

Bidirectional gene flow on a mangrove river landscape and between-catchment dispersal of *Rhizophora racemosa* (Rhizophoraceae)

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Received: 17 August 2016 / Revised: 16 October 2016 / Accepted: 17 October 2016 / Published online: 27 October 2016
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Abstract Understanding how landscape structure shapes the genetic structure of populations of keystone species is important for their long-term management. We tested the unidirectional dispersal hypothesis on the linear river landscape of the Wouri River and the one catchment-one gene pool hypothesis on red mangrove (*Rhizophora racemosa*) populations of the Cameroon Estuary complex. Therefore, we conducted release–recapture experiments in the field, and sampled 649 adult trees for plant material for genetic analyses. This allowed for estimating genetic diversity and structure, as well as dispersal directionality. Genetic diversity in populations downstream did not differ significantly from upstream populations and the molecular variance of populations did not correlate with their position on the linear landscape. Contemporary and historical migration estimates indicated bidirectional dispersal, i.e. in both the downstream and

the upstream direction along the Wouri River. This was confirmed by the propagule dispersal directions derived from our field experiments. Bayesian clustering analysis assigned all individuals of this estuary into one cluster, suggesting high inter-catchment connectivity. River flow currents, tides, and wind plausibly operate together to ensure the high genetic connectivity within this complex estuary.

Keywords Mangroves · Microsatellites · Population genetics · Connectivity · Hydrochory · Cameroon Estuary complex · Wouri River

Introduction

Mangroves are a taxonomically diverse group of woody plants and shrubs occurring in the intertidal area of tropical coasts. Mangrove species are adapted to the dynamic environmental conditions prevailing in tropical intertidal zones. Some species have salt-excreting glands in their leaves, which allow them to cope with fluctuating salinities, while unique root morphologies render respiration possible under low oxygen conditions of water-logged soils (Kathiresan & Bingham, 2001). *Rhizophora racemosa* G. Mey. is the most widespread mangrove in Cameroon (Corcoran et al., 2007), making up about 90% of the mangrove cover, and also the most exploited by local communities (Feka & Manzano, 2008; Din et al.,

Handling editor: K. W. Krauss

Electronic supplementary material The online version of this article (doi:10.1007/s10750-016-3021-2) contains supplementary material, which is available to authorized users.

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2008; Nfotabong-Atheull et al., 2009, 2011, 2013). *Rhizophora* has ambophilous flowers (Menezes et al., 1997; Sánchez-Núñez & Mancera-Pined, 2012) and *R. racemosa* are predominantly outcrossing (unpublished data). Gene flow among red mangrove populations is mediated via pollen transfer (primarily through wind pollination and secondarily through insect pollination) and through hydrochorous propagule dispersal. The hydrochorous propagules of *R. racemosa* are smoother, more slender, and longer, when compared to other sibling, sympatric species such as *Rhizophora mangle* L. and *R. harrisonii* Leechm.

Rhizophora racemosa propagules may float both horizontally and vertically over the course of their dispersal trajectory, propagated by the interaction of ambient ocean currents and winds (Van der Stocken et al., 2013, 2015a). Hydrochory allows for the colonization of new favourable habitats and the connectivity between existing populations, both close and remote, except in the presence of obvious oceanic and land barriers (Wee et al., 2014; Cerón-Souza et al., 2012, 2015; Ngeve et al., 2016). Understanding how plant dispersal occurs along rivers and other water bodies can provide insights on how landscapes are being colonized by plants and predict how they would respond to changes in the climate (Nilsson et al., 2010). Hydrochory is known to be more effective compared to other dispersal mechanisms. This is observed in the lower differentiation among metapopulations of riparian habitats compared to terrestrial habitats, reflecting higher effective gene flow and connectivity of these populations (Kinlan & Gaines, 2003; Chen et al., 2007). Hydrochorous dispersal facilitates gene flow, while restraining differentiation among populations over reasonably long distances (Pollux et al., 2007).

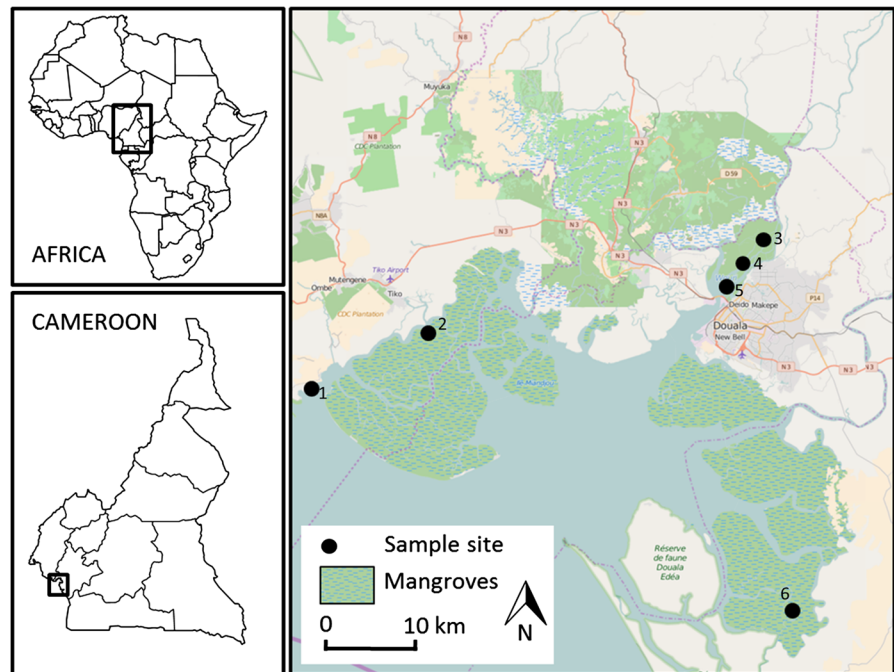
Dispersal in riparian plants may vary greatly according to the specific environmental conditions of different rivers (Pollux et al., 2007). High water flow velocities may impede local stranding and establishment of propagules, transporting them over longer distances (Nilsson et al., 2010). Propagule dispersal distance increases with increasing discharge, which determines water flow velocity (Nilsson et al., 2010). Pollen transfer via both wind and insects has been shown to be less effective in patchy and heterogeneous landscapes such as mangroves (Wee, 2013). Estimates of gene flow may not reflect actual dispersal fluxes as these estimates only reflect dispersal of propagules

that successfully established and thrived (Kinlan & Gaines, 2003). The use of genetic data alone is insufficient for estimating demographic connectivity, which goes a step further to assess growth rates of immigrants versus local recruitment (Lowe & Allendorf, 2010). This implies that several physical processes can interfere with actual dispersal and leave their imprint on the dispersal signatures as derived from genetic data. Nevertheless, genetic indices of connectivity remain indispensable for assessing gene flow and unveiling the evolutionary outcomes of dispersal (Lowe & Allendorf 2010).

