Genetic divergence among Mexican populations of red mangrove (*Rhizophora mangle*): geographic and historic effects

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**ABSTRACT**

The Panamanian Isthmus uplifted about 3.5 million years ago, isolating plant and animal populations distributed in what today are the Pacific and Atlantic coasts. The red mangrove, *Rhizophora mangle*, is one of those species in which gene flow was interrupted by this geological phenomenon. Here, we measure the extent of genetic divergence among Mexican populations of *R. mangle*, both between and within coasts, and explore the evolutionary processes responsible for their genetic structure. Fourteen populations of *R. mangle* were sampled and individuals were screened for multi-locus genotypes using isozymes. We detected a marked genetic differentiation among populations ($F_{st} = 0.287$) and high inbreeding ($F_{is} = 0.428$) in *R. mangle*. Inferred gene flow among populations of the Atlantic coast ($Nm = 0.738$) was lower than that observed among the Pacific populations ($Nm = 3.174$). As indicated by the low values of gene flow ($Nm = 0.433$), and by the presence of alleles restricted to Pacific populations, the two coasts are isolated from each other. Gene flow does not follow the expectation of the model of isolation by distance, and reflects a complex pattern of migration among populations. The loss of one allele is documented for the northernmost population on the Pacific coast. Our results suggest that genetic drift may have played a major role in the population differentiation of red mangrove found in this study.

**Keywords**: genetic drift, isolation by distance, mangroves, Mexico, Panamanian Isthmus, population genetics, red mangrove, *Rhizophora mangle*.

**INTRODUCTION**

Genetic differentiation among populations of a species results from the interaction of selection, drift and gene flow (Wright, 1978; Slatkin, 1987). The extent and tempo of genetic divergence can be affected by mating system, life history and other ecological features (Slatkin, 1987; McCauley, 1991; Harrison and Hastings, 1996; Hamrick and Godt, 1996). For instance, plant species with selfing, a colonizing habit and a short generation time are...
expected to show greater population differentiation than outcrossing and long-lived plants (Hamrick and Godt, 1996). Similarly, the turnover of populations (local extinction and recolonization) affects genetic differentiation (Slatkin, 1987; McCauley, 1991; Harrison and Hastings, 1996). If a population’s local extinction event is followed by recolonization by many individuals, this is equivalent to gene flow and no differentiation is expected (Slatkin, 1987). In contrast, if few migrant individuals find the new population, it creates the opportunity for random differentiation and elevated levels of inbreeding. Natural selection may also promote genetic differentiation through local adaptation if gene flow does not overcome the local selective regime, or if immigrants have reduced fitness (Haldane, 1924). Gene flow may also enhance adaptive evolution and local differentiation (phase III of the shifting balance theory of Wright, 1977; Harrison and Hastings, 1996) through the spread of favourable variations to other populations (but see Gavrilets, 1996, for a discussion of the necessary conditions for phase III to take place).

Population differentiation is also influenced by the geographical distribution of populations (for a review, see Linhart and Grant, 1996) and by the time elapsed since they were first isolated (see Reeb and Avise, 1990; Karl and Avise, 1992; Bermingham and Lessios, 1993; Linhart and Grant, 1996; Weller et al., 1996; Strand et al., 1996). The effect of the geographic distribution of populations has been studied extensively, but the unknown history of populations or age of individuals has resulted in a marked paucity of empirical studies of the effects of time on genetic divergence (but see Hossaert-McKey et al., 1996). Genetic divergence can take place in a few years or generations, as in the case of *Phacelia dubia* (Del Castillo, 1994), *Agrostis* (Linhart and Grant, 1996) and *Lathyrus sylvestris* (Hossaert-McKey et al., 1996), or it may involve thousands of years in the case of long-lived organisms (see Bermingham and Lessios, 1993).

Well-dated historical events, such as the change of a river’s course, the emergence of a peninsula or the origin of islands, offer the opportunity to analyse the effects of temporal isolation on genetic divergence among subdivided populations (see Reeb and Avise, 1990; Weller et al., 1996; Afree et al., 1997). Depending on the type of historical event, ecological or geographic barriers can arise, producing a reduction in gene flow among populations and thus increasing the opportunity for divergence.

The formation of the Panamanian Isthmus in Central America about 3.5 million years ago restricted gene flow among populations of several species, thus offering an opportunity to study the consequences of a relatively well-known geological event on the population genetic structure of formerly continuous populations. Here, we evaluate the amount of genetic differentiation among Mexican populations of *Rhizophora mangle* (Rhizophoraceae) that have been isolated since the late Pliocene.

