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Genetic population structure of red mangrove (*Rhizophora mangle* L.) along the northwestern coast of Mexico

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ABSTRACT

This study contributes to our understanding of population genetic diversity and connectivity in the red mangrove of northwestern Mexico. We evaluated the population genetic structure and the patterns of gene flow of the red mangrove, *Rhizophora mangle*, along the northwestern coast of Mexico. We analyzed samples taken from the northern Pacific distribution range limit, discuss the main mechanisms that probably have shaped the current genetic composition. The results show significantly reduced genetic diversity in northern populations, which can be attributed to very low effective population size, inbreeding, and high environmental pressure at distribution margins. Private alleles were found to be present at very low frequency on both the Pacific coast of Baja California and inside the Gulf of California, which suggests heterogeneity in genetic composition. Although we detected gene flow that was apparently associated with marine currents, we believe that the Baja California peninsula and the convergence of marine currents at the mouth of the Gulf of California are acting as effective barriers that limit gene flow. Cluster analysis supports this hypothesis, showing two main areas of genetic composition (inside and outside the Gulf of California). These findings were confirmed by significant genetic differentiation (F_{ST} = 0.26; R_{ST} = 0.50) between these regions; the findings also show that *R. mangle* presents low genetic diversity at margins without a random distribution and is present in at least two genetic population units along the northwestern Mexican coast.

1. Introduction

The biology of populations at range margins is of increasing interest because it is these marginal populations that will be affected most by future climate change, either as "leading edge" potentially expanding populations and ranges, or as "trailing edge" populations that become increasingly marginal as the habitat changes. Species with distributions that are more or less linear over large latitudinal distances provide good examples for testing hypotheses concerning demographic processes at range margins. Salt marsh communities are limited to a narrow coastal fringe and where they are distributed along north–south trending coastlines, populations at the range limits will constitute the most important gene pools for range expansions and contractions. Mangrove communities along the Pacific coast of America are such an example. On a broad geographic scale, trailing edge populations are of minor importance because they are anchored in the tropics, but the leading edge populations are where potential colonization and range expansion may occur. At more local scales, populations are not continuous because of lack of suitable substrate, or anthropogenic loss that has been a major threat to mangroves in the past (Duke et al., 2007). The latitudinal limit of mangroves is at the 16 °C isotherm, where range is not limited by other physiographic factors (Gilman et al., 2008) and so an anticipated increase in winter temperature of 2–6 °C in southern California over the next century (Hayhoe et al., 2004), suggests a potential northward shift in the distribution of mangroves as has been projected for endemic vegetation of the California Floristic Province (Loarie et al., 2008). Colonization will

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be dependent on the success of propagule dispersal and the availability of suitable substrate that may be a major constraint under rising sea levels (Gilman et al., 2008).

Peripheral populations of mangroves commonly show reduced genetic diversity and higher genetic structure than core populations. This has been variously attributed to low effective population size, dispersal limitations (Maguire et al., 2000), pollinator scarcity (Giang et al., 2003) and higher environmental pressures (Arnaud-Haond et al., 2006). Of importance in leading edge populations with relatively low genetic diversity, is whether this is a result of recent colonization through founder events, or allele fixation through genetic drift in small populations lacking gene flow. The former would support the notion that colonization could be successful given suitable habitat and the latter might be interpreted as a sign that populations may be less responsive to climate change.

In this work we test the hypothesis that discontinuous northern populations of the red mangrove (*Rhizophora mangle*) are low in genetic diversity as the result of recent colonizations and not of genetic drift. We expect that under a process of colonization, propagules are most likely to have come from nearby source populations and that a strong signal of connectivity and gene migration can be detected. On the other hand, if low genetic diversity in the discontinuous populations is a result of genetic drift, we would expect a strong pattern of population structure and a low signal of migration. The Gulf of California, Mexico, is a semi-enclosed marginal sea where the exchange of surface waters between regions occurs only at the mouth of the Gulf of California via cyclonic circulation (Castro et al., 2000). Most of the surface water entering the Gulf of California is tropical surface water that flows north from the equator along the mainland of the continent. It has abundant endemic species and is considered to be among the most biologically diverse regions on Earth (Brusca, 1980; Álvarez-Borrego, 2002). Phylogeographical studies have demonstrated that the tropical waters and current patterns of the southern Gulf of California have presented a barrier to gene flow and migration since the end of the Pleistocene which is reflected in high genetic divergence between the Gulf of California and the Pacific Ocean (Terry et al., 2000; Stepien et al., 2001; Bernardi et al., 2003; Muñiz-Salazar et al., 2005; López-Vivas et al., submitted for publication). Thus, we test also, whether populations of *R. mangle* along the Pacific coast of the Baja California peninsula show a signal of divergence from those of the Gulf of California.

