Evolutionary Ecology Research, 2002, **4**: 1049–1064

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

Juan Núñez-Farfán,* César A. Domínguez, Luis E. Eguiarte, Amelia Cornejo, Martin Quijano, Jesús Vargas and Rodolfo Dirzo

Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, A.P. 70-275, Mexico Distrito Federal, C.P. 04510, Mexico

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

* Author to whom all correspondence should be addressed. e-mail: farfan@servidor.unam.mx

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 *Rhizhopora 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 1. Sampled localities of *Rhizophora mangle* on the Atlantic and Pacific coasts of Mexico

No.	Locality	State	Coast	Longitude (W)	Latitude (N)	Mean annual temperature (° C)	Mean annual precipitation (mm)
1	Bahía Kino	Sonora	P	111°54′	28°47′	22.1	179.6
2	Topolobampo	Sinaloa	P	109°07′	25°36′	24.3	244.1
3	San Blas	Nayarit	P	105°17′	21°30′	25.2	1436.0
4	Coyuca	Guerrero	P	100°02′	16°57′	27.5	1360.3
5	Chacahua	Oaxaca	P	97°33′	15°58′	28.0	905.5
6	Chantuto	Chiapas	P	92°45′	15°09′	26.5	1578.4
7	Punta Allen	Quintana Roo	AC	87°26′	19°48′	29.9	1106.3
8	Ría Lagartos	Yucatán	AC	88°03′	21°35′	25.6	550.1
9	Celestún	Yucatán	AG	90°20′	20°52′	26.4	725.5
10	Cd. del Carmen	Campeche	AG	91°44′	18°38′	26.7	1540.4
11	Centla	Tabasco	AG	92°27′	18°28′	26.6	1560.2
12	Sontecomapan	Veracruz	AG	95°00′	18°33′	24.3	2016.9
13	Tuxpan	Veracruz	AG	97°24′	20°57′	24.8	1352.4
14	Tamiahua	Veracruz	AG	97°25′	21°21′	24.8	1352.4

Abbreviations: AC = Atlantic, Caribbean Sea; AG = Atlantic, Gulf of Mexico; P = Pacific Coast.

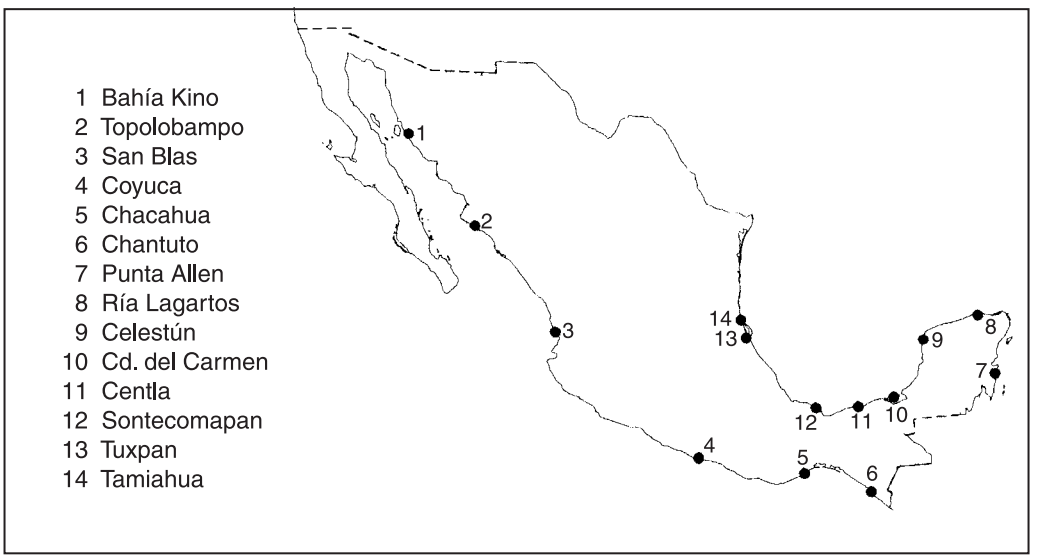


Fig. 1. Sampled populations of *Rhizophora mangle* on the Pacific and Atlantic coasts of Mexico. Numbers of populations correspond to those given in Table 1.