Mangrove communities are ubiquitous along the coasts of Mexico and have been present at least since the mid-Miocene, about 10–15 million years ago (Graham, 1993; Palacios-Chavez and Rzedowski, 1993). The Pacific and Atlantic mangrove communities of Mexico and Central America were separated about 3.5 millions years ago with the completion of the uplift of the Panamanian Isthmus, which, in turn, changed ancient patterns of sea currents and interrupted migration of organisms between previously continuous populations (see Collins et al., 1996). Evidence exists documenting coincidental changes in diversity of marine animals, including molluscs, sea urchins, foraminifera and reef corals (Bermingham and Lessios, 1993; Collins et al., 1996). In contrast with animal species, the impact of the uplift of this barrier on the genetic structure of plant species and their further diversification has been poorly documented to date (but see Macmillan, 1986).
Mangroves are also interesting for population differentiation studies because of their mating system. In contrast with most tropical trees, in which self-incompatibility is well represented (Bawa and Opler, 1975), a proportion of mangroves, shrubs and trees are hermaphroditic, self-compatible species (85% of 54 species surveyed; Primack and Tomlinson, 1980). Self-compatibility is commonly associated with colonizing species that usually, although not exclusively, are herbs (Baker, 1955; Hamrick et al., 1979). Given that mangrove species share some characteristics with colonizing species (Primack and Tomlinson, 1980), we expected this habit to be reflected in the genetic structure of the red mangrove.

In this paper, we present a population genetics analysis of red mangrove (*Rhizophora mangle*) from the Pacific and Atlantic (Gulf of Mexico and Caribbean sea) coasts of Mexico. The amount of genetic differentiation due to the isolation of both coasts (the uplift of the Panamanian Isthmus) was compared with that found within each coast, a situation in which gene flow was relatively unaffected. We hypothesized that the $F_{st}$ value between populations in the Atlantic and Pacific coasts of Mexico would be related to the isolation produced by the uplift of the Panamanian Isthmus, and would be higher than the $F_{st}$ values within coasts.

**MATERIALS AND METHODS**

**Study species**

*Rhizophora* is one of the most widely distributed genera among mangrove taxa in the tropics and it includes eight species (Tomlinson, 1994). *Rhizophora mangle* occurs mainly in the neotropics, from the northern Gulf of Mexico through southeastern Brazil on the Atlantic coast, and from Baja California, Mexico, through Ecuador on the Pacific coast (Ricklefs and Latham, 1993; Tomlinson, 1994; Dinerstein et al., 1995). It also occurs in Africa (Ricklefs and Latham, 1993; Tomlinson, 1994). *Rhizophora mangle* is the most abundant species in the mangrove communities of Mexico, although there are some localities of the Yucatan peninsula where *Laguncularia racemosa* (Combretaceae) is more abundant (Núñez-Farfán et al., 1996). This is also the case at the northern limit of mangrove communities on both coasts of Mexico (Tamaulipas and Baja California), where *Avicennia germinans* (Avicenniaceae) is the most important species (Núñez-Farfán et al., 1996).