We chose *R. mangle* because it is the most widespread mangrove species in northwestern Mexico, the Peninsula of Baja California and the Gulf of California that represents the northern limit for this species along the eastern Pacific coast (Pacheco-Ruiz et al., 2006).

2. Materials and methods

2.1. Plant material and DNA isolation

Leaf tissue was collected from 305 *R. mangle* individuals in ten wild populations along the Pacific coast of Baja California and the Gulf of California during the winters of 2006 and 2008 and the summer of 2009 (Table 1; Fig. 3). To minimize consanguineous samples and to maximize the probability of collecting diverse genotypes, samples were taken from specimens separated by at least 30 m. At Bahia de los Angeles (RBA) and Bahia de Kino, (RKI) where there were only small mangrove patches, samples were separated by at least 10 m. Leaf samples were dehydrated and stored in silica gel until DNA extraction. Total genomic DNA was isolated from approximately 200 mg (dry weight) of tissue using a modified CTAB/PVP method (Muñiz-Salazar et al., 2005). Tissue was crushed to a rough powder using a homogenizer and dry ice, re-suspended in approximately 1.5 volumes of CTAB/PVP and incubated at 65 °C for 6 h. The homogenate was extracted with approximately 0.6 volumes of chloroform/isoamyl alcohol (24:1 v/v) and centrifuged at 13,000 rpm for 10 min to separate phases. DNA was precipitated at −20 °C overnight with 0.7 volumes of isopropanol and then centrifuged at 13,000 rpm for 20 min. The DNA was resuspended in 100 μL of TE 1× and stored at −20 °C until further analysis.

2.2. Microsatellite analysis

Individuals were genotyped at six DNA microsatellite loci (Rm7, Rm11, Rm19, Rm21, Rm38 and Rm46) designed for *R. mangle* by Rosero-Galindo et al. (2002). The forward primers were fluorescent-labeled with FAM, VIC, PET and NED (Applied Biosystems Inc). Amplifications were performed in 20 μL PCR reactions containing 1× Buffer (10 mM Tris HCl, 50 mM KCl, pH 8.3, SIGMA),

Table 1
Sampling sites, geographic location and measures of gene diversity and Hardy–Weinberg Equilibrium (HWE). N, sample size; A, allele number; G, multilocus genotypes; AR, allelic richness; PA, private alleles; H_E, expected heterozygosity; H_O, observed heterozigosity; F_{IS}, inbreeding coefficient; *statistically significant ($P < 0.05$); ns, non significant.

Population	Code	Latitude Longitude	N	A	G	AR	PA	H_E	H_O	F_{IS}	HWE
Bahía de los Ángeles	RBA	29°02′77N 113°30′12W	48	8	3	1.32	0	0.08	0.05	0.32	*
Bahía Concepción	RBC	26°38′23N 111°49′48W	26	9	6	1.50	0	0.12	0.12	0.00	ns
San Ignacio	RSI	26°47′24N 113°09′08W	30	9	6	1.50	0	0.13	0.08	0.32	*
Bahía Magdalena	RBM	24°45′12N 111°59′06W	30	10	6	1.64	1	0.18	0.18	−0.03	ns
Bahía de Kino	RKI	29°02′28N 112°09′94W	27	9	4	1.49	0	0.09	0.08	0.17	*
Guaymas	RGU	27°57′34N 110°58′51W	28	13	10	2.13	1	0.15	0.13	0.15	ns
El Jitzámuri	RJZ	26°18′09N 109°14′27W	28	12	14	2.00	0	0.25	0.26	−0.01	ns
Bahía Balandra	RBL	24°19′20N 110°19′00W	30	11	7	1.81	1	0.18	0.26	−0.44	ns
Bahía de Altata	RAT	24°37′23N 107°54′38W	29	15	14	2.46	1	0.25	0.27	−0.04	ns
Teacapán	RTP	22°32′10N 105°41′44W	29	14	23	2.33	0	0.31	0.24	0.24	*
Mean			31	11	9	1.82	0.4	0.17	0.16	0.07	ns

1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 1 unit Taq DNA polymerase (SIGMA) and 20 ng of genomic DNA. All microsatellite loci were amplified with the same temperature profile on a MyCycler BIORAD thermal cycler, which consisted of 5 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C, and a final cycle of extension at 72 °C for 30 min. To ensure reproducibility and consistency of PCR amplifications, approximately 5% of the samples were re-amplified. In addition, a negative control was run for each set of PCR reactions and genotyped to check for contamination. PCR products were separated by capillary electrophoresis on a PE Applied Biosystems 310 Genetic Analyzer and all allele calls were done through GeneMarker software (Softgenetics).

