COMPARATIVE STUDY OF THE DISJUNCT ZONATION PATTERN OF THE GREY MANGROVE *AVICENNIA MARINA* (FORSK.) VIERH. IN GAZI BAY (KENYA)

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ABSTRACT

The disjunct zonation pattern of *Avicennia marina* (Forsk.) Vierh., frequently observed along the Kenyan coast as a landward and a seaward zone, has been studied on a morphological level and complemented with preliminary genetic data. The objective was to describe the two zones in-depth in order to provide a background ecological setting that may be used in explanations on the emergence of such a bimodal zonation. The vegetation structure in the two zones is described by means of the Braun-Blanquet relevé and PCQ-methods, root- and leaf-morphological characteristics, and environmental variables to provide a background ecological setting. The two zones show considerable differences in physiognomy of *A. marina* trees, and indicate that the differences may constitute an environmental response. Results show that in the landward *A. marina* zone, tree heights, leaf sizes, density, and total length of pneumatophores tend to be smaller as compared to the same attributes of the trees in the seaward zone. Differences in leaf stomatal density and in above- and below-ground pneumatophore length, the latter closely related to the effect of inundation, were observed. This suggests a considerable adaptability and plasticity with respect to environmental conditions. The hypothesis for the genetic analysis was that mangrove fringes as close as 105 m from one another constitute a same genetic pool. Preliminary genetic analysis using 48 amplified DNA fragments (RAPD - Randomly Amplified Polymorphic DNA) from 37 landward and seaward trees revealed significantly different frequencies for four DNA fragments, indicating that there might be less contact and genetic interchange between the two zones than within each zone. Whether this phenomenon, coupled to the different environmental conditions, can lead to further divergence cannot be answered yet.

Of all mangrove zonation patterns described worldwide by various authors, that of *Avicennia marina* (Forsk.) Vierh. provides an incentive for particular analysis because of its disjunct nature (Watson, 1928; Macnae, 1968, 1969; Johnstone, 1983; Smith, 1987a, 1992). In Kenya this species displays a disjunct zonation pattern across the intertidal zone (Dahdouh-Guebas et al., 2002). *Avicennia marina* is present in the most landward as well as seaward fringe of the mangrove forest at certain locations along the Kenyan coast.

The same pattern has been reported for other specific regions of the world; however, it has been described visually or superficially without intensive sampling or in-depth comparisons. Walter and Steiner (1936) described the higher tidal *A. marina* trees as "exceptions" of seaward specimens. Other authors (e.g., Macnae, 1968; Johnstone, 1983; Smith, 1992) reported a "double zonation" of *A. marina*, but did not go beyond stating this observation or mentioning as personal observations that tree height or leaf size varies between the upper and lower intertidal zones. This paper provides a more rigorous comparison of the landward and seaward *A. marina* fringe on a morphological level (such as tree height, leaf size, root density, etc.), as an ecological setting framing the emergence of the two disjunct zones. We also provide preliminary findings on genetic differentiation between the observed zones. Rather than aiming at a generalization from

this type of research, we try to unveil the structure of a local mangrove and comparable forests.

The genus *Avicennia* L. (Avicenniaceae) comprises eight species, occupying diverse mangrove habitats (Tomlinson, 1986; Duke, 1991). *Avicennia marina* is the species with the widest distribution, with phenological trends according to latitude (Duke, 1990), and it is the only representative of the genus in Kenya (Kokwaro, 1985; Tomlinson, 1986). It is often considered to play an important pioneering role in plant succession (e.g., Osborne and Berjak, 1997). Some taxonomic variation within *A. marina* has been proposed in reviews by Bakhuizen van den Brink (1921) and Moldenke (1960, 1967). These authors recognized seven varieties, which are mainly based on geographic segregation (Tomlinson, 1986). Duke (1991) found that there were only three varieties within *A. marina*. Of all varieties, *A. marina* (Forsk.) Vierh. var. *marina* Moldenke is the only one known to have a limited range in East Africa (Tomlinson, 1986; Duke, 1991). However, the identification of *Avicennia* species in East Africa has always been restricted to *A. marina*. For a clear description of the species we refer to Duke (1991).

In Gazi Bay (Kenya), as well as other sites in Kenya such as Lamu (pers. obs.), *A. marina* is observed both in the landward and seaward mangrove fringe (e.g., Dahdouh-Guebas et al., 1998, 2002; Ochieng and Erftemeijer, 2002) and physiognomic differences of trees between these two zones are apparent. The aim of this study was to investigate the disjunct zonation pattern of *A. marina* across the mangrove belt in our study site and to compare the plant attributes from the two zones. Our hypothesis was that phenotypic plasticity within the species may allow diverse physiognomy due to different environmental conditions in seaward vs. landward zones. In the first approach, we compare the rooting system, leaf morphology and physiognomy in the two *Avicennia* zones. Then, we investigate whether the observed differences between the zones correlate with selected environmental factors that may lead to physiological or morphological plasticity. Since the *Avicennia* zones considered in this study are located as close as 105 m from each other, we hypothesized that they are part of only one population, with no significant genetic differences. We do not address the vegetation causes of the emergence of the two zones, but describe the ecological setting as reflected in physiognomy, morphology, and preliminary genetic differentiation.

MATERIALS AND METHODS

STUDY LOCATION.—Gazi Bay (4°25′S, 39°30′E), Kenya, an open estuary located about 50 km south of Mombasa, is fed by two seasonal rivers, the Kidogoweni and the Mkurumuji (Fig. 1). All ten East-African mangrove species can be found in Gazi Bay, including *A. marina*, *Bruguiera gymnorrhiza* (L.) Lam., *Ceriops tagal* (Perr.) C.B. Robinson, *Heritiera littoralis* Dryand., *Lumnitzera racemosa* Willd, *Pemphis acidula* Forst., *Rhizophora mucronata* Lam., *Sonneratia alba* Sm., *Xylocarpus granatum* Koen, and *X. moluccensis* (Lamk.) Roem. However, the species diversity and abundance across the intertidal complex varies with location within and among mangrove forests (Dahdouh-Guebas et al., 2002).