**Study populations**

Fourteen populations of *R. mangle* representing the mangrove communities of both coasts of Mexico were sampled. Our sampling covered a wide climatic and latitudinal range (see Table 1 and Fig. 1; see also Domínguez et al., 1998). For each population, samples of both leaf tissue and propagules (from 6–10 embryos) of 50 mature, reproductive trees (haphazardly chosen along the mangrove line) were collected, labelled and then frozen in liquid nitrogen. The minimum distance between any pair of sampled individuals was set to 100 m, thus avoiding the accidental resampling of the same individual. *Rhizophora mangle* possesses a limited capacity to spread vegetatively (Tomlinson, 1994). Assays with embryos and leaf tissue were carried out to detect enzymatic activity through starch gel electrophoresis (Soltis and Soltis, 1989). Because secondary compounds interfere with the enzymatic activity and banding patterns in *R. mangle*, we used the extraction buffer that Goodall and Stoddart (1989) found to minimize these problems in *R. stylosa*.
<table>
<thead>
<tr>
<th>No.</th>
<th>Locality</th>
<th>State</th>
<th>Coast</th>
<th>Longitude (W)</th>
<th>Latitude (N)</th>
<th>Mean annual temperature (°C)</th>
<th>Mean annual precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bahía Kino</td>
<td>Sonora</td>
<td>P</td>
<td>111°54'</td>
<td>28°47'</td>
<td>22.1</td>
<td>179.6</td>
</tr>
<tr>
<td>2</td>
<td>Topolobampo</td>
<td>Sinaloa</td>
<td>P</td>
<td>109°07'</td>
<td>25°36'</td>
<td>24.3</td>
<td>244.1</td>
</tr>
<tr>
<td>3</td>
<td>San Blas</td>
<td>Nayarit</td>
<td>P</td>
<td>105°17'</td>
<td>21°30'</td>
<td>25.2</td>
<td>1436.0</td>
</tr>
<tr>
<td>4</td>
<td>Coyuca</td>
<td>Guerrero</td>
<td>P</td>
<td>100°02'</td>
<td>16°57'</td>
<td>27.5</td>
<td>1360.3</td>
</tr>
<tr>
<td>5</td>
<td>Chacahua</td>
<td>Oaxaca</td>
<td>P</td>
<td>97°33'</td>
<td>15°58'</td>
<td>28.0</td>
<td>905.5</td>
</tr>
<tr>
<td>6</td>
<td>Chantuto</td>
<td>Chiapas</td>
<td>P</td>
<td>92°45'</td>
<td>15°09'</td>
<td>26.5</td>
<td>1578.4</td>
</tr>
<tr>
<td>7</td>
<td>Punta Allen</td>
<td>Quintana Roo</td>
<td>AC</td>
<td>87°26'</td>
<td>19°48'</td>
<td>29.9</td>
<td>1106.3</td>
</tr>
<tr>
<td>8</td>
<td>Ría Lagartos</td>
<td>Yucatán</td>
<td>AC</td>
<td>88°03'</td>
<td>21°35'</td>
<td>25.6</td>
<td>550.1</td>
</tr>
<tr>
<td>9</td>
<td>Celestún</td>
<td>Yucatán</td>
<td>AG</td>
<td>90°20'</td>
<td>20°52'</td>
<td>26.4</td>
<td>725.5</td>
</tr>
<tr>
<td>10</td>
<td>Cd. del Carmen</td>
<td>Campeche</td>
<td>AG</td>
<td>91°44'</td>
<td>18°38'</td>
<td>26.7</td>
<td>1540.4</td>
</tr>
<tr>
<td>11</td>
<td>Centla</td>
<td>Tabasco</td>
<td>AG</td>
<td>92°27'</td>
<td>18°28'</td>
<td>26.6</td>
<td>1560.2</td>
</tr>
<tr>
<td>12</td>
<td>Sontecomapan</td>
<td>Veracruz</td>
<td>AG</td>
<td>95°00'</td>
<td>18°33'</td>
<td>24.3</td>
<td>2016.9</td>
</tr>
<tr>
<td>13</td>
<td>Tuxpan</td>
<td>Veracruz</td>
<td>AG</td>
<td>97°24'</td>
<td>20°57'</td>
<td>24.8</td>
<td>1352.4</td>
</tr>
<tr>
<td>14</td>
<td>Tamiahua</td>
<td>Veracruz</td>
<td>AG</td>
<td>97°25'</td>
<td>21°21'</td>
<td>24.8</td>
<td>1352.4</td>
</tr>
</tbody>
</table>

Abbreviations: AC = Atlantic, Caribbean Sea; AG = Atlantic, Gulf of Mexico; P = Pacific Coast.
Fig. 1. Sampled populations of *Rhizophora mangle* on the Pacific and Atlantic coasts of Mexico. Numbers of populations correspond to those given in Table 1.

**Electrophoresis and genetic analyses**

From 30 enzymes analysed, 10 presented good activity and resolution and were used to run all samples. Five (six loci) were polymorphic and informative. Individuals of *R. mangle* were characterized for 6-loci genotypes using starch gels (12% w/v). We used the LiOH (pH = 8.3) buffer system of May (1992) to assay glutamate oxaloacetate transaminase (*Got*, 2.6.1.1, one locus), leucine aminopeptidase (*Lap*, EC 3.4.11.1, one locus), glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9, two loci) and rubisco (*Rub*, one locus). The tris-citrate (pH = 9) of Miles *et al.* (1977) was used for diaphorase (*Dia*, EC 1.6.4.3, one locus) and menadione reductase (*Mdr*, EC 1.6.99.2, two loci). The morpholine system of Vallejos (1983) was used for acid phosphatase (*Apc*, EC 3.1.3.2, two loci), malate dehydrogenase (*Mdh*, EC 1.1.1.37, one locus) and malic enzyme (*Me*, EC 1.1.1.40, one locus). Iso- and allozymes were numbered to indicate their mobility, with the locus and allele with the highest mobility numbered as 1. On average, 45 and 41 trees per locus were assayed for each population from the Atlantic and Pacific coast, respectively.