2.3. Data analysis

2.3.1. Genetic diversity

The number of alleles (A), unbiased expected heterozygosity (H_E) and observed heterozygosity (H_O) for each locus across all localities were calculated using GDA 1.1 (Lewis and Zaykin, 2001). Allelic richness per locus (AR) and per population were calculated using FSTAT 2.9.3 (Goudet, 2001). Global tests for deviation from Hardy–Weinberg Equilibrium were performed using a Markov chain algorithm and linkage between all pairs of loci was estimated using GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008) with significance levels determined using the Markov chain method. For all Markov chain tests, the default parameters in GENEPOP were used with 100 batches of 1000 iterations each.

2.3.2. Clustering analyses

The Bayesian clustering algorithm implemented in STRUCTURE v2.2.3 (Pritchard et al., 2000) was applied as an exploratory analysis to infer population genetic structure, to assign individuals (probabilistically) without *a priori* knowledge of population units and limits. STRUCTURE uses individual multilocus genotype data to cluster individuals into K groups while minimizing Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups (Pritchard et al., 2000; Falush et al., 2003). STRUCTURE runs were based on 500,000 iterations after a burn-in of length 500,000 and assumed correlated allele frequencies and an admixture model with an estimated proportion α of admixed individuals. To check for Markov chain Monte Carlo (MCMC) convergence, we performed 10 replicates for each K value and checked the consistency of results. The most likely number of clusters (K) was considered to be the K value with the highest $Pr(X|K)$ (Pritchard et al., 2000). The optimal K value was calculated after the ΔK method described by Evanno et al. (2005).

2.3.3. Genetic structure

According to the clusters determined by STRUCTURE v.2.3.3, genetic differentiation was quantified using F statistics (Weir and Cockerham, 1984) and ρ statistics (Michalakis and Excoffier, 1996) using ARLEQUIN v. 3.5. (Excoffier and Lischer, 2010). The distribution of genetic variation was assessed by Analysis of Molecular Variance (AMOVA) at four hierarchical levels: among groups, among populations within groups, among individuals within populations and within individuals (Excoffier et al., 1992). Statistical significance of the variance was tested by 10,000 non-parametric permutations.

2.3.4. Migration rates

The magnitude and direction of gene flow was estimated according to a maximum likelihood approach implemented in MIGRATE 3.2 (Beerli and Felsenstein, 1999; Beerli and Palczewski, 2010). MIGRATE uses a coalescence approach to estimate migration rates (Nm) among populations, assuming a constant per-locus mutation rate. This approach is judged to estimate gene flow more accurately than other F_{ST} methods, especially when multiple loci are employed (Beerli and Felsenstein, 1999). It is common practice to run 10 short chains and then 2 long chains. Using random values for the first parameter set, Beerli and Felsenstein (1999) have found that for a given data set the results converge to a value that is not dependent on the initial parameter values when the total number of sampled genealogies exceeds 30,000. We believe our analysis is more rigorous and long enough as there were a total of 155×10^7 genealogies visited per locus. We used 10 short-chain searches and three long-chain searches over six microsatellite loci. For each locus, the program was run for 10 consecutive exploratory chains with lengths of 5×10^6 genealogy visits to adjust the driving values for both the run and the 3 long chains; the last chain was used to generate the presented results. Each of the long chains visited 5×10^8 genealogies, sampling 5000 after an initial burn-in of 10,000 steps. The program assumes discrete populations and generations, mutation-drift equilibrium, non-selective effects and the stepwise mutation model (Otha and Kimura, 1973) for microsatellite markers.

