heterozygosity (H_o), within-population inbreeding coefficient (F_{IS}, *significant at $p < 0.05$), private alleles (PA), the p-values (p_w) of the 1-tailed Wilcoxon signed-rank test (2-phase mutation model with 95% single-step mutations and 12% variance) and mode shift test (yes or no) to indicate possible recent bottleneck events

Region	Population	Code	N	A_r	uH_e	H_o	F_{IS}	PA	p_w	Mode shift
Core										
Kenya	Lamu Island	Lam	21	3.66	0.52	0.46	0.12	0	0.527	N
	Mida Creek	MC	39	3.42	0.49	0.45	0.08	0	0.230	N
	Vanga	Van	30	3.56	0.54	0.55	–0.03	1	0.125	N
Tanzania	Pemba	Pem	30	3.08	0.35	0.31	0.14	2	0.273	N
	Mbweni	Mb	29	2.66	0.49	0.38	0.24*	0	**0.002**	Y
	Maf	Maf	27	2.83	0.43	0.27	0.38*	4	**0.010**	N
SRL										
South Africa	Saint Lucia	SLu	33	1.85	0.12	0.06	0.52*	0	0.098	N
	Mlalazi	Ml	29	1.33	0.13	0.04	0.71*	0	**0.002**	Y
	Mgeni	Mg	33	1.78	0.17	0.12	0.30*	0	**0.027**	N
	Mngazana	Mng	40	2.40	0.25	0.14	0.44*	0	0.371	N
	Wavecrest	WC	47	1.48	0.12	0.06	0.50*	0	**0.006**	Y
	Nahoon	Nah	30	1.68	0.20	0.07	0.64*	0	**0.002**	Y

Table 3. Pairwise F'_{ST} values. All values are highly significant ($p < 0.05$, 66 000 permutations)

	Lam	MC	Van	Pem	Mb	Maf	SLu	Ml	Mg	Mng	WC
MC	0.122										
Van	0.092	0.126									
Pem	0.408	0.315	0.236								
Mb	0.190	0.193	0.093	0.215							
Maf	0.242	0.343	0.323	0.431	0.391						
SLu	0.622	0.621	0.622	0.662	0.575	0.592					
Ml	0.557	0.418	0.528	0.510	0.530	0.570	0.738				
Mg	0.480	0.391	0.472	0.553	0.421	0.635	0.646	0.469			
Mng	0.560	0.473	0.521	0.408	0.435	0.559	0.475	0.421	0.489		
WC	0.641	0.541	0.619	0.546	0.583	0.555	0.729	0.485	0.654	0.473	
Nah	0.506	0.389	0.471	0.379	0.448	0.562	0.618	0.178	0.395	0.224	0.520

region, 20 were shared among C and SRL regions, and 1 allele occurred only in the SRL region. The standardized number of alleles per locus per population (based on smallest population size N = 21) ranged from 1.00 (monomorphic loci) to 4.48 (Van). The H_o and the uH_e were significantly higher in C compared with SRL populations (MWU: $p < 0.01$, N = 12) and ranged between 0.12 (SLu and WC) and 0.54 (Van) (Table 2). Furthermore, all individuals of the C populations, except 1 from MC, exhibited unique multi-locus genotypes (MLGs), while there were only 106 unique MLGs on 233 sampled SRL individuals, containing 1 to 4 fixed loci out of 8 in the SRL populations. All repeated MLGs were homozygous for each locus and no heterozygous repeated MLGs were encountered. Therefore, we assume these to be the result of inbreeding events and not clonal growth.