For each population, genotypic frequencies were obtained and used to calculate observed mean heterozygosity (*H₀*) and allelic frequencies. Allelic frequencies at each population were used to estimate the mean number of alleles per locus (*A*), the average proportion of polymorphic loci (*P*) and expected mean heterozygosity (*Hₑ*) (Hartl and Clark, 1989). Heterogeneity of allelic frequencies among populations in each coast was evaluated by chi-square tests (Workman and Niswander, 1970). Unbiased estimates of Nei’s (1978) genetic distance (*D*) were obtained using the program TFGPA (Miller, 1997). Average genetic distances were estimated for all populations and within coasts. To assess population genetic structure, estimates of *f*, *F* and *θ* of Weir and Cockerham (1984), the equivalent to Wright’s (1965) *F*-statistics – *Fₚ*, *Fᵢᵢ* and *Fₛᵢᵢ* respectively – were obtained. Because our sampling procedure included a hierarchical arrangement (coasts, populations within coasts,
and individuals within populations within coasts), we followed the procedure outlined by Weir and Cockerham (1984) and implemented in the TFPGA program (Miller, 1997). Finally, to have a thorough understanding of the genetic structure of *R. mangle*, we further estimated the $F$-statistics for each coast separately.

**Gene flow**

To estimate gene flow, we calculated $Nm$, the mean number of migrants between populations per generation, as $Nm = \frac{1}{4}(F_{st} - 1)$, since $F_{st} = (1/(1 + 4Nm))$ (Wright, 1951; Slatkin, 1985). In addition, gene flow was estimated by using the private alleles method (Slatkin, 1985) as $\ln(\bar{p}(1)) = a \ln(Nm) + b$, where $a = -0.005$, $b = -2.440$ and $\bar{p}(1)$ is the average frequency of the alleles found in only one population (private alleles).

Finally, because *R. mangle* populations are distributed along the shorelines, we assessed if populations follow the isolation by distance model (Slatkin, 1993, 1994). We calculated $\hat{M}$, which is an estimate of $Nm$ for pairs of populations. For gene flow in a one-dimensional stepping stone model, $\hat{M}$ is estimated as $\hat{M} = (4Nm)/k$, where $k$ is the geographic distance separating two given populations (Slatkin, 1993, 1994). To estimate $\hat{M}$, we used a program developed by Slatkin (1993), which estimates $\hat{M}$ using both $Gst$ and $\theta$. $\hat{M}$ is not interpreted as the average number of migrants between a pair of populations ($Nm$), but as the number of migrants necessary to account for the observed genetic differentiation between populations, provided migration follows conditions of the stepping stone model (Slatkin, 1993; Hellberg, 1996).

For populations in equilibrium, the expected slope of the regression of log $\hat{M}$ versus log(geographic distance) is $-0.5$ for a one-dimensional pattern of gene flow (Slatkin, 1993, 1994). The geographic distance between pairs of populations of *R. mangle* was obtained using 1:1,000,000 scale maps (INEGI, 1988). We estimated the shortest distance among populations on each coast. A Mantel test of correlation between the matrices of genetic distance and geographic distance among populations within each coast was performed using the program TFGPA (Miller, 1997).

**RESULTS**

**Genetic diversity**

Populations from the Pacific coast had more genetic diversity than those from the Atlantic coast (Table 2). Observed heterozygosities ($H_o$) were lower than expected from Hardy-Weinberg expectations ($H_e$), suggesting the presence of inbreeding in all populations (Table 2).

Despite the overall greater genetic diversity observed at the Pacific coast, the frequency of the most common allele at each polymorphic locus appeared highly heterogeneous among populations from the Atlantic coast (see Fig. 2). Four loci showed significant heterogeneity in allelic frequencies on the Atlantic Coast (*Acp-2, Lap-1, Me-1, Pgi-2*) ($\chi^2 \geq 125$, $P < 0.001$). In contrast, only one of six loci (*Pgi-2*) showed significant heterogeneity in frequency among populations in the Pacific coast (see Fig. 2). Moreover, geographic changes in allelic frequencies did not show evidence of clinal variation with latitude (Fig. 2). Five alleles were present only in populations from the Pacific coast, whereas one was exclusive to the Atlantic coast.
Table 2. Genetic diversity of 14 populations of *Rhizophora mangle* in Mexico (mean ± standard error)