3. Results

3.1. Genetic diversity

All six analyzed loci displayed low levels of polymorphism in all *R. mangle* populations studied. Only 19 alleles were found among the 305 individuals. The total number of alleles detected per locus ranged from 2 (Rm46) to 4 (Rm21, Rm19) and the mean number of alleles per locus per population (observed allelic diversity) ranged from 1.3 at RBA to 2.5 at RAT. Private alleles were observed in both peninsular (RBM, RBL) and continental (RGU and RAT) populations. However, the frequency was very low; only one or two individuals in these populations showed private alleles (Table 1). The average allelic richness (AR) was 1.82, ranging from 1.32 at RBA to 2.46 at RAT.

The observed heterozygosity (H_O) for each locus varied from 0.01 at locus Rm7 to 0.40 at locus Rm11. Average expected heterozygosity (H_E) was slightly larger than the H_O at most loci, but only Rm19 and Rm46 loci showed significant deviations from Hardy–Weinberg Equilibrium (HWE; data not shown; $P < 0.05$). The Rm7 and Rm46 loci were monomorphic in all populations distributed along the Peninsula of Baja California. Furthermore, locus Rm21 was monomorphic for populations located at the northern limit (RBA, RSI, and RKI). The total number of alleles per population over all loci ranged from 8 in RBA to 15 in RAT with an average of 11 alleles by population (Table 1). The global test for heterozygote deficit revealed significant deviations from HWE in the populations

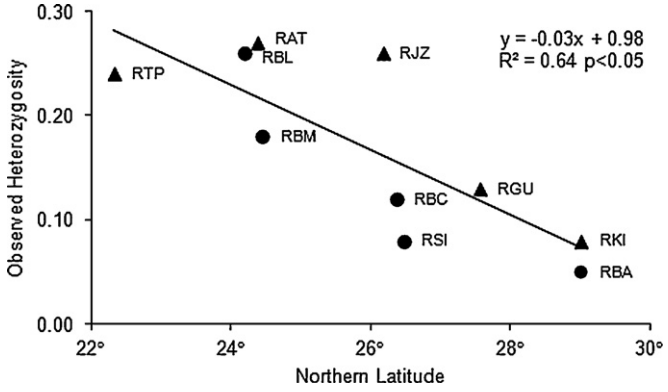

Fig. 1. Genetic diversity decreases in higher latitudes in mangroves from the Baja California Peninsula (circles) and mangroves from the continental coast (triangles).

Table 2
Matrix of pairwise comparisons of population genetic differentiation, F_{ST} below diagonal and R_{ST} above diagonal. Bold values are non significant ($P > 0.05$).

	RBA	RBC	RSI	RBM	RBL	RKI	RGU	RJZ	RAT	RTP
RBA	****	0.305	0.798	0.490	0.703	0.428	0.223	0.350	0.793	0.921
RBC	0.338	****	0.638	0.228	0.505	**0.006**	**0.017**	0.078	0.622	0.807
RSI	0.625	0.443	****	0.249	**0.035**	0.683	0.517	0.437	**0.061**	0.403
RBM	0.496	0.149	0.185	****	0.112	0.274	0.128	**0.066**	0.231	0.484
RBL	0.565	0.325	**0.036**	0.066	****	0.551	0.387	0.298	**0.024**	0.305
RKI	0.453	**0.009**	0.491	0.166	0.364	****	**0.061**	0.104	0.662	0.834
RGU	0.323	**0.007**	0.338	0.106	0.246	**0.040**	****	**0.033**	0.517	0.727
RJZ	0.400	0.114	0.311	0.110	0.217	0.110	0.094	****	0.416	0.624
RAT	0.558	0.373	0.097	0.146	**0.037**	0.414	0.313	0.241	****	0.180
RTP	0.650	0.519	0.347	0.362	0.281	0.547	0.474	0.359	0.145	****

RBA, RKI, RSI and RTP ($P < 0.05$). Both AR and H_O decreased near the limit of population distribution (RBA, RKI, RSI) (Fig. 1) and also showed significant heterozygote deficit (Table 1).

3.2. Clustering analyses

STRUCTURE analysis suggested that the ten localities analyzed cluster into three different groups (highest Ln Pr(X/K), $K = 3$). However, only two groups were inferred when applying Evanno's method (data not shown), probably due to the large associated variances of the data. The first group includes populations inside of the Gulf of California (RBA–RBC–RKI–RGU–RJZ) and the second group comprises populations outside and around the entrance of the Gulf of California (RSI–RBM–RBL–RAT–RTP) (Figs. 2 and 3). The mean percentage of individual assignment into the groups was larger than 70%. Some individuals showed more than 90% assignment. However, some individuals seem to be admixtures by gene flow, mainly at entrance of Gulf of California, as several individuals had a large proportion of their genotype assigned to a different cluster than the one in which they were sampled or had nearly equal Q values in two clusters (Fig. 2).