The unidirectional dispersal hypothesis (drift paradox) in linear landscapes suggests that in riparian species, genetic diversity will increase with decreasing altitude due to the flux of alleles from upstream populations to receiving populations downstream (Pollux et al., 2007, 2009; Honnay et al., 2010; Nilsson et al., 2010; Love et al., 2013). Hydrochory should result in genetic divergence between populations in different catchments, due to the reduced chances of propagules crossing catchments via water. This implies that populations in catchments that have remained isolated over many generations should be characterized by unique gene pools, except when connectivity is maintained by other vectors (e.g. wind, water birds) (Werth & Scheidegger, 2014). Hence, wetland configuration may not have changed strongly over ecological and geological timescales. This is known as the “one catchment-one gene pool” hypothesis (Werth & Scheidegger, 2014).

Several studies have assessed the genetic diversity of *Rhizophora* species in the ACEP biogeographic region, primarily from the Atlantic coast of South and Central America, and the Caribbean. A majority of these studies are on the more widespread *R. mangle* (Cerón-Souza et al., 2010, 2012; Takayama et al., 2013; Sandoval-Castro et al., 2014; Cerón-Souza et al., 2015; Albrecht et al., 2013). However, detailed studies on populations from the eastern Atlantic shores are limited. Therefore, using microsatellite markers, we investigated the “one catchment-one gene pool” hypothesis in *R. racemosa* populations of the Cameroonian Estuary complex (CEC). The key question here was whether there is inter-catchment connectivity. We also tested the unidirectional dispersal hypothesis with samples from the Wouri River, in order to reveal its influence on connectivity of mangroves of this estuary complex. Although these hypotheses have

Fig. 1 Cameroon Estuary complex showing 6 study areas (black circles). The colour study area map (Sentinel-1 data) was downloaded from the European Space Agency (ESA) website through the Data Hub System 0.10.3-4 (<https://scihub.copernicus.eu/dhus/>) (Site numbers indicated as in Table 1). *Study sites*: Mabeta (1 transect), Tiko (5 transects), Bonamoussadi Upstream Wouri (5 transects), Akwa-Nord midway Wouri (3 transects), Bonaberi Downstream Wouri (4 transects), and Mouanko Reserve (3 transects)



predominantly been tested on riparian species (Pollux et al., 2007; Honnay et al., 2010; Love et al., 2013; Werth & Scheidegger, 2014), *Rhizophora racemosa* presents an interesting case study to test these hypotheses in mangroves which are largely intertidal. *Rhizophora racemosa* has the lowest tolerance to salinity compared to sibling sympatric species like *R. mangle* and *R. harrisonii*, and therefore inhabits large catchments where fresh water input is high (Duke & Allen, 2005; Menezes et al., 2008; Cerón-Souza et al., 2015). This species is therefore strongly subjected to riparian influence. Hence, this presents an interesting case to explore the relative influence of both riverine and oceanic factors, in shaping populations. We hypothesized that gene flow is predominantly unidirectional, from upstream to downstream of the Wouri River channel. To support genetic data, we carried out release-recapture experiments using mature propagules in the field. Our objectives were to assess (1) the genetic structure and connectivity of *Rhizophora racemosa* in the entire estuary (i.e. to tests for “one catchment-one gene pool”) and (2) the directionality

of gene flow along the linear landscape of a river (i.e. to test for unidirectional dispersal hypothesis).

Materials and methods

Study area and sample collection

The Cameroon Estuary complex (CEC) (Fig. 1) is located in the Bight of Bonny of the Gulf of Guinea (3°56'53" N; 9°35'24" E). It has the second largest extent of mangrove cover in Cameroon, after the Rio Del Rey Estuary, estimated at about 880–1100 km², and is the most human-disturbed mangrove area along the country's coastline (Alemagi et al., 2006; Corcoran et al., 2007; Nfotabong-Atheull et al., 2009, 2011, 2013; FAO, 2011; Ngeve et al., 2015). Its mangroves stretch from the Bimbia River in the north to the fewer stands at the mouth of the Sanaga River, in the south (Corcoran et al., 2007; FAO, 2011). The semi-diurnal tidal regime is characterized by a pronounced asymmetry, and tidal amplitudes range between 1.35 m and 3 m (Nfotabong-

Table 1 Descriptive statistics of genetic diversity for the different transects

Sampling area	Site No.	Pop ID	N	At	Ae	Ar	Ho	uHe	Fis	Or
Unprotected sites										
Mabeta (North of the CEC)	1	Mab*	31	27	1.4	2.2	0.284	0.254	-0.121	1.275
		TikI	33	27	1.6	2.2	0.347	0.301	-0.155	1.367
		TikII*	31	33	1.7	2.5	0.334	0.337	0.009	0.982
	2	TikIII*	34	32	1.6	2.4	0.283	0.302	0.063	0.881
		TikIV	12	24	1.5	2.2	0.258	0.287	0.106	0.808
TikV		16	26	1.6	2.3	0.3	0.317	0.055	0.896	
Bonamoussadi (Upstream Wouri Channel)	3	DLaIV*	21	29	1.6	2.4	0.329	0.323	-0.019	1.039
		DLaV	24	26	1.5	2.2	0.246	0.265	0.071	0.867
		DLaVI	43	32	1.7	2.5	0.321	0.328	0.021	0.959
		DLaVII	31	30	1.6	2.3	0.314	0.31	-0.011	1.022
		DLaVIII*	46	31	1.5	2.2	0.263	0.278	0.054	0.898
Akwa-Nord (Midway Wouri Channel)	4	DLaI*	52	32	1.5	2.3	0.271	0.281	0.036	0.931
		DLaII*	27	30	1.6	2.4	0.33	0.3	-0.101	1.225
		DLaIII*	22	27	1.6	2.2	0.285	0.307	0.072	0.866
Bonaberi (Downstream Wouri Channel)	5	DLaIX*	30	32	1.6	2.5	0.344	0.322	-0.07	1.151
		DLaX*	36	31	1.6	2.4	0.374	0.337	-0.111	1.25
		DLaXI*	27	31	1.7	2.4	0.421	0.34	-0.242	1.639
		DLaXII*	29	34	1.7	2.5	0.335	0.341	0.016	0.969
Protected sites										
Mouanko Reserve (South of the CEC)	6	RSV1	29	26	1.5	2.1	0.268	0.259	-0.038	1.079
		RSV2	32	24	1.5	1.9	0.267	0.218	-0.231	1.601
		RSV3	37	25	1.5	2.1	0.253	0.268	0.06	0.887
Total C.E.C			31	29	1.6	2.5	0.306	0.299	-0.026	1.052

CEC Cameroon Estuary complex, * indicates transects with significant ($P < 0.05$) bottleneck following SMM from the standardized difference test, N sample size, At total number of alleles, Ae number of effective alleles, Ar allelic richness, Ho observed heterozygosity, uHe = unbiased expected heterozygosity, Fis inbreeding coefficient, Or apparent outcrossing rate. Transects from the Wouri River channel are shaded in grey

Atheull et al., 2013; Onguene et al., 2015). Tidal influence strongly favours mangrove growth several kilometres inland (Corcoran et al., 2007).

