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Genetic diversity and relatedness of the mangrove *Rhizophora mangle* L. (Rhizophoraceae) using amplified fragment polymorphism (AFLP) among locations in Florida, USA and the Caribbean

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Abstract *Rhizophora mangle* L. is a widespread mangrove species in the Western Hemisphere. Mangrove habitat loss and their importance to coastal and reef ecosystems make greater understanding of their genetic structure useful for conservation and management. An amplified fragment polymorphism (AFLP) analysis was performed on samples from Florida and the Caribbean to discover the genetic structure present. *R. mangle* had variable genetic diversity not related to latitude; P ranged 7 %–92 %. Some other factor, perhaps human impact, has caused low genetic diversity in some populations. Across Florida *R. mangle* populations varied in genetic diversity with less diversity (G_{st}=0.195) and greater gene flow on the Atlantic coast (Nm =2.07) than on the Gulf coast (G_{st}=0.717, Nm=0.197). Gene flow between Caribbean islands was low (Nm=0.386) compared to continental populations (Nm=1.40), indicating that long distance dispersal is not common between islands. Analysis of molecular variance (AMOVA) analysis showed significant deviations from Hardy-Weinberg expectations at the level of region among subpopulations and overall genetic difference among subpopulations for *R. mangle*. One implication for management is that small continental populations and island populations may be genetically isolated and distinct from each other.

Keywords Mangrove · AFLP · Florida · Caribbean

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Introduction

Mangroves are woody trees or shrubs that occur only on tropical intertidal muddy shores between latitudes 25°N and 30°S (Valiela et al. 2001), requiring minimum winter temperatures of >0 °C (Ellison 2002). About 35 % of the world's mangrove habitat has disappeared in the last 25 years (Hogarth 2007), and more recent work indicates even this may be an underestimate (Giri et al. 2011). More than 40 % of vertebrates endemic to mangroves are threatened (Luther and Greenberg 2009). Mangrove forests are linked to the health of seagrass beds and coral reefs through nutrient cycling, nursery services, and sediment flow, as well as providing habitat for many fish, invertebrates, reptiles and amphibians, and terrestrial vertebrates (Luther and Greenberg 2009; Valiela et al. 2001). Mangroves are species assemblages of high economic and ecological value (Coastanza et al. 1997; Hogarth 2007). Aburto-Oropeza et al. (2008) estimate the mangrove fishery to be worth $37,500/ha annually in Mexican and UNEP-WCMC (2006) estimates additional ecosystem services ranging from $2,000/ha to $9,000/ha annually. Tourism can add value as well, and be greater than fishery value for certain areas (Conservation International 2008). Mangroves also reduce damage caused by natural disasters such as cyclones (Das and Vincent 2009).

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Currently approximately 6 % of mangroves are protected worldwide (Hogarth 2007). Conservation efforts may benefit from a clear understanding of the genetic structure of mangrove populations (Salas-Leiva et al. 2009a). Knowledge of the genetic structure allows conservation efforts to support existing genetic diversity, prevent inbreeding depression, and avoid mixing genetically different populations unintentionally. Conflicting genetic variability in mangroves has been reported, and this may be partly due to differences in study design such as geographical area sampled, number of samples taken, mangrove species studied, or genetic analysis methods used (Lakshmi et al. 1997; Chiang et al. 2001; Schwarzbach and Ricklefs 2001; Dodd and Rafii 2002; Duke et al. 2002; Castillo-Cárdenas et al. 2005; Arbeláez-Corte et al. 2007; Proffitt and Travis 2010). This study was undertaken to characterize the genetic variability within and between populations of red mangrove (*Rhizophora mangle* L.) from Florida and the northern Caribbean. By using an increasingly common modern genetic methodology we assessed the genetic health and relatedness of sampled populations and to test the patterns of geographic mangrove colonization of the Caribbean proposed by Ricklefs and Lantham (1993) and Plaziat (1995). We also modified published methods of DNA extraction to get high quality samples from this species.

Amplified fragment length polymorphism (AFLP) was used to characterize *R. mangle* mangrove populations. AFLP uses DNA to characterize inter- and instraspecific genetic variation (Vos et al. 1995; Clark et al. 2007; Krumm et al. 2008; Alamalakala et al. 2008). AFLP uses total genomic digestion, PCR, and selective amplification to form DNA "fingerprints" of samples. AFLP produces anonymous multilocus DNA profiles using many loci. Bensch and Åkesson (2005) consider AFLP superior to other methods including microsatellite, SNP, and multigene DNA sequencing.