STRUCTURE OF THE VEGETATION.—In an undisturbed part of the mangrove forest along the major creek that connects the Kidigoweni with the bay, the width of the mangrove belt was surveyed using a transect between the landward terrestrial vegetation and the creek, hereafter called "Transect 1" (Fig. 1). Both on aerial photography and on the field, this transect proved to be representative for the disjunct pattern of *A. marina* (Fig. 1C). The Braun-Blanquet relevé method (Westhoff and Van der Maarel, 1978; Van der Maarel, 1979) was used in 5 × 5 m quadrats at 10 m intervals between quadrats to generate a qualitative description of the vegetation. Along the same transect the Point-Centered Quarter Method (PCQM) of Cottam and Curtis (1956) was used to describe
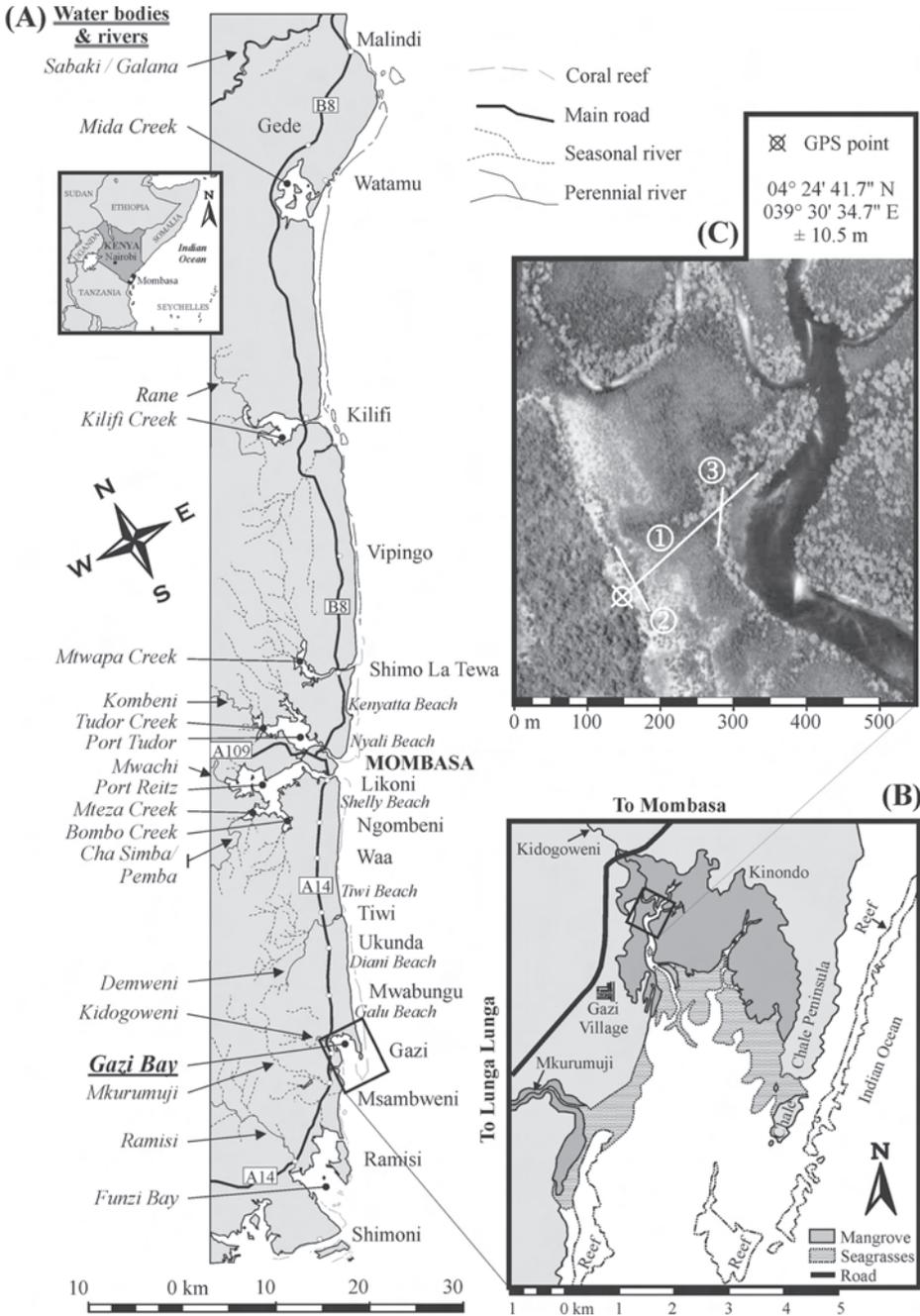
Figure 1. A) Map of the Kenyan coast showing B) a close-up of our study site Gazi Bay; and C) an aerial photograph from 1992 showing the location of Transects 1, 2, and 3. Basic coastal features were redrawn from Dahdouh-Guebas et al. (2000) and Slim (1993).

the vegetation quantitatively (cf. Cintrón and Schaeffer Novelli, 1984). The diameter D_{130} [term according to Brokaw and Thompson (2000), but formerly referred to as DBH, the diameter at breast height] of trees with a D_{130} greater than 2.5 cm was measured. Common mangrove tree

anomalies (prop roots, forking stems) that inhibited a normal D_{130} measurement were dealt with as described by Cintrón and Schaeffer Novelli (1984). The tree height was measured using a clinometer. The topographic gradient was determined at 10 m intervals along the transect by use of a theodolite, with the known maximum water height above datum, as indicated in tide tables available from the Kenya Port Authority, as a benchmark. Elevations were expressed as "meters above datum" and the error of the theodolite was experimentally recorded as 3 cm. Each zone was additionally sampled by a transect running approximately parallel with the water line, entirely within each of the zones: one, 100 m transect, hereafter called "Transect 2," was placed in the most landward *Avicennia* zone at an average of 3.16 m above datum, the other of 80 m length, hereafter called "Transect 3," in the most seaward *Avicennia* zone at an average of 1.32 m above datum. Along these, vegetation analysis was carried out as before.