Twenty-one (21) transects (populations), parallel to the direction of river flow, were established in different mangrove areas of the CEC, encompassing a total sampling area of about 33 km². Twelve (12) of these transects were from the mangrove patches along the Wouri River (Fig. 1, Table 1). On average, leaf tissue was collected from 30 trees per population. A minimum distance of 10 m was deliberately considered between the trees sampled, in order to avoid sampling the same individual. Sampling locations were characterized by natural mangrove stands, and no efforts on reforestation or afforestation had taken place prior to our sampling.

DNA extraction and amplification

Genomic DNA extraction was carried out from 20 to 30 mg of dry plant leaf tissue, using the E.Z.N.A SP

plant DNA Mini Kit (Omega bio-tek). Eleven (11) polymorphic microsatellite markers (Rrace1, Rrace3, Rrace5, Rrace6, Rrace7, Rrace12, Rrace15, Rrace17, Rrace18, Rrace20, and Rrace24), isolated from *R. racemosa* from Cameroon (Tiko), were used in a multiplex PCR to amplify DNA samples. These markers have been developed from a microsatellite-enriched genomic library in the Ecology and Biodiversity laboratory of the VUB, following an enrichment procedure with the combined vector-biotinylated magnetic bead-capture technique (see details in Triest et al., 2015). The 11 microsatellite markers have been archived online by Ngeve, Sierens, and Triest in GenBank (Isolation and characterization of 11 polymorphic microsatellite markers for the red mangrove *Rhizophora racemosa* (Rhizophoraceae), with all accession numbers given in Table 2. Multiplex PCR was performed with the following constituents for each PCR reaction: 2.5 µl of H₂O, 6.25 µl of the master mix (Qiagen multiplex pcr kit master mix), 1.25 µl of the primer mix (forward primers were

Table 2 Genetic diversity measures and characteristics of each microsatellite locus from populations of the Cameroon Estuary complex

Loci	GenBank Accession No.	Primer sequence (5′–3′) (hex-labelled forward primer (F) and reverse primer (R))	Repeat motif	Tan	Size	N	At	Ae	Ho	He	Fis	Fit	Fst
Rrace1	KT455433	F: 6-FAM-GCAGTCTCTCTGCCCTATG R: ACATGGTTGATAGCCCTGGA	(AC)11	56	224–236	30.6	2	1.1	0.103	0.063	−0.649	−0.054	0.360
Rrace3	KT455434	F: PET-CAGCCTCGACTCTCTTCCTG R: CAGCCCTTCGTTCAATCAAT	(AC)12	56	200–226	30.9	7	1.6	0.371	0.368	−0.007	0.051	0.057
Rrace5	KT455435	F: VIC-TTTCCTTCTCATGTGATGCTG R: AGCAACCAATGGCTACATGC	(AG)16	56	130–154	30.4	8	2.7	0.532	0.615	0.136	0.173	0.043
Rrace6	KT455436	F: 6-FAM-ACACACGAATCCACTGGTCA R: CCGGAGGGAAGAGGGATATT	(TG)14(AG)16	56	196–212	30.9	3	2.2	0.590	0.535	−0.103	−0.06	0.039
Rrace7	KT455437	F: PET-AGCCTGTAGTCAGGGGCTC R: CAATGTGGTGCATGGATTA	(CA)12	56	143–149	28.8	5	1.2	0.191	0.147	−0.303	−0.012	0.223
Rrace12	KT455438	F: VIC-AGATTGCAAGCGAAACCAAC R: CTCGATCAAACTCAACGCAGA	(GA)18	51.5	233–251	30.9	6	1.8	0.461	0.388	−0.189	−0.109	0.068
Rrace15	KT455439	F: PET-CCGTGCATCTTATACCAAAAA R: TGGACACGATAGGCACAAAA	(AC)15	56	118–136	30.9	8	1.4	0.253	0.248	−0.021	0.008	0.029
Rrace17	KT455440	F: NED-GTTCGAGAAGGATGGGAACA R: AACAAAGGACATGGGTGGAG	(TC)12(AC)9	56	142–148	30.9	3	1.6	0.392	0.366	−0.07	−0.011	0.055
Rrace18	KT455441	F: VIC-TCCATGTGTCATGTGGTCTCT R: GCCGCTTCTCCACTTACAC	(TG)9(AG)9	56	115–119	30.9	3	1.1	0.047	0.054	0.142	0.167	0.029
Rrace20	KT455442	F: NED-TTTCATCTTGCCACCAAGT R: TTGTTACACAAAGCCATTACATGA	(TG)11	57.4	98–100	30.7	2	1.0	0.008	0.008	−0.049	−0.004	0.042
Rrace24	KT455443	F: NED-TCACCGGAGGTCTGGTAAAG R: TCCAAATCGCAACATTCAAA	(TC)10TT(TC)5	56	218–224	30.8	3	1.8	0.419	0.435	0.037	0.064	0.028
Mean						30.6	4.5	1.6	0.306	0.293	−0.098	0.019	0.088
SE						2.1	0.7	0.1	0.033	0.021	0.068	0.027	0.032

Tan annealing temperature, N mean sample size, At total number of alleles, Ae number of effective alleles, Ho observed heterozygosity, He expected heterozygosity, Fis inbreeding coefficient, total inbreeding (Fit), subpopulation differentiation (Fst), ± weak amplification, + strong and clear amplification – no amplification

fluorescently labelled with 6-FAM, VIC, NED or PET), and 3 µl of DNA, in a final volume of 13 µl. The PCR reaction conditions were as follows: initial denaturation of 95°C for 15 min followed by an extension of 30 s at the same temperature. Annealing was then allowed at a temperature of 57°C, followed by an elongation at 72°C. Subsequently, the initial steps were repeated (34 cycles) and this was followed by a final elongation time of 30 min at 60°C and a cooling to 4°C for 1 min in a Bio-Rad thermal cycler (MJ research PTC-200 and Bio-Rad MyCycler). Fragment analysis was carried out by Macrogen Corporation (Seoul, South Korea). Scoring to identify alleles was done using GeneMarker (SoftGenetics LLC, State College, USA).