Population genetic structure

F_{IS} values ranged from −0.03 to 0.71 and were significantly higher than expected ($p < 0.05$) in 2 C populations (Mb and Maf) and all 6 SRL populations, indicating inbreeding at the within-population level. Pairwise F'_{ST}-values comparisons between populations were all significant ($p < 0.05$), indicating genetic subdivision between the populations. The lowest differentiation was found among C–C populations (0.092–0.431), but there was much higher differentiation between C–SRL (0.379–0.662) and between SRL–SRL populations (0.178–0.738) (Table 3). A 2-sided comparison of the allelic richness, observed heterozygosity, within-population heterozygosity levels, and F_{IS} and F_{ST} between both groups of populations all showed significant differences (Table 4). These 2 discrete groups were further confirmed by the PCoA at both the population and individual level (Fig. 2). The STRUCTURE analysis on all 12 populations gave $K = 2$ as well as $K = 7$ as the 2 optimal K possibilities (Fig. 1). When running STRUCTURE separately for C and SRL populations, K–values of 3 and 4 were found, respectively. The Mantel tests indicated significant isolation by distance in the whole data set ($p = 0.002$, $R^2 = 0.111$), but not within the C ($p = 0.192$, $R^2 = 0.071$) and SRL ($p = 0.409$, $R^2 = 0.004$) group of populations (see Fig. A1 in the Appendix).

DISCUSSION

Core versus range-limit populations

Comparison between 6 populations of *Avicennia marina* at its range core (Kenya, Tanzania) and 6 populations at its southern range limit (SRL; South Africa) revealed significantly more inbreeding and lower genetic diversity at the range limit compared with the core populations, which is in accordance with the central–marginal hypothesis. Although the overall isolation by distance, considering all 12 popu-

Table 4. Two-sided comparison (10 000 permutations), between core (C) and southern range limit (SRL) mangrove populations, of allelic richness (A_r), observed heterozygosity (H_o), within-population heterozygosity (H_s), within-population inbreeding (F_{IS}) and genetic differentiation (F_{ST}). All comparisons are significant (bold) at $p < 0.01$, except for F_{IS} at $p < 0.05$

	A_r	H_0	H_S	F_{IS}	F_{ST}
C	3.20	0.40	0.47	0.14	0.14
SRL	1.95	0.108	0.19	0.44	0.45
P-value	**0.006**	**0.002**	**0.002**	**0.017**	**0.0001**

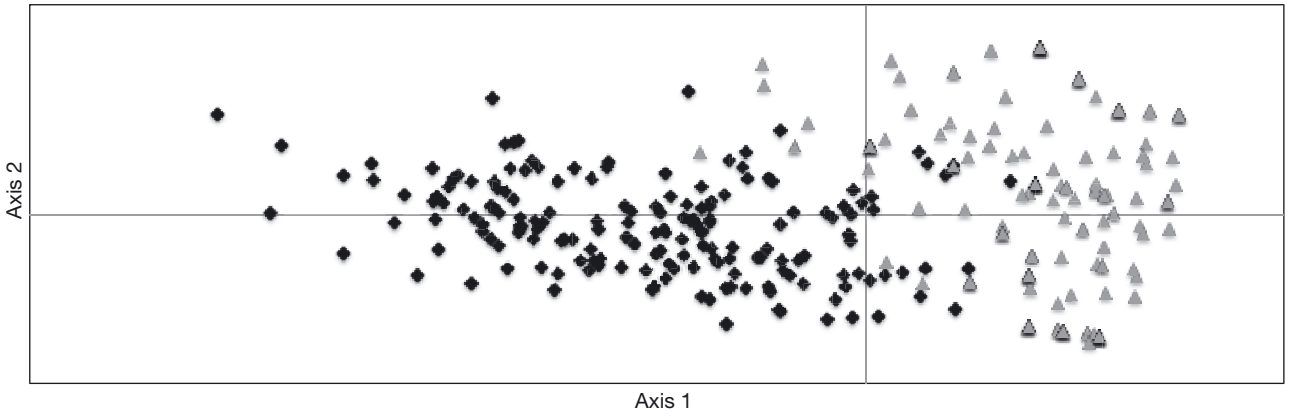

Fig. 2. Principal coordinate analysis of the genetic distances of all sampled *Avicennia marina* individuals. Black diamonds: core populations; grey triangles: southern range limit populations. The first and second axes explain 28.4% and 18.9% of the variation, respectively