Materials and methods

Mangrove leaves were collected from locations throughout Florida, the eastern Caribbean, one location in Bonaire, Antilles, and one Oahu, Hawaii site (Fig. 1). In each case, ten leaves each were taken from ten trees located in the same stand of mangrove. The distance between trees from which leaves were collected was at least 5 m. The leaves were wiped clean and frozen. The leaves were shipped overnight to the University of Nebraska at Kearney and maintained at −20 °C. They were then transported to the Insect Genetics Laboratory at the University of Nebraska–Lincoln.

DNA extraction

Thirty samples from each bag of leaves were diced on a glass plate and ground in 250 μL CTAB buffer by hand using a blue plastic pestle. Each sample was cut from a different leaf, and the mean sample weight was 0.034 g. After grinding, an additional 350 μL CTAB was added for a total volume of 600 μL and the leaves were ground again for uniformity. Samples were incubated on a heat block at 65 °C for 1 h, with mixing every 20 min by inverting the tubes. The tubes were removed from the heat block and 15 μL RNase A was added to each tube. Tubes were then incubated at 37 °C on the heat block for 2 h, mixing every 20 min by inverting the tubes. Following incubation, the samples were centrifuged for 5 min at 12,000 rpm and room temperature in an Eppendorf 5417R centrifuge. The supernatant was removed and transferred to new autoclaved 1.5 mL tubes, and 600 μL phenol:chloroform:isoamyl alcohol (24:24:1) was added to the new tubes. Samples were mixed by inverting the tubes, and centrifuged for 20 min at room temperature and 12,000 rpm. The top aqueous phase was transferred to new autoclaved 1.5 mL tubes. The bottom chloroform phase was discarded. This phenol:chloroform: isoamyl alcohol step was then repeated for a second time. The top aqueous phase was removed and transferred to new autoclaved 1.5 mL tubes, and 400 μL chilled (–20 °C) 100 % isopropanol and 90 μL sodium acetate were added. The tubes were rocked until the DNA began to precipitate. Samples were then stored at 4 °C overnight.

The centrifuge was cooled to 4 °C, then the samples were spun for 30 min at 4 °C and 12,000 rpm, after which a pellet of DNA was visible. The supernatant was poured off, and samples were washed with 400 μL absolute ethanol. Tubes were tapped until the pellet broke free from the bottom of the tube. Samples were centrifuged for 5 min at 4 °C and 12,000 rpm, the absolute ethanol was poured off, and 400 μL chilled (–20 °C) 75 % ethanol was added to each tube. They were centrifuged again for 5 min after which a small pellet of DNA remained visible. The ethanol was poured off, and the remainder was removed with a pipette. Samples were air dried for 25 min until the ethanol was evaporated, then resuspended in 50 μL 1X TE buffer. The DNA was stored at 4 °C until analysis continued. The DNA samples from populations 1–18 (extracted by M. Albrecht) and the DNA extracted from the Oahu and Dutch Antilles leaves were tested for quantity and quality on the NanoDrop spectrophotometer (Thermo Fisher Scientific 2009).

DNA analysis

DNA samples were each diluted to 20–100 ng/μL, the optimum concentration for use in Amplified Fragment Length Polymorphism (AFLP) analysis (Vos et al. 1995; Krumm et al. 2008). A restriction digestion mixture was prepared using 7 μL template DNA, 0.125 μL MseI and 0.0625 μL EcoRI restriction enzymes, 0.125 μL Bovine Serum Albumen (New England Biolabs Inc., Ipswich,