Data quality and genetic diversity

We tested for deviations from the Hardy–Weinberg equilibrium (HWE) using the heterozygote deficiency alternative in Genepop v. 4.3 (Rousset, 2008), using default Markov chain parameters settings of 10,000 Dememorization, 20 batches, and 5000 iterations per batch. We also used Genepop to calculate genotypic linkage disequilibrium between each pair of loci and to estimate null alleles (private allele method). At population level, we estimated number of effective alleles (A_e), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) (using GenAlEx), total number of alleles (A_t), allelic richness (A_r), and the fixation index (F_{is}) (using FSTAT). We used the program BOTTLENECK v. 1.2.02 (Piry et al., 1999) to verify whether these populations have undergone recent reduction in genetic diversity (from bottleneck events), due to high anthropogenic pressures in most areas. This was done based on the assumption that microsatellite markers follow a Stepwise Mutation Model (SMM), which is justified since most of the markers used seem to mutate following this model. We grouped transects of the entire estuary into 5 groups—north, upstream Wouri, midway Wouri, downstream Wouri, and south (protected area)—to compare genetic diversity between different areas and the protected area. We also investigated differences in genetic diversity in the areas of the Wouri River following the 3 groups of the populations in this river. These tests were done in FSTAT (Goudet, 1995).

Hypothesis testing 1: one catchment—one gene pool hypothesis

At the estuarine scale, we estimated F_{IT} (CapF), F_{IS} (small f), and F_{ST} (θ) (Weir & Cockerham, 1984) using FSTAT. We also conducted hierarchical analysis of molecular variance (AMOVA-Fst) and estimated pairwise standardized genetic differentiation (F'_{st}) for all population pairs using GenAlEx (Peakall & Smouse, 2012). The hierarchy introduced was based on the aforementioned grouping of populations into 5 regions based on their location in the estuary—north, upstream Wouri, midway Wouri, downstream Wouri, and south (protected area). We tested for isolation by distance (IBD) among populations (transects) of the entire estuary through a Mantel test of pairwise genetic differentiation (F'_{st}) versus \log_{10} geographic distance (km) (direct flight) using GenAlEx. To visualize the spatial relationship among populations, a principal coordinate analysis (PCoA) was performed at population level using GenAlEx, based on Nei's genotypic distances (Nei, 1978). Additionally, a Bayesian clustering analysis based on the clustering of individuals was conducted using STRUCTURE v.2.3.4 (Pritchard et al., 2000), by testing K values ranging from 1 to 10 (with 10 runs per K value) without any prior indication of population origin. The length of burn-in period was set at 10^5 and number of Markov chain Monte Carlo (MCMC) repeats, repeats after burn-in, at 10^6 . The program minimizes deviations from the HWE and linkage equilibrium, and was run by assuming the admixture model. The results of K values were obtained from STRUCTURE HARVESTER online (Earl & von Holdt, 2012) and the best K value was determined with the highest ΔK value following the Evanno method (Evanno et al., 2005).

Hypothesis testing 2: unidirectional dispersal hypothesis

Zooming in on transects from the Wouri River channel, we investigated genetic structure by carrying out another AMOVA using only samples from this river. A spatial autocorrelation for multiple populations was also carried out to investigate fine-scale genetic structure along this river. Ten (10) even distance classes of 25 m were adjusted in 9999 permutations and 9999 bootstrapping for the spatial autocorrelation in GenAlEx. In addition, we tested for

IBD in populations from the Wouri River channel. This was done on pairwise genetic differentiation ($F'st$) versus direct flight geographic distances (km), through a Mantel test (9999 permutations).

For investigating gene flow directionality, Pearson correlation between the within transect molecular variance and the positions of transects was calculated. The within transect molecular variance was calculated by AMOVA sum of squares divided by $n-1$ (where n is the population size) (Fischer & Matthies, 1998). Also, we performed Spearman rank correlation of the observed heterozygosity of transects versus the position of transects. These were done in order to investigate whether there was an accumulation of genetic diversity in downstream populations due to unidirectional gene flow via propagules dispersed by the river current. This relationship is positive if there is evidence of downstream accumulation of genetic diversity (Honnay et al., 2010).

To test for dispersal direction along the Wouri River, we used the model comparison technique using Bayes factor, available in Migrate-n (Beerli & Palczewski, 2010). We compared 5 migration models to determine which model best explains the historical migration along this river landscape. For this analysis we pooled transects into three populations, following their location on the river—upstream, midway, and downstream. The tested hypothesized models include the following: (1) full migration model, (2) downstream $\leftarrow \rightarrow$ midway $\leftarrow \rightarrow$ upstream (bidirectional, stepping stone dispersal), (3) upstream \rightarrow downstream (unidirectional downstream dispersal), (4) downstream \rightarrow upstream (unidirectional upstream dispersal), and (5) panmixis. The same parameter settings were used for all runs to enable comparison. This run assumed mutation rate of microsatellites to follow the stepping stone model (Brownian microsatellite option), with mutation rates set to vary per loci (crudely estimated from data). It ran four replicates of four heated chain searches (1.0, 1.5, 3.0, and 10^6) and one long Markov chain over all the studied loci; with 1 concurrent chain with 10^6 genealogy visitations after an initial burn-in of 10,000 steps. The Bayes factor for each model was calculated using the following formula:

$$LBF = 2 \times [\ln(\text{ml}(\text{model 1})) - \ln(\text{ml}(\text{model 2}))] \quad (1)$$

(see Beerli & Palczewski, 2010). The Bezier approximation to the marginal likelihood was used to test for the best model and the best dispersal model was

selected using the range of model elimination/selection guidelines as outlined by Kass & Raftery (1995). Contemporary dispersal directions and rates were also estimated for the Wouri River using BayesAss (Wilson & Rannala, 2003). This software uses individual multilocus genotypes to estimate rates of contemporary immigration (last 3 generations) among the studied populations following a Bayesian approach. It also assumes that genotype frequencies can deviate from the HWE proportions within populations. The program settings for the runs were 3×10^6 MCMC iterations with 10^6 burn-in and 2000 sampling frequency.

We carried out field experiments of propagule dispersal along this river to complement genetic data and to unveil the dynamics of the river on propagule dispersal from the mangroves in it. This was done through mark–release–recapture experiments using 600 mature propagules and subsequent calculations of dispersal distances and directions, similar to Van der Stocken et al. (2015b). Propagules were considered mature when they released effortlessly when slightly shaking the branches. Propagules were painted in four colours (150 propagules per colour) and released at four different locations in the river channel: an upstream location (at the mangrove limit upstream), two midway locations (1 closer to upstream and another closer to downstream), and a downstream location. Propagules were released at high tides (on 25/01/2016) and retrieved every subsequent high tide for 5 high tides (morning and evening on 26/01/2016, morning and evening on 27/01/2016, morning high tide of 28/01/2016). Propagule dispersal distances (d) were calculated using the spherical law of cosine and the longitude and latitude data of each retrieved propagules

$$d = \arccos[\sin(Y_1) \sin(Y_2) \cos(X_1) \cos(X_2) + \cos(X_1 - X_2)] * R \quad (2)$$

where R is the radius of the earth (6,370,000 m) and X_1 , Y_1 and X_2 , Y_2 are the coordinates of the release and retrieve locations, respectively.