COMPARISON OF HEIGHT AND SALINITY.—Soil salinity was measured with a precision of 1 at 10 m intervals along Transect 1 using an ATAGO refractometer. The mean height of *A. marina* trees per PCQM sample point, which consists of four quadrants in which the closest tree was surveyed (see Cintrón and Schaeffer Novelli, 1984), was then compared to the salinity.

COMPARISON OF ROOT SYSTEM.—In each of the *A. marina* zones along Transect 1, quadrats of 1 m² were established with an interspace of 25 cm (n = 12 in the landward zone, and n = 10 in the seaward zone). Within each quadrat the number of pneumatophores was counted and their aboveground length was recorded. In addition, four randomly chosen pneumatophores per quadrat were excavated down to the cable root to measure their belowground length. Since little variation in belowground length was observed within these areas of 1 m², the average of the four measurements was added to each of the aboveground length measurements to obtain the total pneumatophore length. For each of the above variables and parameters, the correlation with the height above datum was calculated using Spearman's rank correlation coefficient (r_s) as described by Sokal and Rohlf (1981) and Kent and Coker (1992).

Because of the non-normality within the data set, the significance of the observed differences in root characteristics between the landward and seaward *A. marina* zones for each of these factors was tested with the Mann-Whitney U-test (Sokal and Rohlf 1981; Kent and Coker 1992).

COMPARISON OF LEAF MORPHOLOGY.—One thousand leaves were collected haphazardly from all the trees in each *A. marina* zone to investigate leaf morphology (n = 1000 in the landward zone, and n = 1000 in the seaward zone). For each leaf the following characteristics were measured: petiole length, lamina length, and maximal lamina width. Similar to the comparative analysis of the root system, between-zone differences pertaining to leaf characteristics were calculated using the Mann-Whitney U-test (loc. cit.).

Stomatal density was investigated in the two *A. marina* zones in view of the different inundation regimes and salinity levels. For this purpose, leaves from 16 trees were taken in each zone. On each tree the fifth leaf pair of a branch was sampled and one of the two leaves on this position was collected, thus avoiding young, not fully developed leaves. Following collection in the field, leaves were frozen and further treated in the laboratory within 2 mo of collection. Leaf epidermis was removed by first boiling the leaves for 1 min in a 10% potassium hydroxide solution (Sidhu, 1975), then transferring them into cold water in a Petri dish. The epidermis strips were then washed in sodium hypochlorite to bleach impurities. Stomatal densities could thus be investigated using the epidermis strips from the abaxial leaf sides. The stomatal density was calculated by counting the stomata within the cells of a grid with known dimensions and visualized within the ocular of the microscope. Since it is known that within one species stomatal density can vary due to environmental conditions (Al-Farrajii, 1983), and it may even vary on the surface of one leaf (e.g., distance to central vein), the epidermis strips were always taken from the same location on a leaf.

GENETIC DIFFERENTIATION.—The genetic identity of a total of 37 randomly sampled *A. marina* trees from the landward (n = 17) and seaward zone (n = 20) of the forest was studied to test for genetic differences between zones. Leaf samples were transported to the laboratory in Brussels for DNA analysis within 48 hrs of collection. DNA extraction was carried out according to Abeysinghe et al. (1999) and amplification conditions according to Williams et al. (1993). Genomic DNA

of four individuals from *A. marina* were amplified using 45 arbitrary decanucleotide primers obtained from Operon Technologies, Inc. (U.S.) to find out which primers show polymorphism. A final selection of 11 primers was made on the basis of their reproducibility. The reproducibility of all amplified products was tested by repeating the same reaction at least twice. Since RAPD markers (Randomly Amplified Polymorphic DNA) dominate, a particular DNA band (locus), which is generated from the genome of one individual but absent from a second individual of the same species, represents a polymorphism. The amplification products for all samples were compared to each other and screened for the presence or absence of specific markers. To assess the genetic structure within and among *Avicennia* populations, all 48 amplified DNA fragments were compared at relative frequencies. Genetic parameters (allele frequencies, Fst and genetic distance) were calculated with the software program RAPD-SURV (now named AFLP-SURV; Vekemans, 2002; Vekemans et al., 2002). This program computes, for each population and locus, the fraction of individuals in the population with the marker (1-x) and without the marker (x), then the estimated frequency of the marker allele (1-q) and of the null allele (q). All estimators are from Lynch and Milligan (1994).

RESULTS

STRUCTURE OF THE VEGETATION.—The disjunct zonation pattern of *A. marina* can clearly be observed as a landward (0–90 m distance along Transect 1) and a seaward fringe (195–235 m distance along Transect 1) with a gap of 105 m between them, where this species is absent (Fig. 2). This large gap also justifies the distinction between a "landward" and "seaward" zone. *Ceriops tagal* and *R. mucronata* display wide zones along Transect 1 with high Braun-Blanquet coefficients (≥ 5). Together with *A. marina*, these species constitute the bulk of the forest cover in the area and contribute most to the total cover-abundance. All other species appear in small zones, and of these only *S. alba* scores 4 on the Braun Blanquet cover-abundance scale. The drop in total cover-abundance at about 60 m along Transect 1 is due to the presence of an open space. The total cover-abundance is considerably lower in the first ca. 80 m along Transect 1 as compared to the following ca. 180 m.

Avicennia marina, *C. tagal*, and *R. mucronata*, are the most important species along Transect 1 (Table 1). Density, basal area, and frequency do not necessarily contribute equally to the importance value of a species. For instance, *C. tagal* has a very low relative basal area, whereas seaward *A. marina* has the highest, but a rather low relative density and frequency (Table 1). The high degree of monospecificity of the landward *A. marina* fringe is a less apparent result in the qualitative Braun-Blanquet analysis (Fig. 2), but becomes conspicuous in Table 2A. The high importance of *A. marina* in the seaward fringe and its recognition as a true zone is also corroborated by the results in Table 2B. Comparison between the two *A. marina* fringes reveal a higher mean density in the landward fringe, but a much smaller mean basal area as compared to the seaward fringe.