lations (C and SRL), was sinificant, as could be expected due to the lack of intermediate geographic distances, no smaller-scaled significant relationship with distance was observed within either the C or the SRL regions. The non-significance was caused by low levels of differentiation irrespective of distances within the C region and high levels of genetic differentiation (even at short distances) in the SRL region, reflecting more drift or inbreeding in the latter. The overall strong genetic differentiation detected among the studied *A. marina* populations suggested poor connectivity between the C and SRL regions. The South Equatorial Current that bifurcates at the level of the southern part of the Tanzanian coastline (Lutjeharms & Bornman 2010) could be an important factor explaining the observed genetic differentiation between the C and SRL populations, as it may create a barrier for dispersing propagules. As gene flow over long distances in mangroves is mediated by propagule dispersal, the present distribution of discrete *A. marina* subpopulations could be the result of poor propagule dispersal capacity (short viability and buoyancy period), the (ocean) current system, environmental constraints and (past) environmental conditions (Maguire et al. 2000b, Arnaud-Haond et al. 2006).

In contrast to the open, more accessible coastline of Kenya and Tanzanya (C region), the South African coastline (SRL region) is typically sandy or rocky with steep dune formations that only get breached by rivers at a few locations along the shore. Hence, the potential habitat for mangroves is limited to these landward river or estuary fringes, hampering gene flow between different mangrove sites. Additionally, river mouths in South Africa often close for various periods of time as a result of sediment accumulation, which could result in a reduction or disappearance of mangrove populations (Harrison et al. 2000). Only 17 of the 76 South African estuaries are permanently open, clearly illustrating the low availability of suitable establishment opportunities for dispersing and seaborne propagules (Harrison et al. 1999). Breen (1969) and Bruton (1980) found mangrove die-off after 5 to 8 mo of constant pneumatophore submersion in 2 South African mangrove systems. Recently, a die-back of approximately 90% of the *A. marina* trees in the southernmost natural mangrove forest Kobonqaba was observed as a result of river mouth closure, which caused high water levels in the estuary that submersed the pneumatophores and 'drowned' the trees (Adams et al. 2010). Subsequent die-off of a substantial part of the mangrove population because of recurrent opening and closing of a river mouth could have induced repeated bottleneck events in history, thereby contributing to the current overall low genetic diversity, heterozygote deficiency and high differentiation. The bottleneck events found in the SRL region may thus be due to historical founder events, with one or a few having taken place, without subsequent gene flow between inland, river-fringed populations (Table 2). In the C region (Mb and Maf, Table 2) bottlenecks may have originated either from very small effective population sizes or from an inadequate sample size. However, we think that the latter is less likely than the former, because the sample size is expected to be adequate (around 30 individuals per population) according to Peery et al. (2012).

On the one hand, in the SRL, sand banks can temporarily or permanently close the estuary mouth. This could be due to upstream draining of freshwater for agricultural and development purposes, with sub-

sequent decrease in river discharge, sometimes in combination with sand deposition during sea storms (Rajkaran et al. 2009). On the other hand, an increase of approximately 40% of South African mangrove area cover was observed (Saintilan et al. 2014), indicating that the dynamic coastline opens up possibilities for establishment of new mangrove forests within its current range. For example, Steinke & Ward (2003) observed a colonization by *A. marina* along the Kwelera River, some 60 km south of the most southern natural population at Kobonqaba. Also, in some of the larger estuaries in South Africa there has been an increase in mangrove area. At Richards Bay, the artificial establishment of an estuary mouth and availability of new intertidal habitat led to rapid colonization by *A. marina* (Bedin 2001). This again shows that although *A. marina* is a pioneer species (Osborne & Berjak 1997) with good dispersal potential of its propagules, the geomorphology of the coastline is a major limiting factor of the effective dispersal rates in South Africa.