Significance of directionality in the dispersal direction data was tested with Rayleigh's test of uniformity in Oriana 4.01. Following the bimodal distribution of our data, we therefore designated two groups of dispersal directions based on the mean directions of all dispersal direction data, following the approach of Van der Stocken et al. (2015b). Group 1

consisted of directions in the range from $>136^\circ$ to $<316^\circ$ and Group 2 consisted of directions in the range from $>316^\circ$ to $<136^\circ$. Directionality plots were made using Oriana 4.01.

Results

Data quality and genetic diversity

The total allele diversity for all loci was 50 and the mean per locus was 4.5. Observed heterozygosity (H_o) per locus varied from 0.008 to 0.59 (mean 0.306), while the expected heterozygosity (H_e) ranged from 0.008 to 0.615 (mean 0.293) (Table 2). Significant departure from HWE was detected only for Race5 ($P < 0.001$). Marginally significant allelic associations ($P < 0.05$) were observed for Race3 \times Race5, Race5 \times Race7, and Race5 \times Race17, in the overall test. However, in the within-sample tests, there was no significant linkage disequilibrium, so all loci were used in further analyses. Private alleles were detected by Race3, Race15, and Race24, although at low frequencies (<0.017).

There were no significant deviations from the HWE ($P < 0.001$) nor linkage disequilibrium between any pair of loci in the populations. Null alleles could be present at some loci albeit at very low frequency (Table S1). The total number of alleles (A_t) per population (transect) ranged from 26 to 34 in the study area. Douala XII had the highest number of alleles, while the lowest was Tik IV and RSV2 (Table 1). Average allelic richness ($A_r = 2.5$), effective number of alleles ($A_e = 1.6$), and heterozygosity ($H_o = 0.306$ and $uH_e = 0.299$) revealed moderate genetic diversity in the CEC (Table 1). There was no significant F_{is} in any transect (Table 1). However, evidence of recent bottleneck was observed in 11 out of the 21 transects (52%). Heterozygosity (H_o) and gene diversity (H_s) were the highest in the seaward Bonaberi (downstream Wouri) area, although transects within this region had the lowest genetic differentiation ($F_{st} = 0.006$) (Table S2). The lowest allelic richness, heterozygosity, and gene diversity were observed in samples from the protected area, while the inbreeding coefficient (F_{is}) was low in all groups (Table S2). The grouping of transect of the Wouri River channel (upstream, midway, and downstream) indicated slightly higher allelic richness (A_r), heterozygosity

(H_o), and gene diversity (H_s) in downstream populations (Table S2).

Hypotheses 1 and 2: one catchment—one gene pool and unidirectional dispersal hypotheses

Jackknifing across loci indicated that there was no inbreeding ($f = -0.023$, $SE = 0.054$), low differentiation ($\theta = 0.050$, $SE = 0.017$), and a low relatedness ($Rel. = 0.096$, $SE = 0.036$) for the entire estuary. Hierarchical AMOVA— F_{st} for the whole estuary (assuming the afore-mentioned groups) revealed 4% differentiation among regions ($F_{rt} = 0.044$), resulting in high gene flow ($N_m = 3.5$) (Table S3). Comparison of the AMOVA's of the entire estuary and that of the Wouri River channel revealed that higher levels of gene flow ($N_m = 9.3$) occur within the river (Table S3). Spearman rank correlation was non-significant for the molecular variance versus transect position ($P > 0.05$, $R = 0.2$) and was significant for the observed heterozygosity versus position of transect ($P < 0.05$, $R = -0.7$). Contemporary and historical migration estimates indicated dispersal in both the downstream and the upstream directions (Table S4). The best dispersal model, from the model comparison test, was downstream \leftrightarrow midway \leftrightarrow upstream (bidirectional, stepping stone dispersal). The very low log likelihood scores and the calculated Bayes factor all strongly support the model, resulting in a probability of 1 (Table 3). Release–recapture experiments also strongly support the bidirectionality of propagule dispersal along this river landscape (Fig. 2, Table 4). The maximum distance covered for the retrieved propagules was 5 km, although the majority of the retrieved propagules hardly dispersed farther than 1 km (the reverse is true for the upstream population) (Fig. 2). However, it is important to note that due to the strong dynamics of the river currents, only 10% of the 600 propagules were retrieved (Table 4).

PCoA showed some level of spatial patterning of transects across the entire estuary. It is important to note that the clustering according to PCoA results is unique from the earlier mentioned grouping we performed, based on location of the sample sites, to compare genetic diversity between different areas. According to PCoA, Mabeta and TikI at the north of the CEC are clustered together (1st cluster), followed by a clustering with high admixture of other populations from the Wouri

Table 3 Bayes factors and log marginal likelihoods of hypothetical dispersal models for the Wouri River

Model	Bezier lmL	Harmonic lmL	LBF	Choice (Bezier)	Model probability
1: Full migration model	−94,266.09	−74.12	−20,579.83	2	0
2: Bidirectional stepping stone model (True model)	−83,976.26	−166.17	0.00	1 (best model)	1
3: Upstream to downstream unidirectional model	−100,862.67	−238.12	−33,772.82	3	0
4: Downstream to upstream unidirectional model	−100,928.62	−225.33	−33,904.52	4	0
5: Panmixia	−106,246.07	−281.95	−44,539.62	5	0