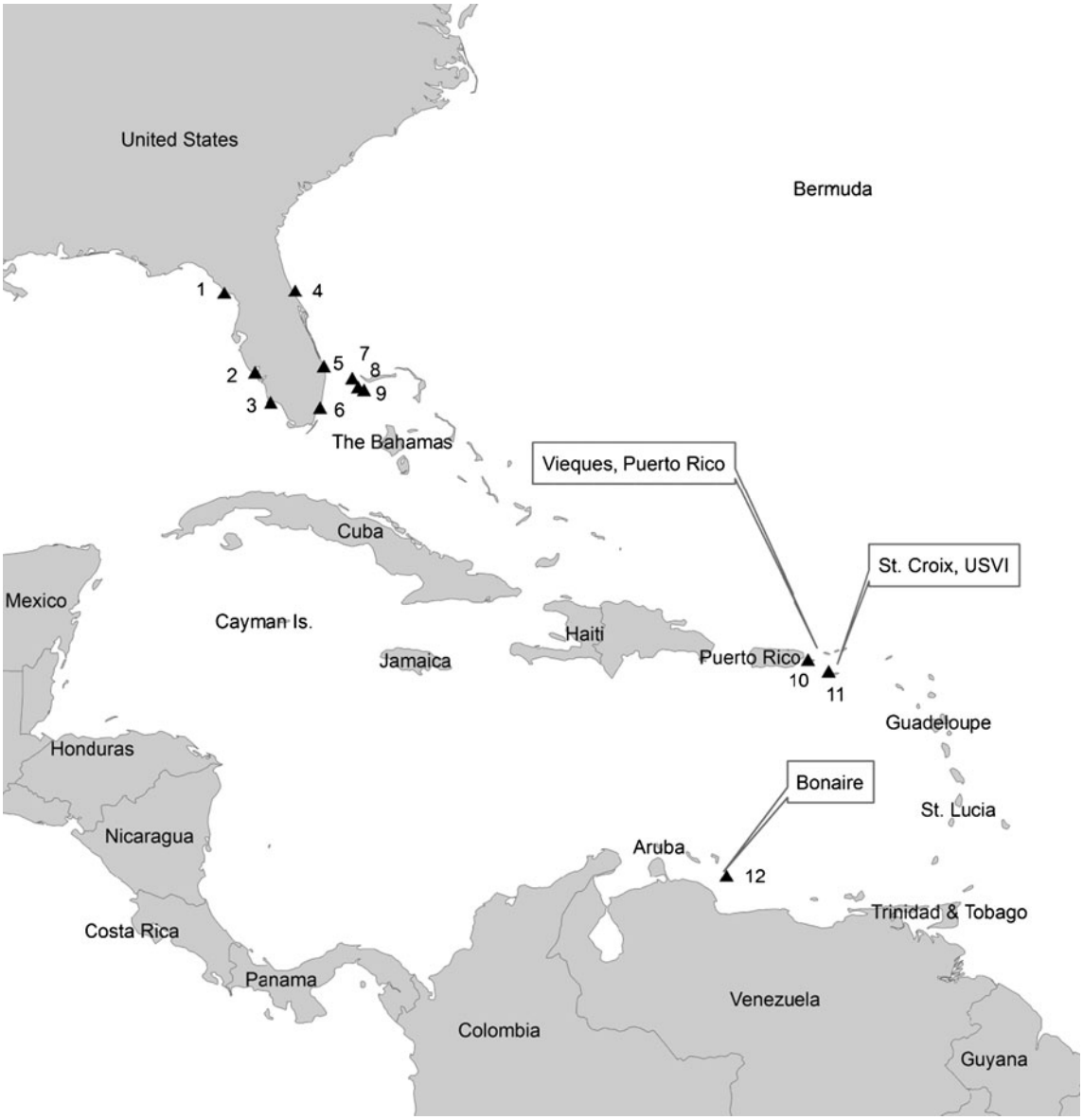

Fig. 1 Samples sites of mangrove collection, except Oahu, Hawaii

MA), 1.25 μL 10X One Phor All buffer (Amersham Parmacia Biotech Inc., Piscataway, NJ) and Nanopure H_2O for a total volume of 12.5 μL. The mixture was aliquotted into 0.2 mL PCR tubes and incubated on a PTC-200 thermal cycler (MJ Research, GMI Inc., Ramsey, MN.) at 37 °C for 2 h, with a final cycle of 70 °C for 15 min and a 4 °C soak. An adapter ligation mix was prepared (0.5 μL EcoRI adapter, 0.5 μL MseI adapter, 0.15 μL T4 DNA Ligase, 0.5 μL T4 DNA Ligase buffer, 3.35 μL Nanopure H_2O) and 5 μL of the mix was added to each sample. Samples were incubated at 25 °C for 8 h. on the PTC-200 thermal cycler and left overnight at 4 °C.

Samples were removed from the thermal cycler and 135 μL 1X TE buffer was added to each tube for a 1:10 dilution. A pre-amplification mix was prepared (10 μL pre-amplification mix II, 1.25 μL 10X PCR buffer, 0.75 μL 15 mM $MgCl_2$, 0.25 μL 5U/mL Taq polymerase), transferred in 12.25 μL aliquots to new PCR tubes, and 1.25 μL of the diluted ligation reactions were added to each tube. This pre-amplification mix was run on the same thermal cycler for 20 cycles of: 94 °C for 30 s, 56 °C for 1 min. and 72 °C for 1 min. followed by storage at 4 °C. The pre-amplification product was diluted 1:20 and a selective amplification PCR mix was prepared (1.2 μL 10X PCR buffer, 0.72 μL 15 mM $MgCl_2$, 0.08 μL 5U/mL Taq polymerase, 4.1 μL dH_2O, and the primers M-CTG and E-ACT). Added 8.6 μL selective amplification mix and 2.0 μL of diluted pre-amplification product to new PCR tubes and ran on the thermal cycler for 1 cycle of: 94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, followed by 12 cycles of: 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min. Added

2.5 μL stop solution to each tube, then ran on thermal cycler at 94 °C for 1 min. for denaturation. The PCR product was stored at 4 °C.