COMPARISON OF HEIGHT VS SALINITY.—A shift in soil salinity and tree height can be discerned at about 100 m along Transect 1 or about 2.54 m above datum (Fig. 3A). In the landward *A. marina* fringe the appearance of the trees is rather shrub-like, from 2.5–5.5 m (mean height = 3.2 m). *Avicennia marina* mangroves in the seaward fringe are robust trees, ranging between 7–12 m (mean height = 10.2 m).

COMPARISON OF ROOT SYSTEM.—For the landward *A. marina* fringe, the pneumatophore density varied between 4–250 m^{-2}, with an average of 121.79 m^{-2}, whereas for the seaward fringe the range was between 44–1950 m^{-2}, with an average of 1091.28 m^{-2} (Fig. 3C). This statistically significant difference (U = 16; $U_{critical}$ = 34; n_1 = 12; n_2 = 10; P < 0.02) may indicate that there is a higher pneumatophore density with longer inundation
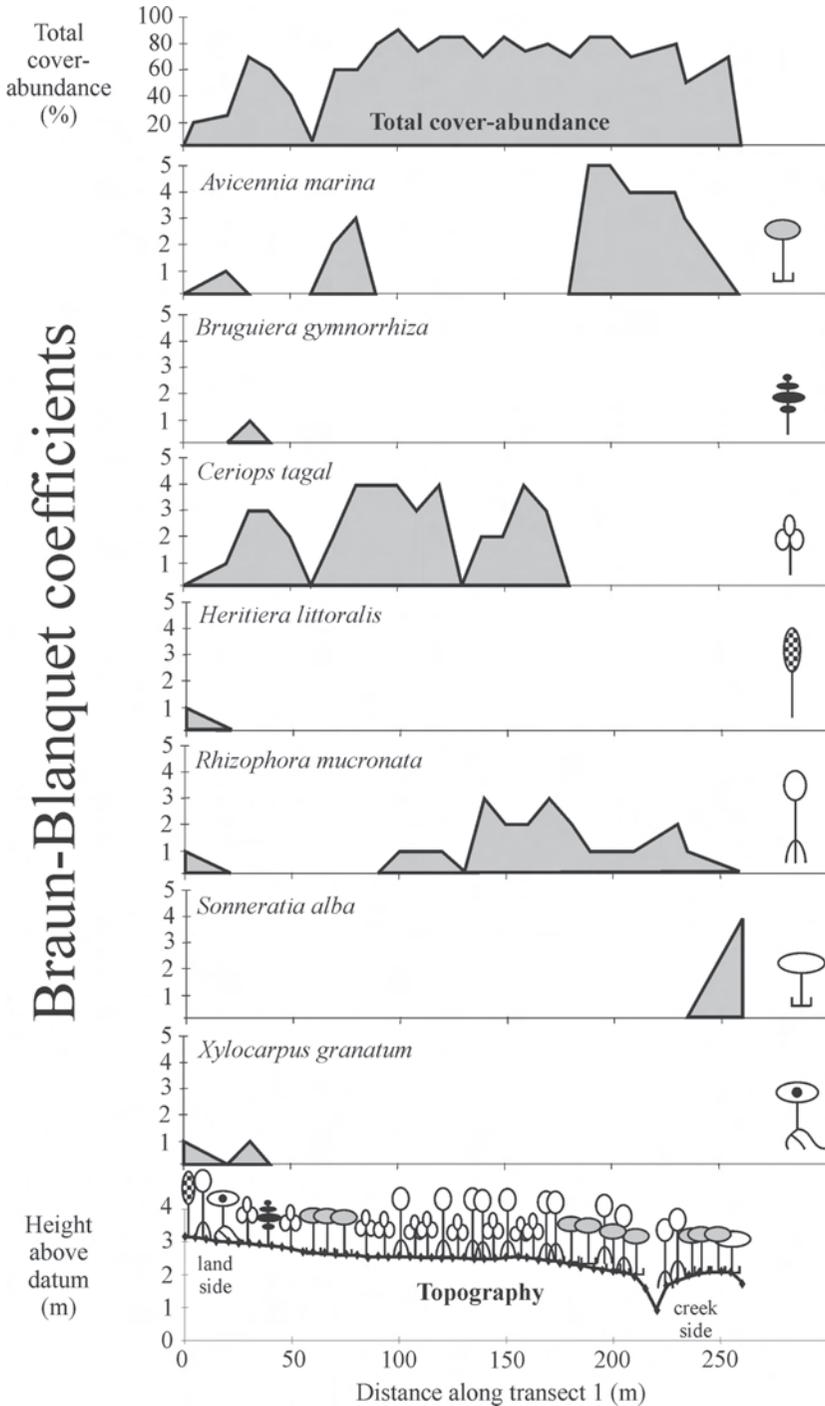
Figure 2. Description of the topography and vegetation according to the Braun-Blanquet method along Transect 1. Note that the Braun-Blanquet method is a qualitative method based on estimated percentage cover (Kent and Coker, 1992), and that true quantitative importance of species is given in Table 1 for Transect 1, and in Table 2 for the landward and the seaward *Avicennia* zones separately.

Table 1. Mean density, mean basal area, absolute frequency and importance value (IV) of Curtis (1959) using PCQM data for Transect 1. Relative values are indicated between brackets.