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 (H_o) 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 (H_e) (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 – F_{it}, F_{is} and F_{st}, 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 $Nm \approx \frac{1}{4}((1/F_{st}) - 1)$, since $F_{st} \approx (1/(1 + 4Nm))$ (Wright, 1951; Slatkin, 1994). 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 G_{st} and θ. $\hat{M}$ 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*) ($\chi^2_7 \geq 125$, $P < 0.001$). 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)

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

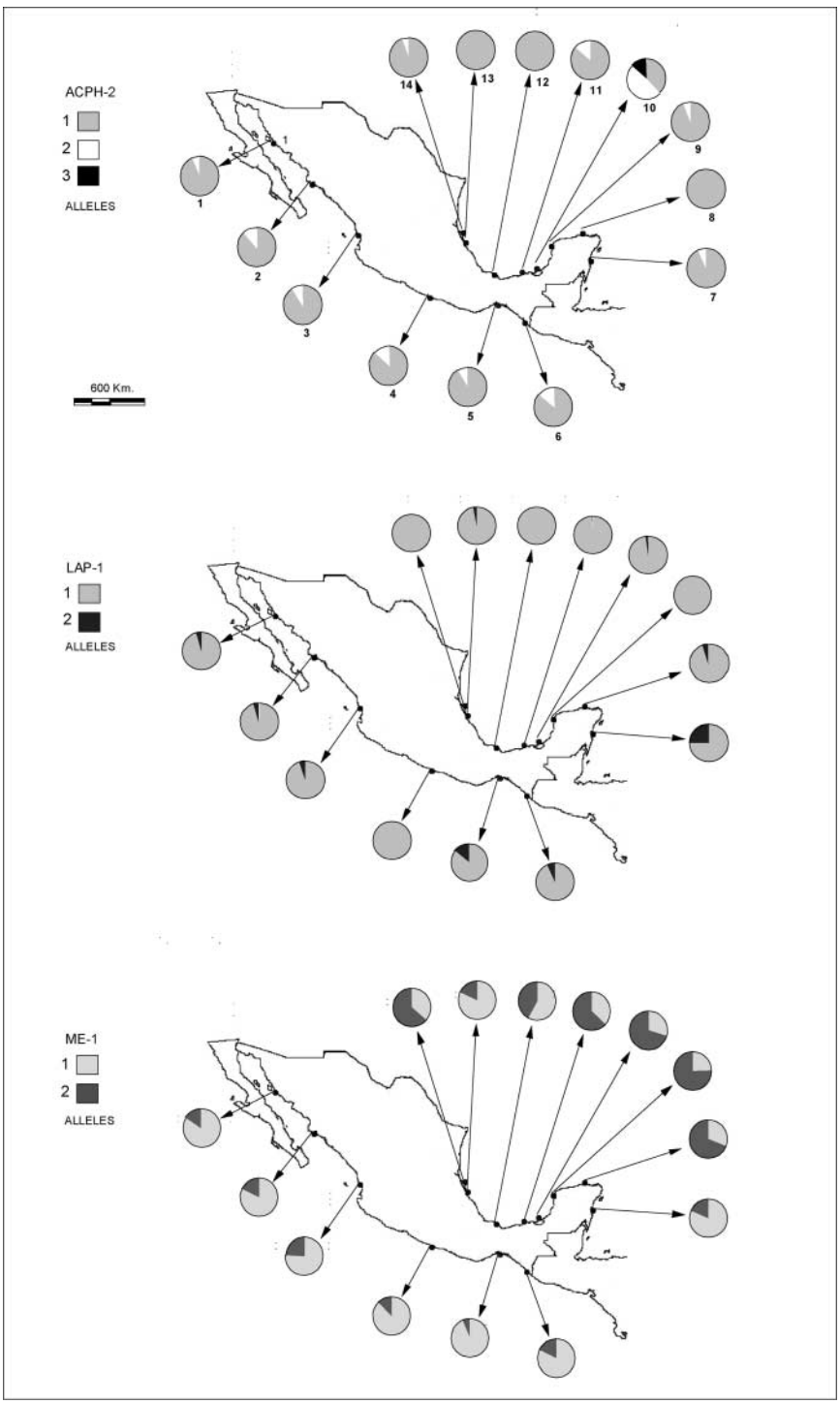
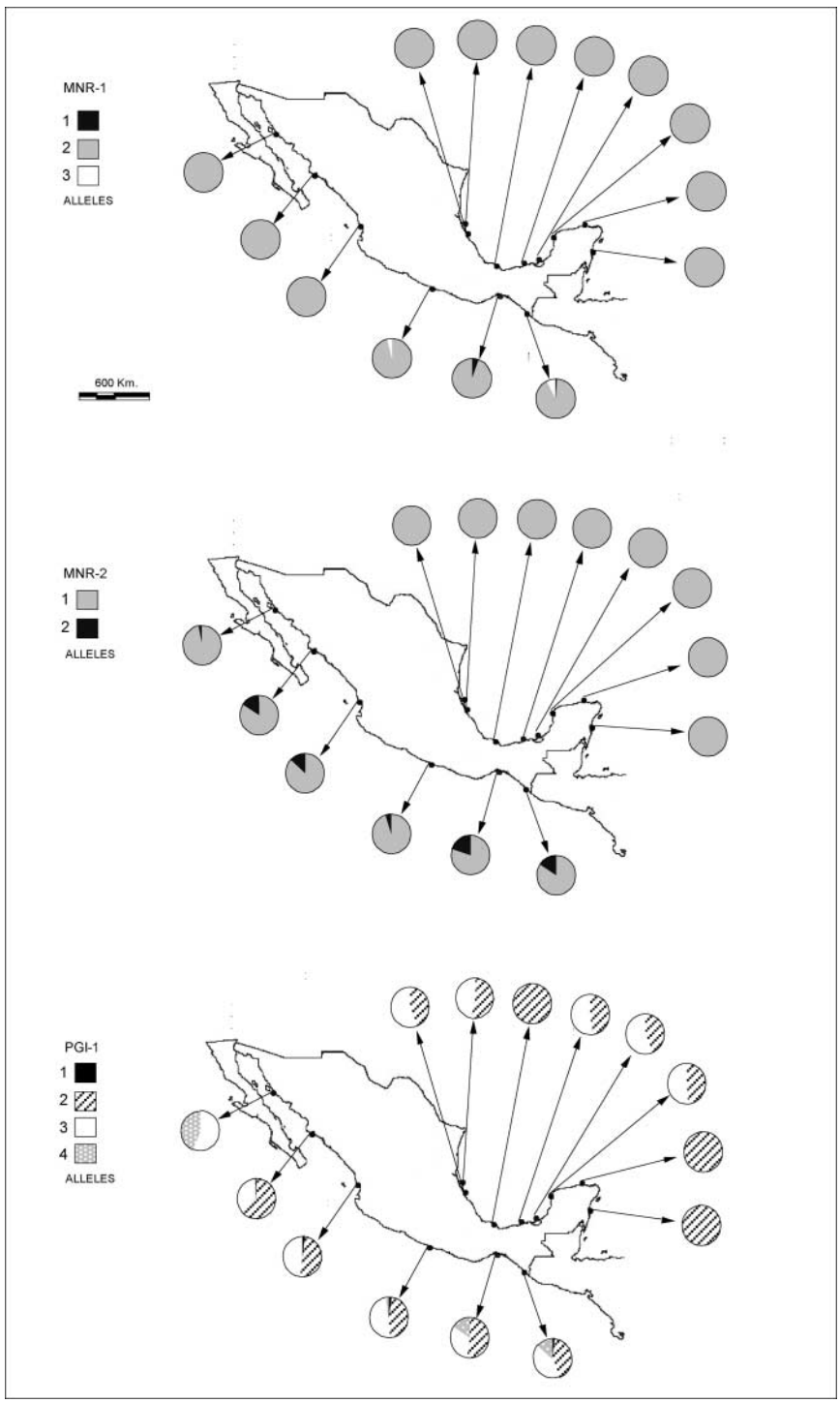