The samples were electrophoresed on KB^{Plus} 6.5 % polyacrylamide gel (LICOR) for 2 h and the image was saved for scoring and analysis. Subsequently, the entire process was repeated using the primer pair M-CTG/E-ACA.

The finished AFLP product was run on acrylamide gels in a LI-COR Gene Reader 4200 (LI-COR, Lincoln, NE). Gels were scored for the presence or absence of bands using the SAGA software package (LI-COR, Lincoln, NE). DBOOT v. 1.1 (Coelho 2001) was used to assess whether the number of loci used were sufficient. The resulting Boolean vectors were analyzed using PopGene (Yeh et al. 1997). Hardy-Weinberg equilibrium was assumed (F_{is}=0) and the populations were analyzed for the percent of polymorphic loci as well as Nei's genetic diversity (Gst). PAUP v.4.01b was used to construct dendrograms using UPGMA, a modified distance method. *Zea mays* (L.) was used as an outgroup. Arlequin v. 3.1 (Excoffier et al. 2005) was used to assess the genetic structure of populations using the analysis of molecular variance (AMOVA) (Excoffier et al. 1992). For the analyses, populations were grouped geographically and by species (Table 1). Mantel tests were performed by the Arlequin software on each species separately.

Results

Thirteen locations were sampled for this study: six sites in Florida, three in the Bahamas, and one each from Vieques, Puerto Rico, St. Croix, U.S. Virgin Islands, Lac Cai, Bonaire, and Oahu Hawaii (Fig. 1). We sampled the *R. mangle* of Florida in a grid pattern to compare the Gulf of Mexico coast to the Atlantic coast at approximately the same latitudes (Fig. 2). A total of 95 loci were identified and used for analysis. DBOOT analysis (Fig. 3) revealed that approximately 90 % of the genetic variation in these populations is accounted for with our markers, indicating that the markers used are sufficient for further analysis.

Table 1 gives the location, genetic analysis group, disturbance level, proportion of polymorphic loci (P), and Nei's (Nei 1978) genetic diversity (h) of *R. mangle* samples. The mean value of P was 46 % (SE ±7.25), and the mean h value was 0.1503 (SE ±0.0233). The Nei genetic diversity h values varied widely. The G_{st} values were similar for the Bahamas and Florida Gulf Coast regions (G_{st}=0.6166 and G_{st}=0.7174 respectively), but much lower for the Florida Atlantic Coast region (Table 2). These G_{st} values indicate genetic isolation between the Bahamas and Gulf Coast locations, but not among the Atlantic Coast locations. Likewise, only the Atlantic coast locations show enough gene flow to counteract genetic drift (Nm=2.0691).

Nei's genetic identity and genetic distance measures calculations are given in Table 3 (Nei 1978). The Caribbean samples, Vieques Puerto Rico, St. Croix USVI, Bimini Bahamas, show high genetic identity numbers with the Florida sites of Punta Gorda and Rookery Bay. These are the two southern sampling sites on the Gulf Coast of Florida. Values are much lower when the Caribbean sites are compared to the other sites in Florida. Comparisons to Grand Bahama, Oahu Hawaii, and La Cai Bonaire are intermediate. The same pattern is reflected in the genetic distance values.

The AMOVA results indicate 12 % of the genetic variation seen was between geographic groups, 52 % was between

Table 1 *R. mangle* (red mangrove) sample sites, latitude, longitude, size of mangrove areas, AMOVA group, proportion of polymorphic loci (P), and Nei's (1978) gene diversity index (h)