3.3. Genetic structure

In general, overall pairwise values of F_{ST} were lower than R_{ST} but significantly different from zero ($P < 0.05$; Table 2). Almost all population pairs were significantly different for both F_{ST} and R_{ST} ($F_{ST} = 0.04$–0.65, $R_{ST} = 0.04$–0.92; $P < 0.05$), revealing a strong genetic structure among populations. Exceptions were RBC-RKI and RBC-RGU for both F_{ST} and $R_{ST;}$ and RJZ-RGU and RAT-RBL for R_{ST} (Table 2).

Genetic structure was also assessed by an AMOVA at four different hierarchical levels using F_{ST} and R_{ST} (Table 3). The greatest variation was observed among individuals (39–58%), followed by the variation among populations within groups (20–32%) and the variation among groups (17–18%; $P < 0.05$; Table 3). Lower values were observed among individuals within populations (0–3%, $P > 0.05$). Thus, most of the genetic structure was at higher spatial scales (Table 3).

3.4. Migration rates

Maximum likelihood estimates based on Markov Chain Monte Carlo simulations performed using MIGRATE suggest that gene flow is predominantly southward along the west coast of the Gulf of California and mainly northward along the east coast (Fig. 3, Table 4). On the other hand, migration was bi-directional along the west coast of the peninsula of Baja California. The southernmost populations (RAT, RPT) showed a northward gene flow, generating migrants to populations along the Gulf of California and the west coast of the peninsula of Baja California (RBM, RSI).

4. Discussion

We set out to test whether mangroves, represented by the common range-limit species *R. mangle*, at the northern limit of their range in Mexico are in a stage of recent expansion, or of contraction. Our data on genetic diversity strongly support reduced

Table 3
Hierarchical analysis of molecular variance of 10 populations of *Rhizophora mangle* at the northwestern Mexican coast calculated by the Infinite Allele Model (IAM) and the Stepwise Mutation Model (SMM). Degrees of freedom (d.f.) and percentage molecular variation (% var.) are explained by the hierarchical level. *Statistically significant ($P < 0.05$). The number of groups were established by STRUCTURE, $K = 2$. Group 1: RBA–RBC–RKI–RGU–RJZ and Group 2: RSI–RBM–RBL–RAT–RTP.

Source of variation	d.f.	IAM		SMM	
		% var.	F_{ST}	% var.	R_{ST}
Among groups	1	27	0.26*	50	0.50*
Among populations within groups	8	16	0.21*	10	0.20*
Among individuals within populations	295	3	0.05	0	0.00
Within individuals	305	54	0.45*	43	0.56*

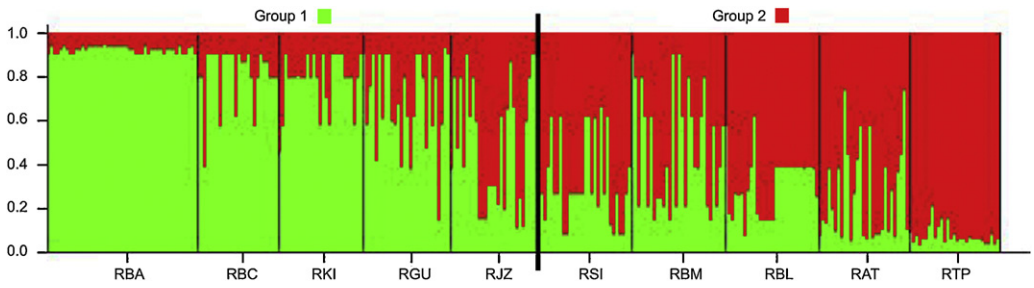

Fig. 2. STRUCTURE plot of *Rhizophora mangle* along the Gulf of California. Vertical bars represent individuals whose genotypes have been apportioned out into 2 clusters (Group 1 and Group 2).