Fig. 2. Allele frequencies at six loci in 14 populations of *Rhizophora mangle* on the Atlantic and



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

Locus	F	$\theta_{\text{among pops within coast}}$	$\theta_{\text{between coasts}}$	f
Mnr-1	0.191	0.056	0.020	0.309
Mnr-2	0.084	0.164	0.139	−0.095
Lap	0.966	0.069	−0.014	0.963
Pgi-2	0.206	0.287	−0.070	−0.114
Acp-2	0.944	0.168	0.180	0.932
Me	0.953	0.358	0.266	0.928
Mean	0.597	0.287	0.137	0.428
Standard deviation	0.291	0.050	0.083	0.378
95% confidence interval	0.206 to 0.947	0.121 to 0.329	−0.008 to 0.256	−0.044 to 0.928

Note: Mean values of estimators were obtained through the jackknife method and confidence limits were obtained through bootstraping (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

Locus	$F = F_{it}$	$\theta = F_{st}$	$f = F_{is}$
Lap	0.935	0.143	0.925
Pgi-2	0.020	0.348	−0.504
Acp-2	0.912	0.315	0.871
Me	0.946	0.172	0.934
Mean	0.603	0.253	0.520
Standard deviation	0.377	0.073	0.551
95% confidence interval	0.103 to 0.945	0.164 to 0.342	−0.504 to 0.934

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

Locus	$F = F_{it}$	$\theta = F_{st}$	$f = F_{is}$
Mnr-1	0.175	0.037	0.143
Mnr-2	−0.062	0.029	−0.094
Lap	1.000	0.013	1.000
Pgi-2	0.263	0.124	0.158
Acp-2	1.000	−0.016	1.000
Me	0.915	0.005	0.915
Mean	0.430	0.073	0.396
Standard deviation	0.215	0.048	0.246
95% confidence interval	0.196 to 0.929	−0.001 to 0.106	0.127 to 0.929

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

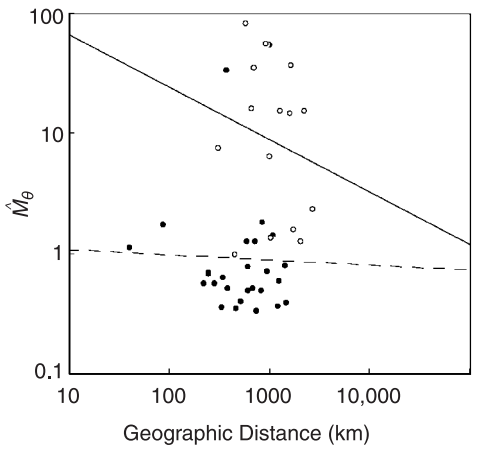


Fig. 3. Values of log ($\hat{M}$) as a function of distance separating pairs of populations of *Rhizophora mangle* on the Atlantic (●) and Pacific (○) coasts of Mexico. Neither slope was significantly different from zero (see 'Results').

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 ($Nm = 0.621$ and $Nm = 0.433$ 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 ($Nm = 3.174$). In contrast, a restricted gene flow was detected for populations established on the Atlantic Coast ($Nm = 0.738$). Gene flow among populations of *R. 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 *R. 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 *R. mangle* in Mexico. As indicated by fossil evidence (Graham, 1993; Palacios-Chavez and Rzedowski, 1993), populations of *R. mangle* were well established in both coasts of Mexico before the

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.

ACKNOWLEDGEMENTS

We thank S. Sinaca and R.I. Martínez for their help in the field. J. Fornoni helped with the figures and N. Pérez-Nasser and G. Furnier helped with various aspects of the electrophoretical work. This study was financed by the National Commission for Biodiversity (CONABIO, project No. B-214), and we appreciate the support of Jorge Soberón.