In comparison to all other SRL populations, the Mngazana and Mgeni populations have the highest diversity and lowest within-population inbreeding. This could partly be explained by the fact that they are among the larger mangrove areas in South Africa and have continuously open river mouths. Mngazana, for example, is home to the third largest mangrove area in South Africa (145 ha in 1999; Adams et al. 2004). In contrast, the large mangrove population at St Lucia has extremely low levels of heterozygosity and high within-population inbreeding (Table 2), which could be the result of historical and recent river mouth closure (Hoppe-Speer et al. 2013).

We expect high within-population pollen and propagule flow in the SRL and C populations, resulting in the mixing of genes within populations. This is because the flowers of *A. marina* are visited by a wide variety of insects (no pollinator limitation), which is attributable to its nectar (Raju et al. 2012). Many healthy propagules were observed on trees, dispersing in the water column and stranded along the intertidal area (Fig. 3). Moreover, protandry, a reproductive mechanism in which the anthers release their pollen before the stigma of the flower becomes receptive, could be an effective mechanism to reduce selfing in *A. marina* trees from Kenya (D. J. R. De Ryck & T. Van Der Stocken unpubl. data). However, protandry only adds to local gene flow as it causes higher cross-fertilisation rates, but it will not contribute much to the level of genetic diversity, as pollinators are unlikely to be able to cross the gap between populations (Fig. 1). Even under high levels

Fig. 3. Large numbers of viable, stranded *Avicennia marina* propagules along the coast of South Africa (just south of St Lucia). This site is not suitable for establishment

of pollen flow, an original population low in genetic and allelic diversity will remain that way unless new alleles arrive by means of propagules from other distant populations.

We conclude that there is high level of historical gene flow in the larger C mangrove populations in contrast to a very limited gene flow in the inland, river-linked and often secluded SRL populations. The resulting strong pattern of differentiation with low allelic richness and recent bottleneck events at the southern range edge most likely originated because of single successful dispersal events with subsequent inbreeding and dispersal limitation caused by coastal geomorphology as well as the dynamics of the South African river systems (open/closed). The overall lower allelic richness thus most likely results from distributional range effects, whereas local inbreeding reflects an effect of geomorphology.

Our case study, comparing core and range-limit populations along the east African coast, revealed a special situation of dispersal limitation at the southern range edge. The very low genetic diversity and gene flow that we found at the SRL suggests that the suitable mangrove habitat, i.e. available intertidal area in estuaries that are permanently open to the sea, is limited and hard to reach, even though *A. marina* propagules possess the dispersal capacity to overcome the distance between different mangrove populations in South Africa (Fig. 3) and belong to the most cold-tolerant mangrove genus. The mangrove plantation in the Nahoon Estuary, south of the 'natural' mangrove range limit (Wavecrest), with healthy, fast-growing *A. marina*, *Rhizophora mucronata* and *Bruguiera gymnorrhiza* trees as well as forest expan-

sion, gives a direct indication that the species' range limit is not in equilibrium with the climate-induced or physiological limit (Steinke 1999, Quisthoudt et al. 2013, Saintilan et al. 2014). Given the right dispersal conditions, *A. marina* (but also *B. gymnorrhiza* and *R. mucronata*) may be able to extend further southward, beyond their current natural distribution. Other studies have also shown that *Avicennia*, the most cold-tolerant genus, seems highly adaptive and can readily expand as extreme cold events decrease (Pickens & Hester 2011, Cavanaugh et al. 2014, Saintilan et al. 2014).

Conservation

The geomorphological outline of the South African coastline in combination with increasing human-induced pressure poses several conservation issues for the mangroves. If a mangrove forest is being reduced in area or disappears, there is a very low possibility of recruiting new alleles or propagules, respectively, because effective dispersal between different mangrove systems is low. This is indicated by the low allelic richness and heterozygosity, high within-population inbreeding (potential for further fixation of alleles) and high differentiation between populations at the range limit. South African mangroves provide an ideal system to contribute further to the discussion on the importance of genetic diversity on adaptive potential. The healthy and expanding planted mangroves in Nahoon, notwithstanding its low genetic diversity, could be proof of the potential for expansion of the East African mangrove range.