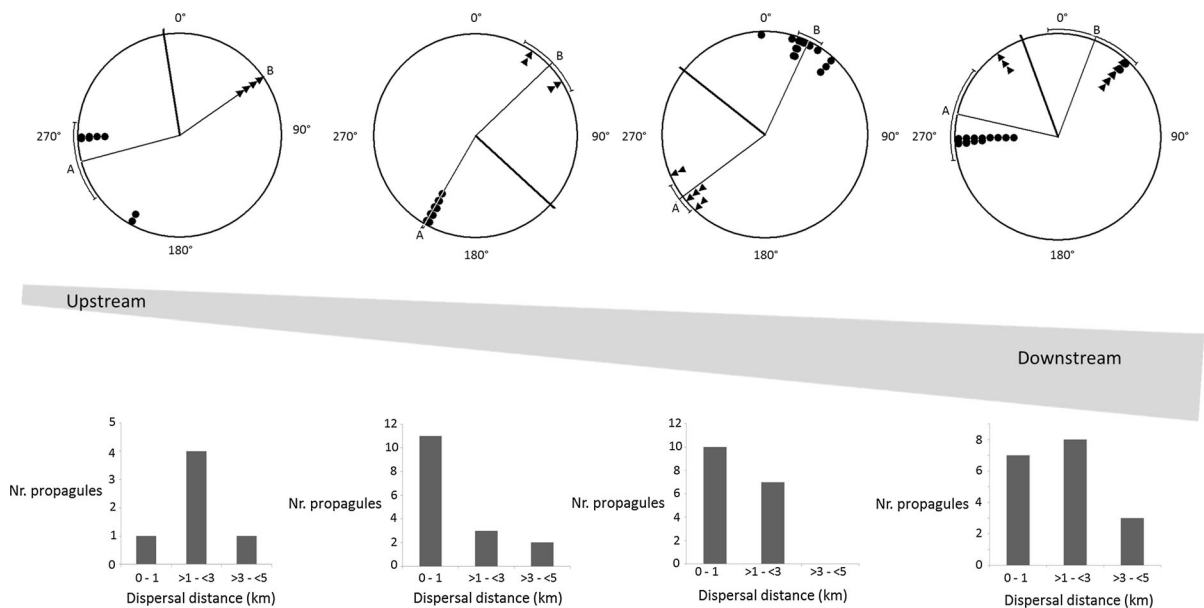


Fig. 2 Dispersal directions (*circular representation, top*) and dispersal distances (*bar charts, bottom*) for the *Rhizophora racemosa* propagules released at 4 locations along the Wouri River (upstream to downstream). In total, 600 propagules were released (i.e. 150 propagules at each of the 4 sites) at high tide on 25 January 2016 and retrieved subsequently over a period of 5 high tides. As our data were bimodal, we defined 2 groups of dispersal directions based on the average directions of all data.

Group 1 (*circles*) consisted of directions in the range from $>136^\circ$ to $<316^\circ$ and Group 2 (*triangles*) consisted of directions in the range from $>316^\circ$ to $<136^\circ$. Thin lines from the centre to the periphery indicate the averages of the different direction groups with the 95% confidence interval outside the periphery and the bold line represents the grand mean. The bar charts indicate the dispersal distance of the propagules released at the 4 respective release locations

(upstream and midway) and the other transects from Tiko (2nd cluster). However, the downstream transects were somewhat isolated from this larger clustering (Fig. 3). Transects from the Mouanko Reserve are also clustered apart from this larger (2nd) cluster (Fig. 3). However, Bayesian clustering analysis (STRUCTURE) grouped transects into 1 group, i.e. the whole estuary is made up of one genetic cluster (Fig. S1). We found a significant ($P < 0.001$) IBD pattern in populations of

the entire estuary (data not shown), following a Mantel test, which was rather non-predictive ($R^2 = 0.19$). On the other hand, stronger IBD ($R^2 = 0.43$, $P < 0.001$) was observed for samples along the Wouri River channel (Fig. 4). We also observed fine-scale spatial aggregation of individuals at short distance classes of 25 m within the Wouri River channel ($r = 0.072$, $P < 0.001$) following a spatial autocorrelation analysis (Fig. 5).

Table 4 Descriptive circular statistics of propagules retrieved from field capture–release experiments after 5 high tides (3 days)

No. of observations	Group 1 ($>136^\circ$ to $<316^\circ$)				Group 2 ($>316^\circ$ to $<136^\circ$)			
	Upstream 4	Midway 4	Midway 3	Downstream 12	Upstream 2	Midway 12	Midway 14	Downstream 6
Mean angle	244.8	202.6	233.7	291.9	55.2	67.7	19.7	28.1
SD	25.5	13.0	9.8	57.3	0.1	18.7	15.8	27.5
Rayleigh test (P)	0.025	0.01	0.038	0.009	0.137	*	*	0.003
Rayleigh test (Z)	3.281	3.799	2.913	4.416	2	10.792	12.981	4.416

A total of 600 propagules were released at four different locations (150 per location) along the river channel from upstream to downstream. Dispersal directionality was bimodal so we used the mean dispersal direction of all data to group data into two groups. Circular statistics was applied on this grouping and significance at $P < 0.05$ (bold) and <0.001 (*) are indicated below. *SD* standard deviation

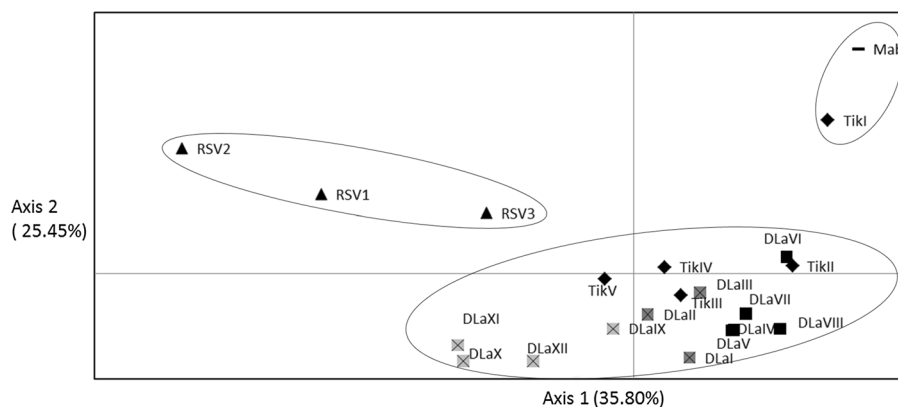


Fig. 3 Principal coordinate analysis (PCoA) of 21 transects into three groups: a major group of transects of the main Wouri River channel and its northern catchments (DLA I—DLA VIII and TikI—TikV), a second group of transects from the

protected area of the Douala-Edea (Mouanko) Reserve (RSV1–RSV3), and a third group of two highly connected transects of the northern end of the estuary (Mab and TikI)

Discussion

Moderate genetic diversity of red mangroves in the CEC

We observed moderate allelic richness and heterozygosity in the highly disturbed mangroves of the Cameroon Estuary complex (CEC). This diversity pattern is comparable to those reported for other *Rhizophora* species from different areas along the Atlantic and Pacific coasts (Ceron-Souza et al., 2012; Sandoval-Castro et al., 2014; Yahya et al., 2014; Ng et al., 2015; Wee et al., 2015). Heterozygosity dearth can arise due to reduced genetic diversity at range limits of species (Dodd et al., 2002; Arnaud-Haond et al., 2006), inbreeding (Dodd et al., 2002), the presence of null alleles (Arbeláez-Cortes et al., 2007), Wahlund's effect (Ng et al., 2015), and in mangroves probably due

to strong selection in founders due to the harsh environment of the intertidal zone (Triest, 2008). The CEC is centrally located in the Cameroonian and West African mangrove range, and inbreeding signals were absent in all studied populations. This indicates that the low heterozygosity observed could have arisen due to the biology of the species (Wee et al., 2015). Populations of downstream Wouri were the highest in genetic diversity, most likely due to the input of alleles from upstream and via ocean currents from mangrove populations in nearby coastal areas. However, genetic diversity was the lowest in the protected area, probably highlighting the impact of historical pressures in this protected area. Historically, large quantities of timber were extracted around this area for processing in the country's pioneer timber mill located in Monaco (Saenger & Bellan, 1995). Alternatively, low diversity in this protected area could be due to restricted gene