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean sample size per locus</th>
<th>Mean no. of alleles per locus</th>
<th>% Polymorphic loci</th>
<th>$H_o$</th>
<th>$H_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atlantic coast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Punta Allen</td>
<td>46.60 ± 2.5</td>
<td>1.30 ± 0.2</td>
<td>33.30</td>
<td>0.016 ± 0.016</td>
<td>0.091 ± 0.050</td>
</tr>
<tr>
<td>Ría Lagartos</td>
<td>49.30 ± 0.6</td>
<td>1.20 ± 0.1</td>
<td>22.20</td>
<td>0.002 ± 0.002</td>
<td>0.059 ± 0.048</td>
</tr>
<tr>
<td>Celestún</td>
<td>47.60 ± 1.4</td>
<td>1.30 ± 0.2</td>
<td>33.30</td>
<td>0.090 ± 0.090</td>
<td>0.111 ± 0.064</td>
</tr>
<tr>
<td>Cd. del Carmen</td>
<td>45.00 ± 0.0</td>
<td>1.60 ± 0.2</td>
<td>33.30</td>
<td>0.064 ± 0.056</td>
<td>0.175 ± 0.085</td>
</tr>
<tr>
<td>Centla</td>
<td>48.30 ± 1.0</td>
<td>1.40 ± 0.2</td>
<td>33.30</td>
<td>0.110 ± 0.097</td>
<td>0.135 ± 0.071</td>
</tr>
<tr>
<td>Sontecomapan</td>
<td>45.30 ± 0.4</td>
<td>1.10 ± 0.1</td>
<td>11.10</td>
<td>0.000 ± 0.000</td>
<td>0.055 ± 0.055</td>
</tr>
<tr>
<td>Tamiahua</td>
<td>39.80 ± 0.6</td>
<td>1.30 ± 0.2</td>
<td>33.30</td>
<td>0.097 ± 0.097</td>
<td>0.118 ± 0.070</td>
</tr>
<tr>
<td>Tuxpan</td>
<td>42.40 ± 1.3</td>
<td>1.30 ± 0.2</td>
<td>22.20</td>
<td>0.086 ± 0.079</td>
<td>0.096 ± 0.061</td>
</tr>
<tr>
<td><strong>Mean ± standard error</strong></td>
<td>45.54 ± 1.20</td>
<td>1.31 ± 0.06</td>
<td>27.75 ± 3.17</td>
<td>0.058 ± 0.017</td>
<td>0.105 ± 0.015</td>
</tr>
</tbody>
</table>

| **Pacific coast**   |                           |                              |                    |             |             |
| Bahía Kino          | 40.10 ± 4.9               | 1.60 ± 0.2                   | 44.40              | 0.074 ± 0.066 | 0.132 ± 0.057 |
| Topolobampo         | 40.10 ± 4.9               | 1.60 ± 0.2                   | 44.40              | 0.089 ± 0.056 | 0.147 ± 0.057 |
| San Blas            | 43.70 ± 1.1               | 1.70 ± 0.2                   | 44.40              | 0.078 ± 0.045 | 0.154 ± 0.064 |
| Coyuca              | 40.00 ± 0.0               | 1.90 ± 0.3                   | 55.60              | 0.067 ± 0.047 | 0.140 ± 0.059 |
| Chacahua            | 44.90 ± 0.1               | 1.70 ± 0.2                   | 55.60              | 0.100 ± 0.060 | 0.147 ± 0.068 |
| Chantuto            | 44.80 ± 0.2               | 2.00 ± 0.3                   | 66.70              | 0.099 ± 0.059 | 0.191 ± 0.067 |
| **Mean ± standard error** | 42.27 ± 1.09             | 1.75 ± 0.07                  | 51.85 ± 4.08       | 0.085 ± 0.006 | 0.152 ± 0.009 |
| **Grand mean ± standard error** | 44.14 ± 0.90            | 1.50 ± 0.07                  | 38.08 ± 4.14       | 0.069 ± 0.010 | 0.125 ± 0.011 |
Fig. 2. Allele frequencies at six loci in 14 populations of *Rhizophora mangle* on the Atlantic and
Pacific coasts of Mexico. Numbers of populations correspond to those given in Table 1.
Genetic distances

The mean genetic distance between all pairs of populations was 0.049 (standard error = 0.0041; \( n = 48 \) pairs). Genetic distances within coasts showed different patterns. While populations from the Pacific coast were highly similar (\( D_{\text{Pacific}} = 0.012 \pm 0.0036 \)), the average genetic distance among populations within the Atlantic coast was three times higher than that of the Pacific populations (\( D_{\text{Atlantic}} = 0.042 \pm 0.0054; t_{12} = 3.82; P = 0.0025 \)) and similar to the average distance between coasts.