REFERENCES

Afree, L., Thompson, J.D. and Debussche, M. 1997. Genetic structure of continental and island populations of the Mediterranean endemic *Cyclamen balearicum* (Primulaceae). *Am. J. Bot.*, **84**: 437–451.

Baker, H.G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, **9**: 347–349.

Bawa, K.S. and Opler, P.A. 1975. Dioecism in tropical trees. *Evolution*, **29**: 167–179.

Bermingham, E. and Lessios, H.A. 1993. Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proc. Natl. Acad. Sci. USA*, **90**: 2734–2738.

Collins, L.S., Budd, A.F. and Coates, A.G. 1996. Earliest evolution associated with closure of the tropical American seaway. *Proc. Natl. Acad. Sci. USA*, **93**: 6069–6072.

Del Castillo, R.F. 1994. Factors influencing the genetic structure of *Phacelia dubia*, a species with a seed-bank and large fluctuations in population size. *Heredity*, **72**: 446–458.

Dinerstein, E., Olson, D.M., Graham, D.J., Webster, A.L., Primm, S.A., Bookbinder, M.P. and Ledec, G. 1995. *A Conservation Assessment of the Terrestrial Ecoregions of Latin America and the Caribbean*. Washington, DC: WWF/The World Bank.

Domínguez, C.A., Eguiarte, L.E., Núñez-Farfán, J. and Dirzo, R. 1998. Flower morphometry of *Rhizophora mangle* (Rhizophoraceae): geographical variation in Mexican populations. *Am. J. Bot.*, **85**: 637–643.

Gavrilets, S. 1996. On phase three of the shifting-balance theory. *Evolution*, **50**: 1034–1041.

Goodall, J.A. and Stoddart, J.A. 1989. Techniques for the electrophoresis of mangrove tissue. *Aquat. Bot.*, **35**: 197–207.

Graham, A. 1993. Historical factors and biological diversity in Mexico. In *Biological Diversity of Mexico: Origins and Distribution* (T.P. Ramamoorthy, R. Bye, A. Lot and J. Fa, eds), pp. 109–127. New York: Oxford University Press.

Haldane, J.B.S. 1924. A mathematical theory of natural and artificial selection. Part II. *Trans. Cambr. Phil. Soc.*, **23**: 158–163.

Hamrick, J.L. and Godt, M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. *Phil. Trans. R. Soc. Lond. B*, **351**: 1291–1298.

Hamrick, J.L., Linhart, Y.B. and Mitton, J.B. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Ann. Rev. Ecol. Syst.*, **10**: 173–200.

Harrison, S. and Hastings, A. 1996. Genetic consequences of metapopulation structure. *TREE*, **11**: 180–183.

Hartl, D.L. and Clark, A.G. 1989. *Principles of Population Genetics*, 2nd edn. Sunderland, MA: Sinauer Associates.

Hellberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution*, **50**: 1167–1175.

Hossaert-McKey, M., Valero, M., Magda, D., Jarry, M., Cugen, J. and Vernet, P. 1996. The evolving genetic history of a population of *Lathyrus sylvestris:* evidence from temporal and spatial structure. *Evolution*, **50**: 1808–1821.

INEGI. 1988. *Atlas Nacional del Medio Fisico*. Aguascalientes, México: Instituto Nacional de Estadística Geografía e Informática.

Karl, S.A. and Avise, J.C. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science*, **256**: 100–102.

Klekowski, E.J., Jr., Lowenfeld, R. and Hepler, P.K. 1994. Mangrove genetics. II. Outcrossing and lower spontaneous mutation rates in Puerto Rican *Rhizophora. Int. J. Plant Sci.*, **155**: 373–381.

Linhart, Y.B. and Grant, M.C. 1996. Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst.*, **27**: 237–277.

Lowenfeld, R. and Klekowski, E.J., Jr. 1992. Mangrove genetics. I. Mating system and mutation rates of *Rhizophora mangle* in Florida and San Salvador Island, Bahamas. *Int. J. Plant Sci.*, **153**: 394–399.