CONCLUSIONS

Our study showed significantly higher inbreeding levels and lower genetic diversity of *Avicennia marina* populations at the species' southern range limit as compared with the core populations, which is in accordance with the central–marginal hypothesis. Despite the fact that *A. marina* propagules have the capacity to disperse between populations, gene flow was moderate to high within the C region but extremely low within the SRL region. A combination of historical single successful dispersal events with subsequent inbreeding because of the geomorphology of the coastline, the dynamics of river systems (open/closed) and other genetic range-edge effects may explain the pattern of differentiation, with low allelic richness at the southern range edge. This study highlights a genetically depauperate situation in peripheral populations, as a consequence of dispersal limitation partly owing to coastal geomorphological features.

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Appendix

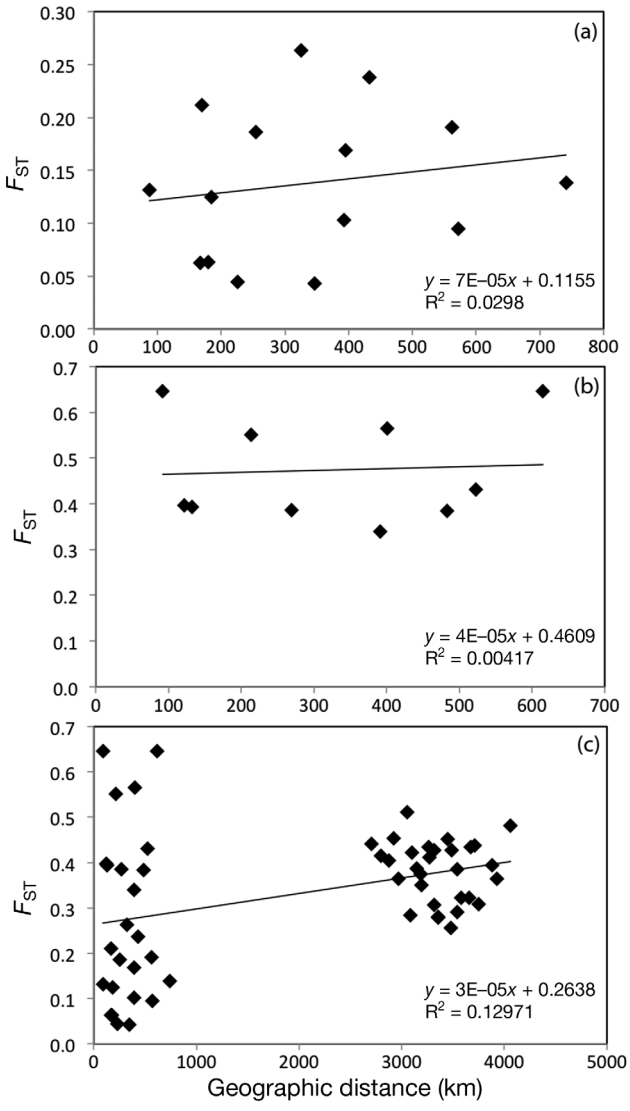

Fig. A1. Mantel tests (9999 permutations) of (a) the core (p = 0.31) and (b) southern range limit *Avicennia marina* populations (p = 0.41) separately, as well as (c) the total of 11 populations (p = 0.003, Nahoon plantation excluded) with the genetic differentiation (F_{ST}) as a function of the geographic distance between each pair of populations. The geographic distance is the shortest path by water between 2 populations (Google Earth, www.google.com/earth/index.html). The equation of the regression line and Pearson correlation coefficient (R) are indicated in each panel

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