Fig. 4 Isolation by distance (Mantel test) for *Rhizophora racemosa* populations from the Wouri River channel (significant at $P < 0.001$)

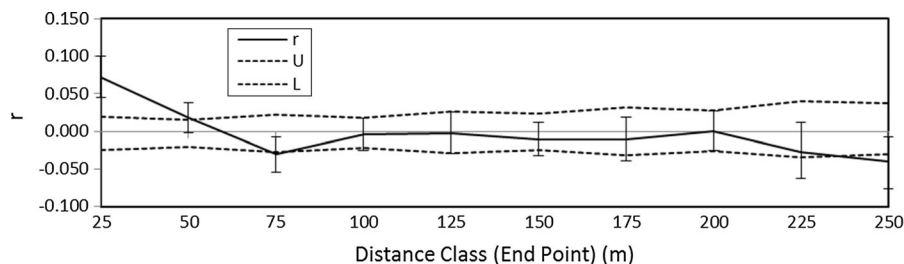
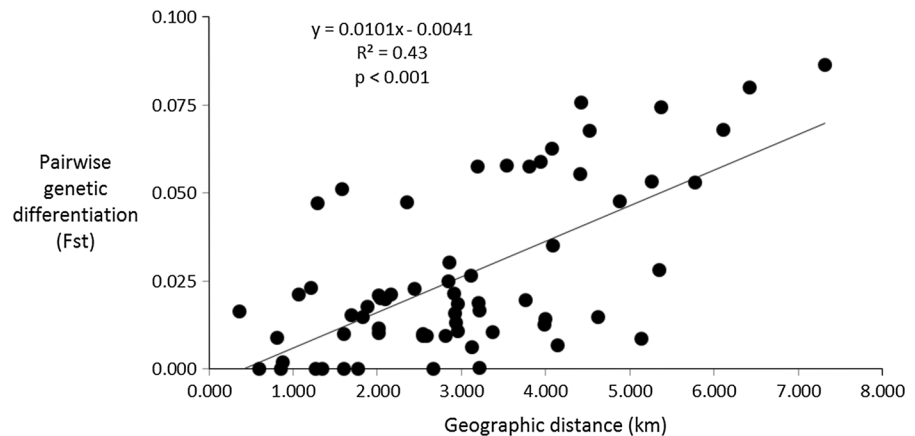


Fig. 5 Spatial autocorrelogram showing fine-scale spatial structure as a function of even short distance classes (25 m at $P < 0.001$) of *Rhizophora racemosa* individuals along a river

flow (reduced input of new propagules) into this area. Ngeve et al. (2016) observed that the mangroves of this protected area may be the source of propagules that colonized the Wouri River channel following Slatkin's "propagule pool" model (Slatkin, 1977). Their results show that this population is the "giving" population, contributing propagules into other populations, especially populations of the river channel, while it rarely receives input from other populations. This, most likely, results from its isolation either due to deforestation in surrounding non-protected areas (cf. Naughton-Treves et al., 2005) or due to its landward positioning.

Despite the wind-mediated and insect-assisted self-pollination potential in *Rhizophora* (Menezes et al., 1997; Sánchez-Núñez & Mancera-Pined, 2012; Nadia & Machado, 2014), high outcrossing was observed in all our study sites. Gene flow based on AMOVA-Fst was high ($Nm = 3.5$). This suggests a system where strong winds and an abundance of insect pollinators operate for effective pollen dispersal, as

linear landscape (Wouri River), r = spatial autocorrelation coefficient; U and L are the upper and lower confidence intervals)

well as effective seed dispersal via hydrochory within this complex estuary, thereby reducing the genetic differentiation among populations (Ismail et al., 2012).

Bidirectional gene flow along a linear river landscape

We observed slightly higher values of heterozygosity, allelic richness, and higher number of alleles in the downstream populations when compared to the upstream populations of the Wouri River. However, our analysis of dispersal models suggests that the best model fit of dispersal along this river landscape is a bidirectional (stepping stone) model. This pattern is supported by our experimental seed dispersal dynamics and estimates of contemporary as well as historical dispersal rates. Also Spearman rank correlations of within-population molecular variance and observed heterozygosity versus the position of the populations

on the river channel indicated that there was no accumulation of genetic diversity in downstream populations. This suggests the rejection of the “unidirectional dispersal hypothesis” and the presence of asymmetrical bidirectional dispersal along this linear river landscape. Several studies carried out to investigate the unidirectional dispersal hypothesis focused on floating and submerged macrophytes, riparian herbs, and shrubs (Pollux et al., 2007, 2009; Honnay et al., 2010; Love et al., 2013; Werth & Scheidegger, 2014). Contrary to *Rhizophora* propagules, these species generally have small hydrochorous seeds, whereas some are clonal and can disperse vegetative units. Many of these studies refuted the unidirectional dispersal hypothesis, attributing dispersal in the upstream direction to other dispersal vectors such as waterfowl (Pollux et al., 2007, 2009; Honnay et al., 2010; Werth & Scheidegger, 2014) (but see Mitsui et al., 2010; Love et al., 2013).

The role of zoochory in mediating dispersal in the upstream direction has been well established for macrophytes, since their seeds can easily be ingested (or cling on feathers) and be passively transported by water birds. However, this is impossible for the very large (>30 cm) hydrochorous propagules of *Rhizophora racemosa*. Although propagules are being depredated by monkeys, gastropods, and decapods (personal observation; Longonje & Raffaelli, 2013; Maxwell et al., 2015), there is little chance that they will be transported to upstream habitats in a viable state by animals. Therefore, the bidirectional dispersal observed suggests processes other than the frequently reported zoochory. This river system, like most others in the Gulf of Guinea, is under tidal influences which facilitate mangrove growth several kilometres inland (Corcoran et al., 2007). The role of tides could be further reinforced by winds to promote upstream dispersal in species with horizontally floating propagules (Van der Stocken et al., 2013, 2015a, b), and wind-borne pollen would also mediate upstream dispersal, and thus counteracting downstream accumulation of alleles over time. Van der Stocken et al. (2015a) found that dispersal of larger propagules, such as those of *Rhizophora* species, is constrained by major tidal currents.