F-coefficients

Hierarchical analysis of F-coefficients showed that \( F \) was significantly greater than zero, indicating a high deficit of heterozygosity among individuals of all populations, but not among individuals within populations, as indicated by the lack of significance of \( f \) (Table 3). In contrast to our expectations, the average genetic differentiation between coasts was not significant, but differences among populations within coasts accounted for almost 30% of the genetic variation (Table 3).

Two independent estimations of F-coefficients, one for each coast, were in agreement with our previous analysis and showed strong differences in the genetic structure of populations from each coast. The differences among populations accounted for 25% of the genetic variance in the Atlantic coast (Table 4). The average \( F_{is} \) value for the Atlantic populations was not significantly different from zero, whereas \( F_{it} \) was high and significant, indicating a deficit of heterozygous individuals in the Atlantic populations as a whole. In contrast, populations from the Pacific coast showed high levels of inbreeding (positive and significant \( F_{is} \) and \( F_{it} \)) and no genetic differentiation (Table 5).

Gene flow

Indirect estimates of gene flow among all populations showed a rather low value (\( Nm = 0.621 \)). Independent estimations for each coast showed that the \( Nm \) values were much lower in the Atlantic than in the Pacific coast (\( Nm = 0.738 \) vs 3.174). The private alleles method also indicated that gene flow is relatively low in the Atlantic coast (\( Nm = 0.433 \)).

The pattern of gene flow among populations within each coast did not follow the expectations of the model of isolation by distance, as indicated by the lack of significance of the slopes (\( P > 0.05 \) in both cases), but showed a complex pattern of gene flow (Fig. 3). For instance, two Atlantic populations separated by only 50 km (Tuxpan and Tamiahua in the Gulf of Mexico) are more differentiated than expected by the isolation by distance model. In contrast, apart from the northernmost population (Kino Bay), high levels of gene flow occurred among all the Pacific populations irrespective of geographic distance (Fig. 3). Accordingly, there was no correlation between the matrices of genetic and geographic distances for each coast (Mantel’s test, \( z = 7.63, r = 0.069, P = 0.391 \) for the Atlantic; \( z = 1.29, r = 0.080, P = 0.347 \) for the Pacific).

DISCUSSION

The results of this study indicate a complex pattern of genetic differentiation among Mexican populations of \( R. \) mangle. Hierarchical analysis showed that populations from
Table 3. *F*-statistics derived from genetic analyses in 14 populations of *Rhizophora mangle* from Mexico: hierarchical analysis considering the effects of coasts and population within coasts

<table>
<thead>
<tr>
<th>Locus</th>
<th>F</th>
<th>θ among pops within coast</th>
<th>θ between coasts</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mnr-1</td>
<td>0.191</td>
<td>0.056</td>
<td>0.020</td>
<td>0.309</td>
</tr>
<tr>
<td>Mnr-2</td>
<td>0.084</td>
<td>0.164</td>
<td>0.139</td>
<td>−0.095</td>
</tr>
<tr>
<td>Lap</td>
<td>0.966</td>
<td>0.069</td>
<td>−0.014</td>
<td>0.963</td>
</tr>
<tr>
<td>Pgi-2</td>
<td>0.206</td>
<td>0.287</td>
<td>−0.070</td>
<td>−0.114</td>
</tr>
<tr>
<td>Acp-2</td>
<td>0.944</td>
<td>0.168</td>
<td>0.180</td>
<td>0.932</td>
</tr>
<tr>
<td>Me</td>
<td>0.953</td>
<td>0.358</td>
<td>0.266</td>
<td>0.928</td>
</tr>
<tr>
<td>Mean</td>
<td>0.597</td>
<td>0.287</td>
<td>0.137</td>
<td>0.428</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.291</td>
<td>0.050</td>
<td>0.083</td>
<td>0.378</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.206 to 0.947</td>
<td>0.121 to 0.329</td>
<td>−0.008 to 0.256</td>
<td>−0.044 to 0.928</td>
</tr>
</tbody>
</table>

*Note:* Mean values of estimators were obtained through the jackknife method and confidence limits were obtained through bootstrapping (see text).