Location	Latitude	Longitude	Group	Disturbance	P	h
South Coast, Vieques, PR	26.1	−65.6	4	Low	74 %	0.2452
Sandy point, St. Croix US VI	17.7	−64.9	5	Low	64 %	0.1898
Oahu, HI	21.4	−157.8	6	Low	60 %	0.1838
Lac Cai, Bonaire	12.1	−68.2	7	Low	92 %	0.3386
Bimini, Bahamas	25.7	−77.3	8	Low	62 %	0.1750
Grand Bahama, Bahamas	26.5	−78.8	8	Medium	21 %	0.0860
Grand Bahama, Bahamas	26.7	−78.9	8	Medium	46 %	0.1797
Punta Gorda, FL	26.9	−82.1	9	Medium	71 %	0.1518
Rookery Bay, FL	26.0	−81.7	9	Medium	23 %	0.0477
Cedar Key, FL	29.2	−83.1	9	High	33 %	0.1381
New Smyrna Beach, FL	29.0	−80.9	10	High	21 %	0.0851
Jupiter, FL	26.9	−80.1	10	High	7 %	0.0267
Cutler Ridge, FL	25.6	−80.3	10	Medium	25 %	0.1061
Means (±SE)					46 % (±7.25)	0.1503 (±0.0233)

Fig. 2 Florida *R. mangle* sample sites showing the approximate grid pattern of sites

sample locations within the geographic groups, and 36 % was within sample locations themselves (Table 4). F_{st} again indicates a relatively high amount of genetic isolation among locations (F_{st}=0.63742).

The Mantel test for *R. mangle* data was not significant (r=−0.0537, p=0.6740), indicating no correlation between genetic and geographic distance. Likewise, the UPGMA dendrogram (Fig. 4) of the *R. mangle* samples shows no relationship between genetic and geographic distance.

Discussion

Amplified fragment length polymorphisms (AFLP) is a genetic analysis technique that combines restriction fragment amplification and PCR. The technique can be applied to any organism and has strong discrimination and reproducibility abilities (Amar et al. 2008). It has been described as a DNA fingerprinting method. AFLP samples a larger proportion of the total genome than other techniques, producing more

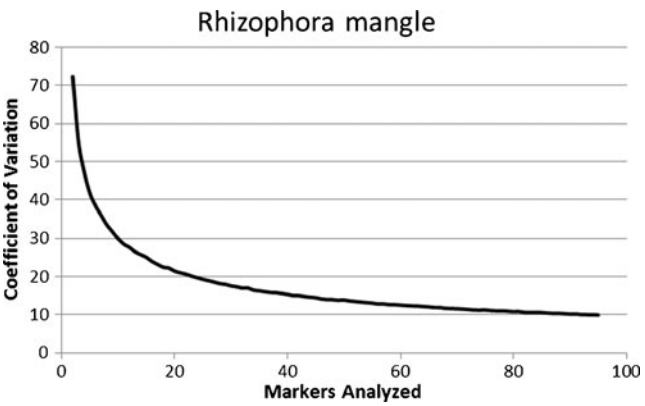

Fig. 3 Coefficient of variation, calculated for *R. mangle* red mangrove

Table 2 Values of G_{st} and *Nm* for *R. mangle* samples (Nei 1978)

Group	Area	G_{st}	Nm
8	Bahamas	0.6166	0.3109
9	Gulf Florida Coast	0.7174	0.1969
10	Atlantic Florida Coast	0.1946	2.0691

detailed information than microsatellite methods for instance (Campbell et al. 2003). In our study for example each sample was marked at 95 polymorphic loci. The resolution of the techniques provided fine-grained genetic information. AFLP has been in use since 1995, and has been use increasingly due to its favorable features (Amar et al. 2008). Papers such as Kelleher et al. (2005) and Schönswetter et al (2004) demonstrate that AFLP has been used successfully for population studies in plants with samples sizes for populations as small as three individuals. This was possible due to the amount of information, and the degree of detail that AFLP obtained, from each organism sampled.

Studies of *R. mangle* in the Caribbean and South America show a variety of values even for a broad parameter such as the proportion of polymorphic loci (P). Any natural variation is likely increased by the use of different molecular techniques and experimental designs. Duke et al. (2002) tracked chlorophyll-deficient mutations in Florida and the Bahamas and determined that self-pollination rates were between 71 % and 95 %, indicating that low genetic diversity would be expected. Núñez-Farfán et al. (2002) examined *R. mangle* populations on the Atlantic and Pacific coasts of Mexico using allozyme analysis. They found an overall polymorphism level of 38 %, hetereozygosity of 0.069, and an Nm of 0.621 (Nei 1978). They found higher levels of all these measures on the Pacific coast than the Atlantic. Núñez-Farfán et al. (2002) pointed out that there is more human development on the Atlantic side of Mexico. Mantel tests were not significant on either coast (Núñez-Farfán et al. 2002). Arbeláez-Corte et al. (2007) investigated *R. mangle* genetic structure on the Pacific coast of Columbia using microsatellite markers. They found 100 % polymorphic loci, an average heterozygosity of 0.493, and Nei (1973) genetic distances between 0.046 and 0.318. They also performed an AMOVA which found 5.58 % of the genetic variance occurring between populations and 94.62 % within populations, which was a significant result. Arbeláez-Corte et al. (2007) performed a Mantel test on Nei's genetic distance versus geographic distance and found no significant result.