Species	Mean density (ind 0.1 ha^{-1})	Mean basal area (m^2 0.1 ha^{-1})	Mean frequency	IV	Rank
Avicennia marina L	28 (9.7%)	0.316 (2.6%)	17 (9.6%)	21.9	4
Avicennia marina S	26 (9.1%)	7.550 (62.5%)	17 (9.6%)	81.1	1
Bruguiera gymnorrhiza	3 (1.2%)	0.342 (2.8%)	2 (1.1%)	5.1	7
Ceriops tagal	133 (46.1%)	0.350 (2.9%)	57 (31.9%)	80.9	2
Heritiera littoralis	2 (1.2%)	0.471 (3.9%)	2 (1.1%)	5.6	6
Rhizophora mucronata	82 (28.5%)	2.948 (24.4%)	36 (20.2%)	73.1	3
Sonneratia alba	10 (3.6%)	0.080 (0.7%)	8 (4.3%)	8.6	5
Xylocarpus granatum	3 (1.2%)	0.028 (0.2%)	4 (2.1%)	3.6	8

L = landward; S = seaward

periods. Within the landward zone, comparison of the pneumatophore density with the height above datum further supports this suggested relationship (test: r_s = 0.76; n = 12; P < 0.001), but this was not observed in the seaward zone (test: r_s = 0.46; n = 10; n.s.).

The total pneumatophore length is greater when closer to the sea (Fig. 3B). Within each of the fringes this relationship is highly significant (landward zone: test: r_s = 0.70; n = 42; P < 0.001; seaward zone: test: r_s = 0.78; n = 38; P < 0.001). In the landward *A. marina* fringe the belowground length is relatively constant, whereas the aboveground length shows large variations (Fig. 3B). Nevertheless, both the below- and aboveground pneumatophore length is significantly higher closer to sea (below-ground length: test: r_s = 0.66; n = 42; P < 0.001; aboveground length: test: r_s = 0.78; n = 42; P < 0.001). Within the seaward fringe, on the other hand, there is a significant increase with decreasing distance to the sea (test: r_s = 0.51; n = 38; P < 0.001), whereas the belowground length shows large variations and is also significantly higher closer to the sea (test: r_s = 0.60; n = 38; P < 0.001).

COMPARISON OF LEAF MORPHOLOGY.—Petiole length, lamina length and maximal lamina width were significantly smaller in the landward fringe than in the seaward fringe (Table 3).

The mean stomatal density for the seaward *A. marina* zone was 151.3 mm^{-2}, which is not significantly different from the 183.8 mm^{-2} measured for the landward zone (U = 90.5; U$_{critical}$ = 165.5; n$_1$ = n$_2$ = 16; n.s.).

Table 2. Mean density, mean basal area, absolute frequency and importance value (IV) of Curtis (1959) using PCQM data for the landward Transect 2 (A) and the seaward Transect 3 (B) located in the *Avicennia marina* fringes. Relative values are indicated between brackets.

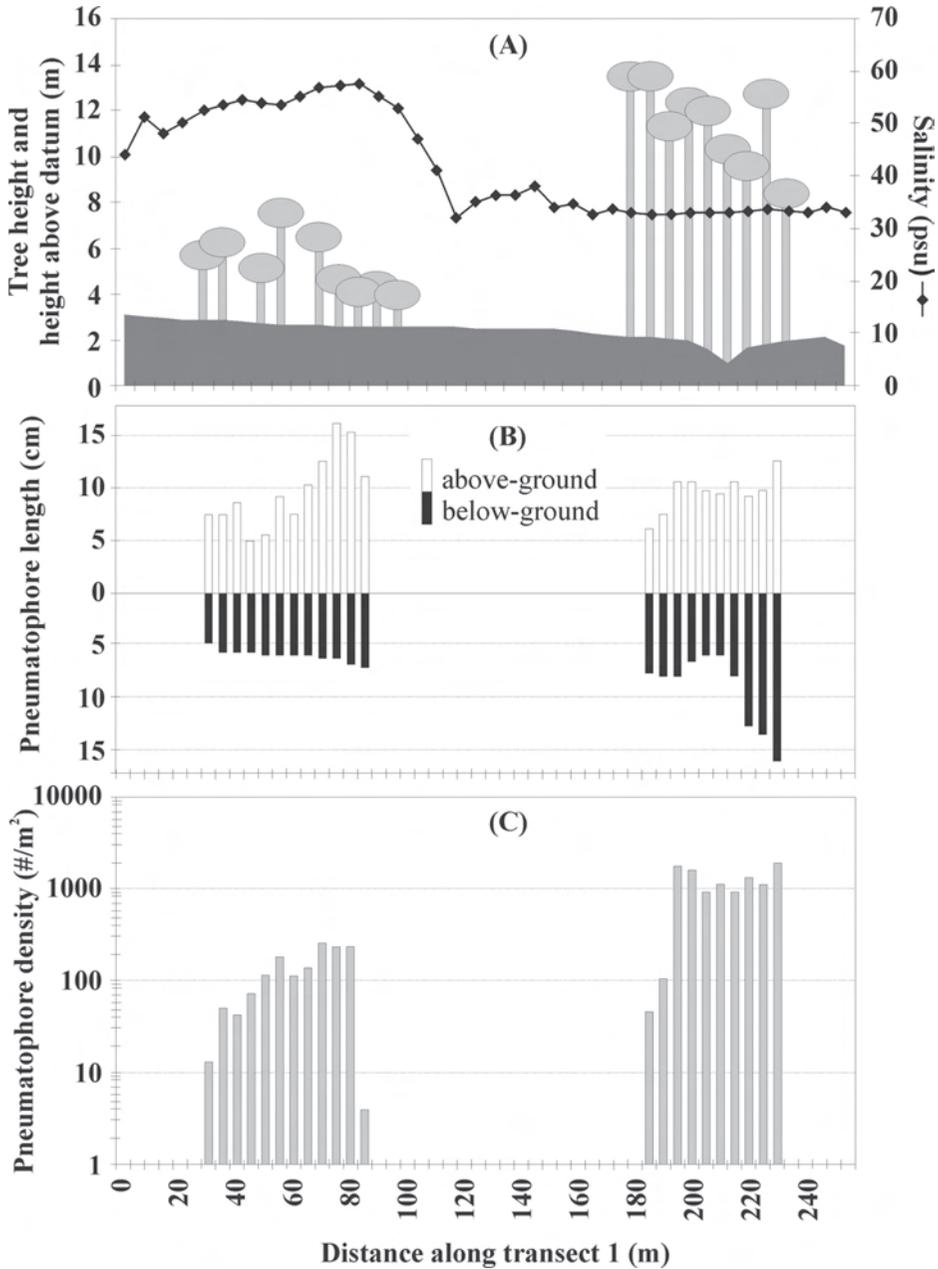| Species	Mean density (ind 0.1 ha^{-1})	Mean basal area (m^2 0.1 ha^{-1})	Mean frequency	IV	Rank
(A) Landward *A. marina* fringe (Transect 2)					
Avicennia marina L	654 (100%)	3.298 (100%)	100 (100%)	300.00	1
(B) Seaward *A. marina* fringe (Transect 3)					
Avicennia marina S	287 (71.4%)	66.697 (98.9%)	87.5 (60.9%)	237.00	1
Rhizophora mucronata	103 (25.7%)	0.661 (1.0%)	37.5 (26.1%)	55.27	2
Sonneratia alba	11 (2.9%)	0.077 (0.6%)	3.6 (8.7%)	8.25	3