Macmillan, C. 1986. Isozyme patterns among populations of black mangrove *Avicennia germinans*, from the Gulf of Mexico-Caribbean and Pacific Panama. *Contrib. Mar. Sci.*, **29**: 17–25.

May, B. 1992. Starch gel electrophoresis of allozymes. In *Molecular Genetic Analysis of Populations: A Practical Approach* (A.R. Hoelzel, ed.), pp. 1–27. Oxford: Oxford University Press.

McCauley, D.E. 1991. Genetic consequences of local population extinction and recolonization. *TREE*, **6**: 5–8.

Miles, M.A., Toyle, P.J., Oswald, S.C. and Godfrey, D.G. 1977. The identification by isozyme patterns of two distinct strain-groups of *Trypanosoma cruzi*, circulating independently in a rural area of Brazil. *Trans. R. Soc. Trop. Med. Hygiene*, **71**: 217–225.

Miller, M. 1997. Tools for population genetic analyses (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetics data. Computer software distributed by the author.

Nei, M. 1978. Estimates of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583–590.

Núñez-Farfán, J., Domínguez, C.A., Dirzo, R., Eguiarte, L.E. and Quijano, M. 1996. Estudio ecológico de las poblaciones de *Rhizophora mangle* en México. Final Report No. B007, National Commission for Biodiversity (CONABIO), Mexico City, Mexico.

Palacios-Chavez, R. and Rzedowski, J. 1993. Estudio palinológico de las floras fósiles del Mioceno Inferior y principios del Mioceno Medio de la región de Pichucalco, Chiapas, México. *Acta Bot. Mex.*, **24**: 1–96.

Primack, R.B. and Tomlinson, P.B. 1980. Variation in tropical forest breeding systems. *Biotropica*, **12**: 229–231.

Reeb, C.A. and Avise, J.C. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica. Genetics*, **124**: 397–406.

Ricklefs, R.E. and Latham, R.E. 1993. Global patterns of diversity in mangrove floras. In *Species Diversity in Ecological Communities*, (R.E. Ricklefs and D. Schluter, eds), pp. 215–229. Chicago, IL: University of Chicago Press.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution*, **39**: 53–65.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, **236**: 787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**: 264–279.

Slatkin, M. 1994. Gene flow and population structure. In *Ecological Genetics* (L. Real, ed.), pp. 3–17. Princeton, NJ: Princeton University Press.

Soltis, D.E. and Soltis, P.S. 1989. *Isozymes in Plant Biology*. Portland, OR: Dioscorides Press.

Strand, A.E., Milligan, B.G. and Pruitt, C.M. 1996. Are populations islands? Analysis of chloroplast DNA variation in *Aquilegia. Evolution*, **50**: 1822–1829.

Tomlinson, P.B. 1994. *The Botany of Mangroves*. Cambridge: Cambridge University Press.

Vallejos, C.E. 1983. Enzyme activity staining. In *Isozymes in Plant Genetics and Breeding* (S.D. Tanksley and T.J. Orton, eds), Vol. A, pp. 469–516. Amsterdam: Elsevier.

Weir, B.S. and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358–1370.

Weller, S.G., Sakai, A.K. and Straub, C. 1996. Allozyme diversity and genetic identity in *Schiedea* and *Alsinidendron* (Caryophillaceae: Alsinoideae) in the Hawaiian Islands. *Evolution*, **50**: 23–34.

Workman, P.L. and Niswander, J.D. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genet.*, **22**: 24–49.

Wright, S. 1951. The genetical structure of populations. *Ann. Eugenics*, **15**: 323–354.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**: 395–420.

Wright, S. 1977. *Evolution and Genetics of Populations. III. Experimental Results and Evolutionary Deductions*. Chicago, IL: University of Chicago Press.

Wright, S. 1978. *Evolution and Genetics of Populations. IV. Variability Within and Among Populations*. Chicago, IL: University of Chicago Press.