Table 4. *F*-statistics derived from genetic analyses in populations of *Rhizophora mangle* from Mexico: independent estimations of *F*-coefficients for populations from the Atlantic coast

<table>
<thead>
<tr>
<th>Locus</th>
<th>F = Fit</th>
<th>θ = Fst</th>
<th>f = Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lap</td>
<td>0.935</td>
<td>0.143</td>
<td>0.925</td>
</tr>
<tr>
<td>Pgi-2</td>
<td>0.020</td>
<td>0.348</td>
<td>−0.504</td>
</tr>
<tr>
<td>Acp-2</td>
<td>0.912</td>
<td>0.315</td>
<td>0.871</td>
</tr>
<tr>
<td>Me</td>
<td>0.946</td>
<td>0.172</td>
<td>0.934</td>
</tr>
<tr>
<td>Mean</td>
<td>0.603</td>
<td>0.253</td>
<td>0.520</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.377</td>
<td>0.073</td>
<td>0.551</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.103 to 0.945</td>
<td>0.164 to 0.342</td>
<td>−0.504 to 0.934</td>
</tr>
</tbody>
</table>

*Note:* Mean values of estimators were obtained through the jackknife method and confidence limits were obtained through bootstrapping (see text).

Table 5. *F*-statistics derived from genetic analyses in populations of *Rhizophora mangle* from Mexico: independent estimations of *F*-coefficients for populations from the Pacific coast

<table>
<thead>
<tr>
<th>Locus</th>
<th>F = Fit</th>
<th>θ = Fst</th>
<th>f = Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mnr-1</td>
<td>0.175</td>
<td>0.037</td>
<td>0.143</td>
</tr>
<tr>
<td>Mnr-2</td>
<td>−0.062</td>
<td>0.029</td>
<td>−0.094</td>
</tr>
<tr>
<td>Lap</td>
<td>1.000</td>
<td>0.013</td>
<td>1.000</td>
</tr>
<tr>
<td>Pgi-2</td>
<td>0.263</td>
<td>0.124</td>
<td>0.158</td>
</tr>
<tr>
<td>Acp-2</td>
<td>1.000</td>
<td>−0.016</td>
<td>1.000</td>
</tr>
<tr>
<td>Me</td>
<td>0.915</td>
<td>0.005</td>
<td>0.915</td>
</tr>
<tr>
<td>Mean</td>
<td>0.430</td>
<td>0.073</td>
<td>0.396</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.215</td>
<td>0.048</td>
<td>0.246</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.196 to 0.929</td>
<td>−0.001 to 0.106</td>
<td>0.127 to 0.929</td>
</tr>
</tbody>
</table>

*Note:* Mean values of estimators were obtained through the jackknife method and confidence limits were obtained through bootstrapping (see text).
the Atlantic and Pacific coasts are not genetically differentiated. Within-coast analyses indicated that 25% of the genetic variation in the Atlantic was accounted for by differences among populations. In contrast, genetic differentiation was not evident among the Pacific populations. This result was unexpected given that populations in the Atlantic coast are more continuously distributed than those from the Pacific (Dinerstein et al., 1995).

Genetic diversity was higher in the Pacific populations. The percentage of polymorphic loci in the Pacific coast was almost twice that in the Atlantic coast, and the expected heterozygosity and the average number of alleles per locus followed the same trend. Overall estimations of gene flow produced very low values ($N_m = 0.621$ and $N_m = 0.433$ for the indirect and private alleles methods, respectively), while independent estimations for each coast showed a contrasting pattern of gene flow between coasts. Populations from the Pacific showed, in general, a higher amount of migration ($N_m = 3.174$). In contrast, a restricted gene flow was detected for populations established on the Atlantic Coast ($N_m = 0.738$). Gene flow among populations of *Rhizophora mangle* did not follow the expectations of the model of isolation by distance.

Overall, these results do not support our original expectations of a higher divergence between coasts than among populations within coasts. Thus, despite the long time populations from both coasts have been separated, we did not find significant genetic differentiation between coasts, suggesting that the uplift of the Panamanian Isthmus did not produce, by itself, an automatic differentiation among populations from both coasts. This result contrasts with a previous study showing that coasts explained almost 30% of the phenotypic variance in the floral attributes of *Rhizophora mangle* (Domínguez et al., 1998), and with empirical evidence collected for other organisms (molluscs, sea urchins, foraminifera and reef corals) revealing a tremendous evolutionary impact of the uplift of the Panamanian Isthmus (Bermingham and Lessios, 1993; Collins et al., 1996).