L = landward; S = seaward

Figure 3. A) Height above datum and tree height vs salinity regimes; B) above- and below-ground pneumatophore length; and C) pneumatophore density for *Avicennia marina* along Transect 1.

GENETIC DIFFERENTIATION.—Forty-five decanucleotide random primers were screened using the template DNA from *A. marina* to identify informative primers that generated polymorphic patterns of PCR.

Most of the primers (62%) produced a monomorphic pattern for *A. marina* rather than a set of discrete amplification products. Out of 45 primers, eleven primers that produced

Table 3. Comparison of *Avicennia marina* leaves from the landward (n = 1000) and seaward fringe (n = 1000). The mean values (bold) from the descriptive statistics' summary were used for the statistical analysis.

Descriptive statistics	Petiole length		Lamina length		Lamina width	
	Land	Sea	Land	Sea	Land	Sea
Mean (cm)	**0.80**	**0.94**	**6.73**	**8.15**	**2.97**	**3.38**
Median (cm)	0.80	0.90	6.60	8.10	3.00	3.40
Standard deviation (cm)	0.43	0.21	1.15	1.35	0.42	0.62
Variance (cm)	0.19	0.04	1.33	1.81	0.18	0.39
Minimum (cm)	0.20	0.40	2.90	0.40	1.90	1.80
Maximum (cm)	1.90	1.80	10.80	12.70	7.40	8.60
Statistical analysis (Mann-Whitney U-test)						
U-value	225,612	774,389	302,556	679,444	223,074	776,926
z-value	−21.25		−15.29		−21.45	
p-value	< 0.05		< 0.05		< 0.05	

consistent reproducible polymorphism, and simple, unique, easily resolved bands were chosen and used to analyze all 37 individuals. A total of 48 different DNA fragments was amplified by these 11 decanucleotide primers. Five primers failed to amplify and the remainder showed amplification without detection of polymorphism.

For 20 DNA fragments, the marker band was present in both populations with a frequency of 50% or more. For calculation of the genetic parameters, the following 11 primers and 48 loci were considered: OPA-07 (7 loci), OPA-10 (4 loci), OPA-12 (5 loci), OPA-19 (2 loci), OPE-1 (5 loci), OPJ-1 (8 loci), OPJ-12 (2 loci), OPJ-17 (4 loci), OPO-6 (2 loci), OPT-12 (5 loci), and OPT-15 (4 loci). Only four loci were monomorph in both populations and none of the alleles was unique for a population. From the observed band frequencies, the allelic frequency of the marker per locus (1-q) was calculated. The majority of the obtained DNA fragments occurred at comparable frequencies in both ecological zones (Fig. 4). The coefficient of determination (r^2) was 0.54 for the correlation of the calculated frequencies for each allele in the landward and seaward zone (d.f. = 46; P < 0.001). This scatter diagram and the linear regression illustrate a trend towards lower frequencies of the very rare alleles in the landward population, whereas the commonest alleles tend to be at higher frequencies than in the seaward population. Two DNA fragments were at higher allelic frequencies in the seaward zone (A10c, A12c), while another two were at higher allelic frequencies (J12a, J17d) in the landward zone (Table 4, Fig. 4), all of which highly significant (P < 0.001). The ability to differentiate between the two zones using the rest of the markers was not significant. The Fst between the two populations was 0.102 (S.E. 0.017) and the Nei's genetic distance calculated after Lynch and Milligan (1994) was 0.04.

Discussion

Morphologic Differentiation.—The zonation pattern of the mangrove belt sampled here is similar to that observed in other studies conducted in Gazi Bay (Beeckman et al., 1989; Gallin et al., 1989; Dahdouh-Guebas et al., 2002). The discrepancy between the qualitative (Fig. 1) and the quantitative (Table 1) investigation illustrates that it is important to not delineate zones based on qualitative cover-abundance, but to do so based on actual counts and calculations of density, basal area and frequency, and the computation
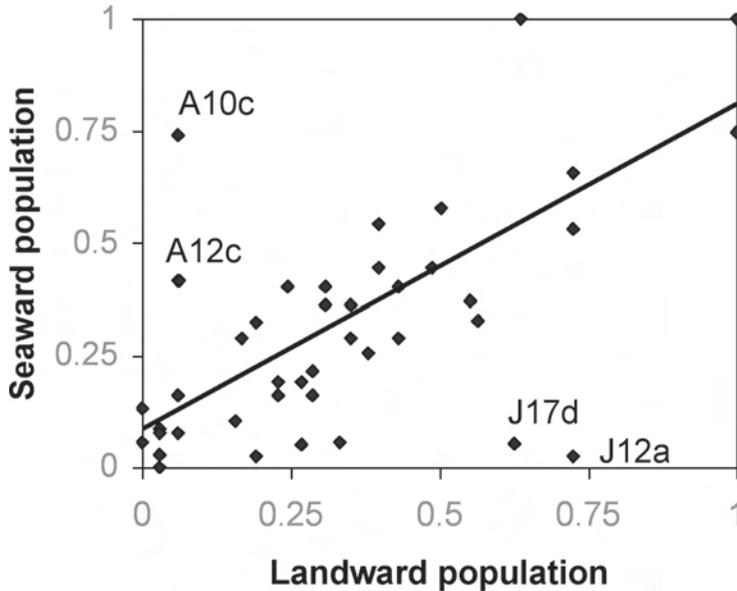
Figure 4. Correlation of the calculated frequencies for each allele in the landward and seaward zone of an *Avicennia marina* population ($r^2 = 0.54$, d.f. = 46; $P < 0.001$). Two DNA fragments were at significantly higher allelic frequencies in the seaward population (A10c, A12c), while two were at significantly lower allelic frequencies (J12a, J17d).