The role of intervening forest between stands of the upstream and downstream limits apparently affects dispersal, causing it to follow a stepping stone dispersal model. We observed significant IBD

via the Mantel test and our model comparison analysis supports a stepping stone bidirectional dispersal to be the best model describing dispersal along this river channel. Dispersal vectors (water in this case) moving through closed mangrove forest landscapes should have lower vector displacement velocities and shorter seed passage time, thereby restricting LDD (jump dispersal) along the landscape (Nathan et al., 2008). The difficulty of propagules to leave and enter dense systems can either favour or limit short distance dispersal (Van der Stocken et al., 2015b). High retention rates by *Rhizophora* roots have been reported by Van der Stocken et al. (2015b). Such processes, in addition to tidal flooding, may contribute in explaining the significant spatial autocorrelation (significant fine-scale structure) observed in this linear landscape.

Dispersal in the upstream and downstream directions can hardly be symmetrical, since upstream populations will be seed-limited because the number of propagules flowing out cannot be compensated by propagules coming in. However, this leaves more recruitment opportunities for propagules that arrive at the less propagule-dense upstream areas to thrive (Honnay et al., 2010). The ambophilous flowers of *R. racemosa* are predominantly wind pollinated, ruling out the effects of unidirectional dispersal (Honnay et al., 2010), especially when populations are not very far apart from each other. Wind-borne pollen dispersal, however, is less effective in patchy landscapes such as mangroves (Wee, 2013), and although we cannot distinguish between gene flow via pollen and seed in this study, this indicates that bidirectional gene flow is predominantly accounted for by (hydrochorous) propagule dispersal. This is supported by the results of our capture–release experiments.

Genetic connectivity among catchments

We found sufficient evidence to refute the “one catchment-one gene pool” hypothesis because of the grouping of all individuals in this estuary into a single genetic cluster. None of the catchments had a unique gene pool. The difference in the separation of transects by PCoA and by Bayesian clustering analysis is due to mixing under the equilibrium assumption, whereas PCoA records show short-term occurrences (Triest et al., 2014). Nevertheless, the results of both analyses reveal connectivity between catchments. Inter-

catchment connectivity was observed for *Mycaria germanica*, mediated by water fowl (zoochory) (Werth & Scheidegger, 2014), and for *Rhododendron ripense* as a result of river captures (Kondo et al., 2009). High inter-river connectivity in our study is likely to be due to the tidal influence on these river systems (Corcoran et al., 2007). A plausible scenario is one in which propagules reaching this complex estuary and the coastal waters are randomly transported by tidal fluxes, back inland into catchments, irrespective of those of their origin, allowing mixtures from different catchments. Also, it is sensible to assume that the admixture results from wind-borne pollen that is being randomly dispersed over the entire complex.

Implications for conservation

Rhizophora racemosa has a ‘least concern’ status but with declining population trends according to the IUCN (2016). However, the species is threatened in West Africa by the spread of the exotic *Nypa fruticans*, and high anthropogenic pressures as indicated earlier (Corcoran et al., 2007; Nfotabong-Atheull et al., 2009, 2011, 2013; FAO, 2011; IUCN, 2016).

Mangroves in Cameroon have been shown to be heavily impacted by climate change-induced sea level rise (SLR) (Ellison & Zouh, 2012). Hydrological changes and spatial shifts in the optimal salinity ranges for *R. racemosa* may occur under SLR. If tidal reach and saline waters would extend up the river (saline intrusion), survival of *R. racemosa* stands along river channels will depend on the species’ ability to colonize upstream localities, their adaptability to changes in hydrological variables such as inundation frequency and hydroperiod (Di Nitto et al., 2014), sediment supply (e.g. Nicholls & Cazenave, 2010; Woodroffe et al., 2016), and the influx of viable propagules in those regions. Additionally, human activities and development may limit the area of potential suitable habitat, hampering upstream expansion.

Downstream populations along the Wouri River already face some of these challenges. Therefore, we advise that these downstream populations, which are also the most genetically diverse in this estuary complex, be considered as a special conservation unit, and recommend the use of their propagules in afforestation programs so as to conserve their unique

diversity. Detection of hotspots of genetic diversity within an area is a good argument, among others, to convince decision makers to conserve genetic diversity (Triest, 2008), as well as guide future conservation efforts. Due to the costly nature of conservation plans, efforts geared at preserving genetic variability is important for a population’s long-term survival. Nevertheless, authorities should strive to maintain all populations since genetic diversity may increase with population density.

The observed moderate genetic diversity, low differentiation among populations, and lack of inbreeding in all the populations, imply that these populations, despite evidences of recent bottlenecks in some, do not face any threat of genetic drift at the moment. However, efforts need to be made to avoid habitat fragmentation through the enforcement of regulations that restrict unsustainable exploitation. Also, measures should be taken to regulate sand extraction from the CEC and abort the removal of (compact) mangrove sediment for its use in land reclamation. Both practices directly affect substrate, limiting the recruitment potential of juveniles. Removal of compact mangrove substrate would also affect the overall long-term hydrology and geomorphology of the Wouri River. As local communities encroach into the river, through land reclamation for the construction of residential homes, the ecosystem’s long-term resilience may be reduced. Developmental projects like the expansion of the Bonaberi (Wouri) Bridge (downstream Wouri) should not undermine the small, but uniquely diverse mangrove populations in its proximity. The relatively small mangrove area protected under the Douala-Edea Reserve has the lowest genetic diversity in the CEC, urging the need for increasing the mangrove protected area coverage in the CEC.

Conclusion

Our results indicate that the 11 polymorphic microsatellite markers developed from *R. racemosa* in this study (Cameroon) have wide applicability and would add to the existing markers for revealing patterns of genetic variability and differentiation for *Rhizophora* spp. From this study, including 21 transects, we conclude that genetic structuring in *R. racemosa* populations of the CEC is maintained by

interacting hydrological factors, namely the flow currents of the Wouri River, tidal fluxes, and coastal currents, coupled with winds. In this linear river landscape, gene flow is bidirectional. Intervening tree stands may favour dispersal following a stepping stone model. We believe that the results presented here are valuable for guiding future management efforts on strategizing restoration and/or rehabilitation efforts of these populations in a bid to conserve optimal genetic variability, and hence the long-term resilience of these mangroves to cope with the various effects of climate change.

Acknowledgments The Rufford Foundation (UK) Small Grant (17335-1) awarded to M.N. Ngeve catered for the field work and field experiments. This study was part of the Vrije Universiteit Brussel—International Relations and Mobility Office (VUB—IRMO) Doctoral Scholarship awarded to M.N. Ngeve. The BAS 42 funding of the VUB also supported the laboratory analyses of this study. We are grateful to Ms. Natalie Kana, Ma Christiana Nalova Ngenye, Mr. Peter Elive Ikome, and Mr. Henri Larry Etonde Kingue, for their logistical and field assistance. We are grateful to the anonymous reviewers for their comments on earlier versions of this manuscript that helped in its improvement.

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