In this study the P values for the populations outside Florida were about twice those seen in the other populations (Table 1). P values from the Gulf Coast of Florida were more than twice as high as those from the Atlantic coast. The *R. mangle* samples showed G_{st} values for the Bahamas and Gulf Coast of Florida that were high. The Nm values

Table 3 Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for *R. mangle* (Nei 1978)

Sample	SV, PR	SP, VI	B, BH	PG, FL	RB, FL	SB, FL	CR, FL	CK, FL	Ju, FL	GB, BH	GB, BH	O, HI	LC, BN
SV,PR	–	0.8596	0.8374	0.9303	0.8946	0.3224	0.3337	0.3805	0.2780	0.3928	0.5123	0.5355	0.7759
SP, VI	0.1513	–	0.8480	0.8028	0.7501	0.3422	0.3643	0.4237	0.3454	0.4445	0.4735	0.4713	0.7702
Bi, BH	0.1775	0.1648	–	0.8408	0.8161	0.3496	0.3658	0.4023	0.3184	0.4348	0.5152	0.4806	0.7881
PG, FL	0.0723	0.2197	0.1734	–	0.9708	0.2295	0.2502	0.2827	0.1787	0.2963	0.4307	0.4820	0.7340
RB, FL	0.1113	0.2876	0.2032	0.0296	–	0.2246	0.2447	0.2999	0.1707	0.3154	0.4444	0.4636	0.7173
SB, FL	1.1319	1.0724	1.0509	1.4720	1.4936	–	0.9785	0.9459	0.9757	0.8720	0.8214	0.7394	0.5968
CR, FL	1.0975	1.0097	1.0058	1.3855	1.4078	0.0218	–	0.9646	0.9744	0.8839	0.8557	0.6997	0.5887
CK, FL	0.9664	0.8588	0.9105	1.2635	1.2044	0.0556	0.0360	–	0.9553	0.9053	0.8657	0.7224	0.6115
Ju, FL	1.2800	1.0631	1.1443	1.7222	1.7680	0.0246	0.0259	0.0457	–	0.8851	0.7980	0.7239	0.5597
GH, BH	0.9344	0.8107	0.8329	1.2163	1.1539	0.1370	0.1235	0.0994	0.1220	–	0.8220	0.6912	0.6026
GH, BH	0.6689	0.7476	0.6633	0.8423	0.8111	0.1968	0.1559	0.1442	0.2256	0.1961	–	0.6343	0.6486
Ou, HI	0.6245	0.7522	0.7326	0.7298	0.7687	0.3019	0.3571	0.3252	0.3232	0.3693	0.4552	–	0.6946
LC, BN	0.2538	0.2611	0.2381	0.3093	0.3322	0.5161	0.5298	0.4918	0.5804	0.5065	0.4329	0.3645	–

were low for the Bahamas region and the Gulf Coast of Florida, but were high for the Florida Atlantic Coast (Table 2). Our analysis indicated the genetic variation of *R. mangle* populations on the Florida Atlantic Coast was low even though gene flow has been high. The Gulf Coast of Florida showed the opposite pattern with high genetic diversity values and low gene flow. The Bahamian region values were between the other regions, but more similar to the Gulf Coast of Florida values.

A latitudinal trend was not seen in the data. In Florida both the highest and lowest G_{st} values were found in the middle latitude samples (Punta Gorda and Jupiter). The G_{st} values from the Bimini, Bahamas were similar to those of Oahu, HI and Sandy Point, St. Croix USVI.

Nei's genetic identify and genetic distance values for *R. mangle* show that the sites from Puerto Rico, St. Croix, Bimini Bahamas, and southwest Florida: Punta Gorda, FL and Rookery Bay were different from the east Florida sites, New Smyrna Beach, Cutler Ridge, Jupiter, Cedar Key in northwest Florida (Table 3). Figure 4 shows these relationships as well. The two southwest Florida sites are more similar genetically to other sites in the Caribbean than to sites on the Florida Atlantic coast or the Bahamas, with the exception of Bimini, Bahamas. Bimini is the closest Bahamian island to Florida, and sits on the edge of the Gulf Stream current. The southwest Florida sites have been more protected from human development than the other sites sampled Florida. The other four sites in Florida were similar to the two sites on Grand Bahamas, Bahamas and the Oahu, Hawaii sample fell in this group as well. The Oahu sample may be similar due to Florida being one source of *R. mangle* introduced to Hawaii (Allen 1998).