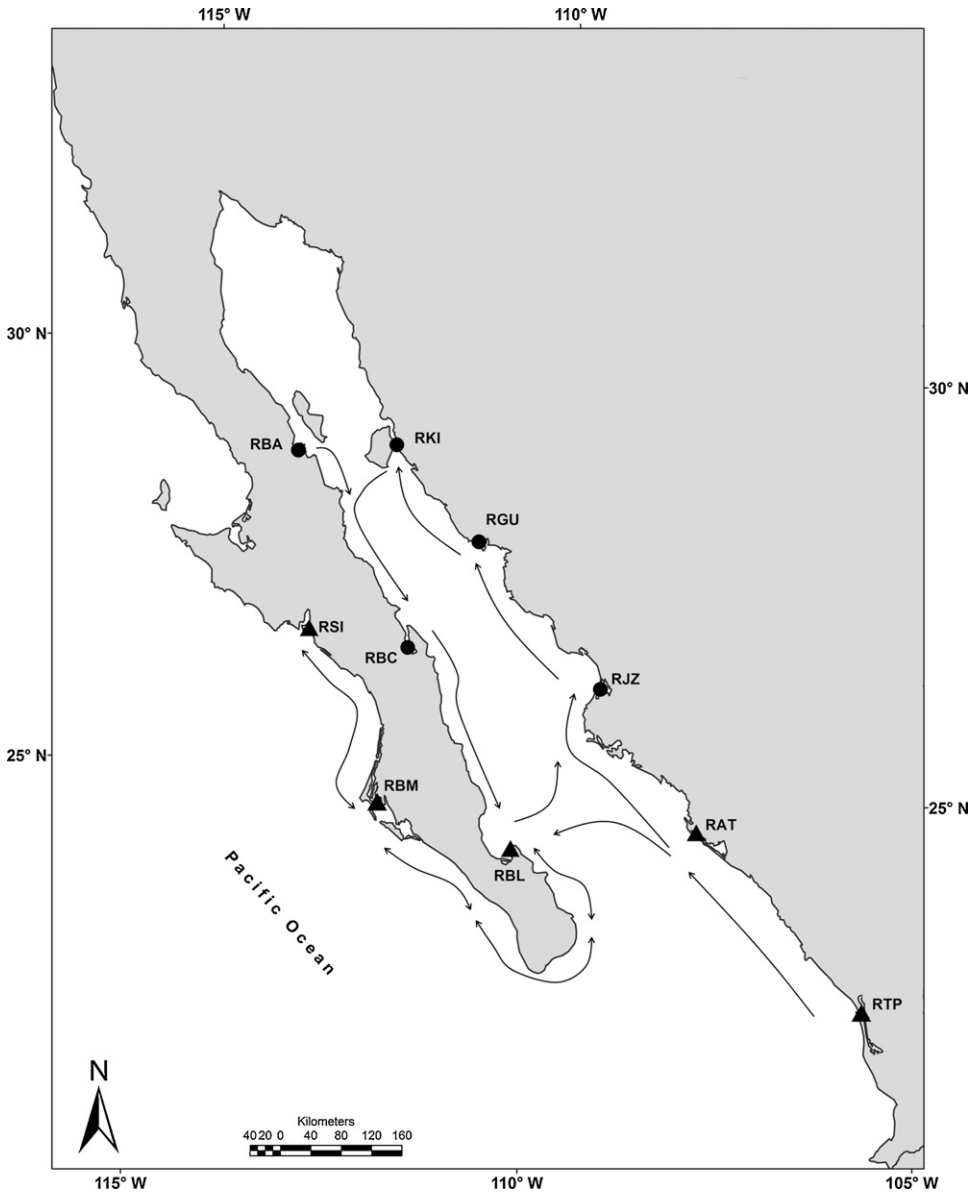

Fig. 3. Gene flow direction and geographic location of *R. mangle* sampling sites along the northwestern Mexican coast. The arrows show gene flow direction according to MIGRATE. The circles (Group 1) and triangles (Group 2) show clustering groups predicted with Evanno's algorithm.

genetic diversity and increased inbreeding in this region relative to expectations for populations from more core regions: for example, Arbeláez-Cortes et al. (2007) reported a three times higher genetic diversity on the Pacific coast of Colombia than the present study found. Our findings are consistent with those reported by Pil et al. (2011) for the southern limit of this species along the Brazilian coast. This pattern of low diversity at range limits has been well documented in widely distributed mangrove species such as *A. germinans*, along the Atlantic East of Pacific (Dodd et al., 2002) and *A. marina* along the Indo-Pacific (Maguire et al., 2000).

Table 4
Migration rates (number of migrants) among analyzed populations of *Rhizophora mangle*. Populations in columns are giving migrants to populations in rows. Numbers in bold indicate unidirectional genetic flow among populations.