of an importance value (cf. Curtis, 1959), preferably along transects within the zone, or alternatively along multiple parallel transects across the entire mangrove belt. The need for such quantitative ground-truthing is also emphasized for remote sensing studies, which sometimes reveal different "mangrove canopy zones" as compared to "mangrove floor zones" on a same geographic location (Dahdouh-Guebas et al., in press).

The observation of the low total cover-abundance in the first 100 m along Transect 1 can be explained by the higher salinity. The landward *Avicennia* fringe, classified under inundation class 4 (according to Watson, 1928) is inundated at spring tides only, soon after which, salt crystals start covering the soil as a result of high evaporation rates and long evaporation periods between two successive inundation events. The different vegetation structure of both *Avicennia* fringes could be a consequence of salt stress: the PCQM calculations indeed show a very low mean basal area, indicating a "dwarf-growth" of *A. marina* in the landward zone, which was later confirmed by the height measurements of the trees. Whereas, *Avicennia germinans* (L.) Stearn dominated zones are characterized by strongly reduced soils and high sulphide concentrations (McKee, 1993), the soil redox potential, sulphide concentration, and pH measured along the same transect (Transect 1) could not be correlated to the distribution of *A. marina* (Matthijs et al., 1999).

In other areas of the Indo-West-Pacific region such as New Zealand, with a tidal range from 0.3–3.2 m (Spalding et al., 1997), mean plant size and density, with respect to tidal position, shows no consistent pattern across forests (Osunkoya and Creese, 1997). Smith (1987b) on the other hand, reported that *A. marina* reaches the largest natural densities in lower intertidal forests. Our results, more in line with Osunkoya and Creese (1997), show the opposite. The mean density in this study is relatively high for the landward zone, possibly because of a lack of permanent interspecific competition and tidal influences,

Table 4. The calculated allelic frequency (1-q) of four dominant RAPD markers that show highly significant differences (P < 0.001) between *Avicennia marina* trees from the landward and seaward zone.

RAPD marker	1-q (Land)	1-q (Sea)
A10c	0.060	0.740
A12c	0.060	0.415
J12a	0.725	0.025
J17d	0.626	0.053

topics for further research. The former is often observed along the Kenyan coast (pers. obs.), whereas the latter does not allow that propagules are easily swept away. However, research on *A. germinans* at their biogeographical limit (Mauritania), under arid conditions, has revealed that sun and air exposure of propagules for 24 hrs is enough to damage the propagules in an unrecoverable way (Dahdouh-Guebas and Koedam, 2001). Nevertheless, in Gazi Bay we found a lot of *Avicennia* seedlings in the landward zone, particularly in exposed sites, the short-term survival of which is probably guaranteed by the moist soil. Clarke and Myerscough (1993) suggest that recently established *A. marina* seedlings reflect spatial variability and older seedlings may reflect temporal variability in the moisture conditions of a salt marsh. Clarke (1993, 1995) further reported on the dispersal and densities of *A. marina* propagules in southeastern Australia, but without paying attention to the possible disjunct zonation pattern that can be observed there (e.g., Clarke and Myerscough, 1993) or elsewhere.

The hypothesis that pneumatophore density tends to be higher near the sea, as observed in this study, seems acceptable since it matches the longer and more frequent inundation periods and resulting hypoxia. The lack of significance in the statistical analysis of pneumatophore density relative to position within the seaward *A. marina* zone along Transect 1, may be due to complete coverage of pneumatophores by mangrove detritus. Deposition of detritus increases closer to the sea or creek, a feature of the seaward fringe rather than of the landward fringe.

The increase in pneumatophore length with a position closer to the sea also matches the longer inundation periods. However the differences between above- and belowground pneumatophore length must be put into the following context. The aboveground length in the seaward fringe is of less importance because every day the root system is flooded entirely (the spring tidal amplitude in Kenya is 3.5 m). Since the seawater is not hypoxic, the function of pneumatophores in the seaward zone can be rather as an adaptation to cope with the hypoxic, muddy substrate (Dale, 1938). An increase in pneumatophore density might solve this problem, where an increase in pneumatophore length is only necessary to reach above the substrate. The latter is the case within the landward fringe, where a significant increase in the aboveground pneumatophore length occurs closer to sea. During the (spring) tidal inundations, which occur every two weeks, the water level does not reach far above the pneumatophores, if at all (F.D.-G., pers. obs.). Therefore, the aboveground pneumatophore length can play a considerable role in coping with temporary hypoxia. The belowground pneumatophore length in the landward fringe is more constant, probably because of the sandy substrate, which allows better gas access.

The smaller values for mean petiole length, mean lamina length, and mean lamina width observed for the landward *A. marina* fringe are in agreement with the high salinity and drought of the environment. *Avicennia* deals with salt stress by actively secreting salt through salt glands (Downton, 1982; Clough, 1984; Tomlinson, 1986). A small leaf area

could thus be an efficient adaptation against evaporation. The significantly shorter petiole in the landward *A. marina* fringe is obvious. A longer petiole increases the mobility of a leaf and can be explained as an adaptation to reduce evaporation. When swinging, the aerial circulation around the leaf may increase and therefore also the evaporation.