Table 4 AMOVA results for *R. mangle* (red mangrove)

Source of variation	D.F.	Sum of squares	Variance components	Percent of variation
Among regions	6	1422.282	2.59574 Va	11.98
Among locations within regions	6	675.000	13.21340 Vb	51.76
Among samples	151	1186.089	7.85489 Vc	36.26
Total	163	3283.451	21.36034	
Fixation Indices				
F_{CT}	0.11982			
F_{SC}	0.58807			
F_{ST}	0.63742			
	P-value			
V_a and F_{CT}	0.16716±0.00992			
V_b and F_{SC}	0.00000			
V_c and F_{ST}	0.00000			

The two sites in southwest Florida were closer to large contiguous stands of *R. mangle*, as well as being in large protected areas. The two northern sites in Florida are near the northern limit for this species (Hogarth 2007). The sites on the Florida Atlantic coast were more disturbed and were from smaller mangrove patches (T. Champeau, Pers. Comm.).

The AMOVA results showed significant variation of individuals versus the total data set (F_{ST}), and when comparing variance within sample locations to the total variance (F_{SC}) but no significance was seen when comparing variations between regions to the total variance present in the data (F_{CT}).

Genetic differentiation of populations is dependent upon the processes of natural selection, founder effect, and gene flow (Wright 1978; Slatkin 1987). Mangrove species typically self-pollinate, are colonizers, and are short lived. These traits lead to greater population differentiation than the opposite traits. Local extinction and recolonization events may or may not lead to greater population differentiation based on the number of migrants.