	RBA	RBC	RSI	RBM	RBL	RKI	RGU	RJZ	RAT	RTP
RBA	–	0.48	0.42	0.12	0.54	0.96	0.67	0.12	4.35	0.36
RBC	0.22	–	0.76	0.56	0.65	**2.70**	1.84	0.22	**7.78**	0.63
RSI	0.40	0.87	–	0.27	1.75	**1.09**	**2.48**	1.54	**7.10**	1.68
RBM	**0.98**	0.63	**2.58**	–	1.98	1.42	0.83	1.24	**9.46**	**2.16**
RBL	**1.43**	0.19	1.12	1.24	–	**1.55**	0.56	0.43	**5.59**	2.36
RKI	1.00	0.36	0.21	0.78	0.78	–	**2.42**	1.49	**1.48**	0.78
RGU	**1.43**	1.14	0.57	**3.92**	**2.10**	0.72	–	0.81	**1.93**	0.81
RJZ	**1.71**	**1.28**	**2.73**	**3.07**	**1.47**	1.19	1.03	–	1.28	1.88
RAT	4.63	2.22	4.92	2.47	3.90	0.72	0.40	1.76	–	**4.38**
RTP	0.34	0.13	1.31	0.52	1.86	1.34	0.77	2.15	1.22	–

We hypothesized that marginal populations that were undergoing genetic drift would show a strong signal of divergence, whereas marginal populations in a phase of colonization would be held together by some common migratory patterns. The latter seems to be the case for *R. mangle* at the northern limit of its range. For example, despite pairwise comparisons (F_{ST}/R_{ST}) suggesting an elevated population genetic structure (Table 2), our Bayesian analysis implemented in the program STRUCTURE showed that the *R. mangle* populations formed only two main groups. The power of Bayesian approaches to determine patterns of population structure is partly due to the fact that they combine information from several loci into a single probability model, as opposed to the simple averaging used, for instance, in traditional F_{ST}/R_{ST} analysis (Corander et al., 2003). The Bayesian approach also allows estimation of the amount of genetic admixture in the population (Fig. 2) as well as level trough pooling of sets of individuals independently of the actual sample structure (Pritchard et al., 2000).

As most gene variation was shared among populations and because the allelic frequencies were somewhat comparable, we believe that our Bayesian analysis yielded the most likely scenario to explain the population genetic structure of *R. mangle* along northwestern Mexico. According to our Bayesian analysis, one group comprises populations situated inside of the Gulf of California (RBA–RBC–RKI–RGU–RJZ) while a second group includes populations located outside and around the entrance of the Gulf (RSI–RBM–RBL–RAT–RTP: Fig. 3). This suggests that the peninsula of Baja California acts as an effective barrier that limits gene flow between populations inside and outside of the Gulf of California as reported for other marine organisms such as fish (Terry et al., 2000; Stepien et al., 2001), marine mammals (Schramm-Urrutia, 2002) and eelgrass (Muñiz-Salazar et al., 2005). In addition, our results are consistent with the geographic isolation reported for both macroalgae (Espinoza-Ávalos, 1993) and microalgae (Santamaría-Del Ángel et al., 1999) in the Gulf of California. Interestingly, RBA and RKI, the two most northern populations of mangrove forest in the Gulf of California, were grouped in the same cluster despite being genetically distinct according to pairwise comparisons (F_{ST} and R_{ST}). The Gulf of California is narrower at this latitude. However, effective gene flow might have been interrupted by the strong marine circulation reported at this latitude in the Gulf of California (Marinone, 2003).

The Gulf of California is a semi-enclosed marginal sea where the exchange of surface waters between regions occurs predominantly at the mouth of the Gulf via cyclonic circulation (Castro et al., 2000). In this area, the fusion between tropical waters from the equator and the California current forms a constant oceanic front that promotes a temperature barrier (Castro et al., 2000) and may block effective propagule dispersal and gene flow among populations of mangrove and many other biota. We found a dispersal pattern among populations that coincides with the general pattern of surface circulation in the Gulf of California. This pattern consists of a system that flows northwards from the equator along the Mexican mainland into the Gulf of California and also swings out along the peninsula coast. The California current flows southward along the Pacific coast and converges with the Gulf of California surface water at the tip of the Baja California Peninsula (Marinone, 2003). The continental and peninsular populations of red mangrove are connected via cyclonic circulation both in the north-central region of the Gulf of California and at its entrance. This circulation pattern is predominant during summer (Marinone, 2003), matching the time of mature propagule production.