Several factors can help to explain the remarkable pattern of differentiation of *Rhizophora mangle* in Mexico. As indicated by fossil evidence (Graham, 1993; Palacios-Chavez and Rzedowski, 1993), populations of *Rhizophora mangle* were well established in both coasts of Mexico before the

![Fig. 3. Values of log ($\hat{M}$) as a function of distance separating pairs of populations of *Rhizophora mangle* on the Atlantic (●) and Pacific (○) coasts of Mexico. Neither slope was significantly different from zero (see ‘Results’).](image-url)
uplift of the Panamanian Isthmus. If genetic diversity was well represented in populations from both coasts and effective population sizes were large, random differentiation would not be expected by the separation alone (Slatkin, 1987). In this sense, it seems that the time elapsed since the uplift of the Panamanian Isthmus has not been long enough to produce significant genetic differentiation between the coasts of Mexico. In a similar study evaluating the genetic consequences of the uplift of the Florida peninsula, Karl and Avise (1992) found a geographic uniformity in allozyme frequencies between populations of *Crassostrea virginica* from the Atlantic coast of North America and those from the Gulf of Mexico. Because population differentiation was apparent when they used RFLP and mitochondrial DNA, they proposed the discordance possibly was due to selection on protein electrophoretic characters that balance allozyme frequencies in the face of severe constraints to gene flow.

Thus, although it is possible that our allozyme-based estimations did not have enough resolution to detect the expected differentiation between the Atlantic and Pacific coasts, we did find high and significant differentiation among populations from the Atlantic coast, suggesting that our results are not a consequence of a poor resolution of allozyme markers. Moreover, unlike the case of *C. virginica*, our results do not seem to be a consequence of stabilizing selection on allozyme frequencies, but a consequence of inbreeding and genetic drift. There are three lines of evidence to support this interpretation. First, results from this study and from other populations in the Caribbean Sea (Lowenfeld and Klekowski, 1992; Klekowski *et al.*, 1994) indicate that inbreeding is a prevalent characteristic in *R. mangle*. Second, although the high constancy in genetic frequencies among populations from the Pacific coast may be interpreted as the result of natural selection favouring a particular allele, this was not the case for the Atlantic populations. Third, a related investigation of the same populations used in this study showed that the patterns of phenotypic variation in flower morphology were not related to clinal variation or geographic location of the sites, thus suggesting that drift has played a mayor role in the evolution of flower morphology in this species (Domínguez *et al.*, 1998).

Overall, our results suggest that the isolation of populations from the Atlantic and Pacific coasts of Mexico about 3.5 million years ago did not result in genetic differentiation, probably because each isolated group of populations contained most of the genetic variance present at that moment. It would appear, however, that isolation favoured a very different evolutionary dynamic in each coast. Although inbreeding is apparent on both coasts, a relatively high gene flow may explain the lack of genetic differentiation among the Pacific populations. In contrast, the complex pattern of genetic differentiation of *R. mangle* on the Atlantic coast seems to be related to the highly dynamic geological history of this coast. With the uplift of the Panamanian Isthmus, sea currents from the south, which during the Pliocene crossed from the Atlantic towards the Pacific through the Central American channel, shifted northwards (Collins *et al.*, 1996), allowing genetic contact among previously separated populations from the Atlantic. Since the peninsulas of Florida and Yucatan emerged during the Pleistocene (see Reeb and Avise, 1990; Graham 1993), this epoch was characterized by changes in seawater temperature and in the shoreline, which, in turn, produced the extinction and recolonization of populations and the opportunity for differentiation by genetic drift (see Slatkin, 1987; McCauley, 1991; Harrison and Hastings, 1996). Moreover, contemporary sea currents in the Gulf of Mexico are seasonally variable in direction in a way that makes it difficult to predict which populations are maintaining gene flow via seedling dispersal. In addition to the previous factors, the rare events of gene
flow from African populations of *R. mangle* (Tomlinson, 1994) may have contributed to the complex pattern of genetic differentiation we observed among Atlantic populations of this species.

Finally, the results of this study support Primack and Tomlinson’s (1980) hypothesis that mangrove species behave as colonizing species and, therefore, high levels of inbreeding and population differentiation are expected. Our results also suggest that conservation efforts directed to maintain genetic diversity in *R. mangle* should focus not only on large areas of a few natural preserves, but also on increasing the number of populations.

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**REFERENCES**


Genetic structure of Rhizophora mangle