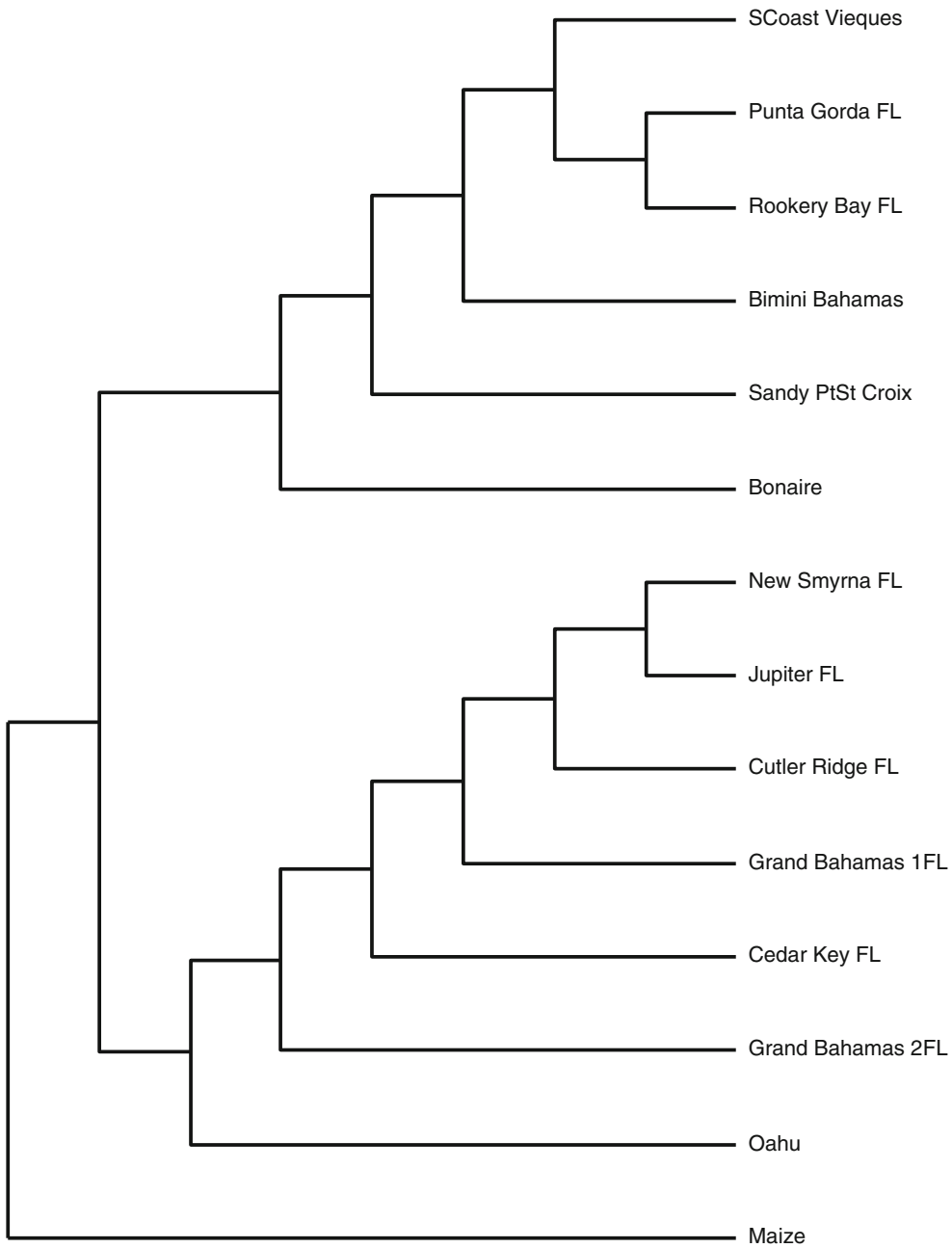

Fig. 4 Dendrogram of genetic similarity by AFLP analysis for sampled populations of *R. mangle*

Geography also has an effect on population differentiation. Generally, the greater the degree of isolation a population experiences will lead greater differentiation. The geographic effects on populations have been widely studied for some species, but mangrove species in the Caribbean only recently (Plaziat et al. 2001; Nettel and Dodd 2007). The Caribbean Ocean was formed approximately 100 Mya ago with the volcanic formation of the Lesser Antilles along the edge of the Caribbean and North American plates. This includes Puerto Rico and St. Croix. Mangrove species are thought to have spread to North and South American from their origins in Asia, as a band of tropical vegetation existed across the separating Pangea during the Early Cretaceous period (Plaziat et al. 2001). The peninsula of Florida was present as early as the middle Jurassic and appears to have come from the northwestern edge of Africa (Ziegler et al. 2001). The result is that for mangrove there has been a separation of land between the coasts of Florida since that time. Plaziat et al. (2001) argues that mangrove were pantropical by the Eocene, and that climate change, not continental drift brought about the evolution of mangrove floras.

The placement of the *R. mangle* from Oahu, Hawaii in the dendrograms was of interest. The date of introduction of *R. mangle* to Hawaii is 1902 and Florida has been suggested as a possible source (Allen 1998). This agrees with our results which point to the Atlantic Coast of Florida or Grand Bahama Island as close to the origin of these founders.

In the last 20 years mangrove ecology has become more clearly understood, leading to a growing recognition of the importance of mangrove in tropical marine ecosystem health (Ricklefs and Lantham 1993; Spalding et al. 1997; Hogarth 2007). Our results showing mangrove from small populations have lower polymorphism and heterozygosity is not surprising, but is troubling. Due to the ongoing reduction of mangrove habitat worldwide conservation and reforestation efforts are under way including projects such as Mangrove Dynamics and Management (MADAM) in Brazil, the "Coastal Habitats and Risk" project and Integrated Mangrove-Aquaculture System (IMAS) in Asia (Berger et al. 1999; Field 1999; Adeel and Pomeroy 2002; Peng et al. 2009). Examples of such efforts in the United States include mangrove nurseries at the at Vieques, Puerto Rico Fish and Wildlife Service National Wildlife Refuge and the St. Croix, Virgin Islands Sandy Point National Wildlife Refuge (Mike Barandiaran, Pers. Comm.) and the Reef Ball Mangrove Solutions Foundation locations in Grand Cayman and Florida (Reef Ball 2011). Other efforts are ongoing in Columbia (Salas-Leiva et al. 2009b). Secretary of State Clinton spoke on the important of mangrove restoration in Port Moresby, Papua New Guinea in November 2010 (Clinton 2010).

There were genetic differences between the populations of *R. mangle* sampled in this study. Factors such as ocean currents, storms, as well as recent human disturbances have given each population a different history. Our results showed that isolation, as demonstrated by higher genetic distance values at the level of sample sites and higher G_{st} values at the regional level were seen in oceanic island sites and sites from areas that are highly impacted by human activity. This indicates that mangrove conservation and restoration efforts need to consider the species involved and the source of propagules. This study identified hotspots of genetic diversity such as Lac Cai, Bonaire. This study also showed that the geographic location of a mangrove area does not predict the genetic diversity of the population well. We suggest that conservation and restoration plans include genetic analysis at both the local and regional levels.

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