Populations located along the west coast of the Baja California peninsula have a bidirectional gene flow, which could be explained by both (1) the predominant California Current that flows southward and (2) the oceanographic phenomenon known as El Niño Southern Oscillation (ENSO). In this condition, a coastal northward flow is generated southwest of the Baja California Peninsula and is advected north of Punta Eugenia (Durazo-Arvizu and Baumgartner, 2002), situated at the northern end of the RSI mangrove population. Thus, propagules could follow northward during ENSO conditions and match the directional patterns of gene flow that we detected with MIGRATE. The same pattern of northward dispersal in this area has been reported by Lavaniegos et al. (1998) for tropical zooplankton, by Coyer et al. (2001) for seaweed species and eelgrass by Muñiz-Salazar et al. (2005) for eelgrasses.

We cannot entirely rule out fragmentation at the northern extreme that has resulted in low genetic diversity and inbreeding. Indeed, effects of the Pleistocene glaciations would be expected to result in a northward loss of genetic diversity in tropical salt-marsh communities. Palynologic studies suggest that the genus *Rhizophora* emerged after a drastic floral change during the Eocene-Oligocene transition (33.7 million years ago) and became dominant during the Miocene (Rull, 1998). Lower latitudes were apparently less affected by glacial events, as Eocene mangrove components (*Pelliciera*) are still present but reduced to small areas in Central America (Rull, 1998) and also because species richness is greatest along the Pacific coast of Colombia, Panama and Costa Rica (Duke et al., 1998). Furthermore, mangrove genetic composition seems to be negatively affected at higher latitudes; results obtained by Arbeláez-Cortes et al. (2007) and Cerón-Souza et al. (2010) confirm a higher genetic diversity in *R. mangle* along the Colombian coast than at southern (Pil et al., 2011) and northern range limits (this study).

In addition, genetic diversity might be affected by processes of hybridization (Mallet, 2005). An example of this is the increased genetic diversity reported by Nettel et al. (2008) in the black mangrove (*A. germinans*) due to an ancient hybridization between *A. germinans* and *A. bicolor*. Similarly, ancient and introgressive hybridization among red mangrove species (*R. mangle*, *R. racemosa* and *R. harrisonii*) reported by Cerón-Souza et al. (2010) on the Pacific coast region of Colombia, Panama and Costa Rica may partly explain the increased genetic diversity of *R. mangle* in this area (Arbeláez-Cortes et al., 2007; Cerón-Souza et al., 2010). Hybridization is most likely a mechanism that supports high genetic diversity in extensive mangrove areas sharing species from the same genus. However, the low genetic diversity discovered in this study is likely due to the small population size (genetic drift) and non-effective interchange of germplasm from southern populations.

According to Tomlinson (1994), *R. mangle* reproduction results mainly from wind-driven pollen and, although cross-pollination might occur, self-pollination appears to prevail. Such non-random breeding may reduce genetic diversity and increase genetic differentiation, particularly in small and fragmented populations. In this study, we detected significant levels of inbreeding for four (RBA, RKI, RSI and RTP) of the ten populations analyzed. The populations RBA, RKI and RSI are located at the northern limit of the *R. mangle* range and their low genetic diversity and deficit of heterozygotes suggest that they may be the result of recent founder events. Inbreeding at RTP, located within the most extensive mangrove forest from the Mexican Pacific, may be an artifact resulting from a restricted sampling effort (Triest, 2008). Sampling at this location was probably not representative of the entire gene pool because all individuals were collected in the northern region of the Teacapán-Agua Brava-Marismas Nacionales lagoon system. A similar phenomenon has been reported in seagrasses in semi-enclosed embayment (Muñiz-Salazar et al., 2006). More extensive sampling in the Teacapán-Agua Brava-Marismas Nacionales lagoon complex will be needed to test this hypothesis.

In conclusion, the genetic diversity in *R. mangle* along the Pacific coast of Baja California and the Gulf of California was low, showing a decreasing trend northwards, where the mangrove forests are more stressed by natural and anthropogenic disturbances. However,

genetic diversity is not randomly distributed; rather, it is significantly structured into at least two genetic units along northwestern Mexico with strong patterns of migration that are consistent with recent colonizations in this region.

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