The stomatal density of *A. marina* has been reported to vary between 175–200 mm^{-2} (Walter and Steiner, 1936). However, it is difficult to compare these values with the ones found in this study since these authors did not indicate for which zones within the mangrove forest the values are valid and the measurement method was not explicit. They most likely referred to landward *A. marina* specimens because they identified this species as characteristic for dry and saline soils. Although no significant difference in stomatal density between the landward and seaward *Avicennia* fringe could be found in this study, evidence of a higher stomatal density in drier and more saline places has been found for other species in Gazi Bay. *Lumnitzera racemosa*, a species thriving in relatively dry conditions only (landward), displayed the highest stomatal density, whereas *Sonneratia alba*, a species thriving in wet conditions only (seaward), displayed the lowest density (Gallin, 1988). Within *Lumnitzera racemosa,* Gallin (1988) also found a difference between specimens occurring on very dry soils (157 stomata mm^{-2}) and specimens occurring on less dry soils (132 stomata mm^{-2}). The investigation of the stomatal apparatus in previous studies revealed no significant difference between stomata dimensions among or within mangrove species, even when a subdivision based on dry or wet conditions was made (Sidhu, 1975; Gallin, 1988). For *A. marina* the reported dimensions of the stomata ranged between 26–28 μm for the length and 16–18 μm for the width. Apart from changes in stomatal density and dimensions for *A. marina*, other adaptations might contribute to a reduction of transpiration as well, such as embedding of stomata in the leaf tissue or the presence of hairs. Ball and Farquhar (1984a,b) also demonstrated that the stomata of *A. marina* remain coordinated with metabolism when challenged by salinity and that stomatal functioning appears consistent with a strategy for allowing minimal water loss relative to carbon gain, even as the leaf metabolism (e.g., photosynthetic CO_2 assimilation) is affected. Although often questioned, several authors state that mangroves have xeromorphic adaptations to the physiologically dry environment in which they can thrive (Chapman, 1976).

GENETIC DIFFERENTIATION.—We expected the *A. marina* fringes to constitute a single population, with a differentiation between the landward and seaward populations of *A. marina* reflecting morphological plasticity. However, the genetic analysis shows that out of the 48 considered RAPD loci, four DNA fragments were able to slightly differentiate in their allelic frequencies between the two fringes. This does not necessarily indicate that an ecological or physical barrier exists because differences in allelic frequencies also can be due for instance to stochastic events, low population and sample sizes, or inbreeding effects. Nevertheless, the observation that allelic differences might occur between *A. marina* trees from contrasting ecological zones in such close vicinity warrants further attention. Also when taking into account all 48 DNA fragments, the obtained Fst of 0.102 and genetic distance of 4% still indicated a slight genetic divergence between the two zones. This preliminary analysis of 17 landward trees and 20 seaward trees thus suggests that there might be less contact or genetic interchange between the two zones than within each zone. Moreover, the trend towards lower frequencies of the very rare alleles in the landward zone also might indicate a slightly diminished gene flow when compared to the seaward zone. If the sample size was locally representative of the whole fringe, then the possibility of some type of barrier to genetic flux between the two fringes

as well as other limiting factors within each zone should be considered (e.g., spatial structure of the trees, temporal structure of flowering and pollination periods, etc.). The type of barrier is probably not physical since tidal processes, associated with propagule dispersal, are unconstrained and connect the two zones. An alternative explanation is that stressful conditions in the high intertidal zone inflict significantly higher mortality on certain genotypes, resulting in some level of genetic differentiation between the two sub-populations.

The reports of Fst values on the basis of dominant RAPD markers in plant populations are not numerous. Mostly descriptive diversity indices and cluster analysis are provided. An attempt to use RAPD in two morphologically distinct populations of *Manilkara zapota* (L.) P. Royen from contrasting habitats in Mexico, failed to genetically differentiate the forest from the swamp ecotypes (Heaton et al., 1999). In the more widespread and ephemeral *Senecio gallicus* Vill. from the western Mediterranean, a moderate level of intraspecific differentiation was observed among populations from coastal and inland regions for both RAPDs and quantitative traits (Comes and Abbott, 2000). RAPDs provided greater resolution and Fst values among populations and clusters thereof varied from 0.106 (coastal versus inland) to 0.372 (total among populations). Therefore, the Fst values for *Avicennia* are comparable to the former. In *A. marina* from India, very low intrapopulational genetic variation has been observed. The percentage of polymorphic RAPD loci varied from 17.8–38.9 (Parani et al., 1997). The observation that in Gazi Bay none of the alleles was unique for a certain zone and that only few loci were monomorph throughout, indicated that gene diversity within each zone remains high. This can probably be related to a predominant outcrossing breeding system. The possible explanation for the locally lowered allelic frequencies in four loci (two in each zone) must be examined further and should consider larger samples sizes, more loci and additional regions. Nevertheless, this preliminary screening highlights the resolving power of molecular markers to indicate sub-structuring in separated mangrove stands in extremely close vicinity. To study the fragmentation of *Avicennia* communities, microsatellites, known to be abundant in the *Avicennia* genome, could also be used (Maguire et al., 2000).

In summary, *A. marina* is sometimes described as a mangrove tree thriving on extremely dry and saline substrates, exemplified as the most landward fringe, or as the highest latitudinal species. However, under less extreme conditions this species can develop into a very robust tree, e.g., in the east African seaward *A. marina* fringe. The present comparative study of the landward and seaward *Avicennia* fringe revealed significant differences in vegetation structure, physiognomy, root system structure, and leaf morphology, the majority of which can be attributed to the differential salinity and tidal inundation periods that occur in the two zones. Preliminary genetic analysis showed that the RAPD method can be used to reveal slight genetic divergence within *A. marina* within a close vicinity. The wide morphological variability shown by *A. marina* individuals is likely a reflection of the different environmental conditions to which they are exposed. The incipient genetic divergence suggests a genetic sub-structuring of both zones. It is not known at which time scale this type of disjunct zonation patterns developed, and at which frequency forest dynamics or catastrophes reorganized the vegetation structure. The combination of environmental pressure and genetic sub-structuring can, however, lead to further divergence. Additional studies of other disjunct zonations of *Avicennia* are needed to corroborate this hypothesis.

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