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ANALYSIS OF GENETIC VARIATION IN *RHIZOPHORA MANGLE* POPULATIONS ON SAN SALVADOR, BAHAMAS, USING MICROSATELLITE DNA MARKERS

Randall E. Cross, Gina Hendrick, and Marilyn Cruz-Alvarez
Department of Biological Sciences
Florida Gulf Coast University
10051 FGCU Blvd. S.
Fort Myers, FL 33965

ABSTRACT

The genetic variation within and between three populations of red mangroves (*Rhizophora mangle*) on San Salvador Island was analyzed using microsatellite markers. Two of the populations are located in marine lakes found in the interior of the island: Stouts Lake and Mermaid Pond, and are not open to propagule influx. The third population is located along the north shore of Pigeon Creek and is open to gene flow because it borders the estuary on the southern end of the island. Five different primer sequences previously used to amplify microsatellite loci in Pacific populations of red mangroves, resulted in successful amplification of DNA from the San Salvador populations. Preliminary results with these primer sequences analyzed for ten individuals from each population indicated a low level of polymorphism. Only one primer sequence exhibited polymorphism within the Stouts Lake and Pigeon Creek populations, but not within the Mermaid Pond population. Although further analysis with a larger number of individuals is necessary, the lack of polymorphism in the Mermaid Pond population is suggestive of limited gene flow into this population.

INTRODUCTION

Mangroves are an important, if not essential, part of the ecology of shallow coastal and estuarine systems in the tropics. Their contribution to net primary productivity of coastal areas is well known, as well as their "nursery habitat" value to larval and juvenile stages of fishes and crustaceans (Nybakken and Bertness 2005). Net primary productivity of these ecosystems can rival that of any other ecosystem on the planet and the physical structure of red mangrove root systems provides a hard substrate for attached organisms, and a refuge for nekton in what is otherwise a soft-substrate system. In addition, the physical structure imparted by mangrove root systems helps to stabilize soft-sediment systems.

Mangrove systems are being altered and reduced in areal extent for a variety of reasons around the planet. Efforts to conserve these valuable habitats are increasing and it is important to understand the genetic history and population dynamics to both preserve and restore mangrove ecosystems. Efforts to preserve and restore mangrove populations are benefitting from molecular tools (Schwarzbach and Ricklefs 2001) and DNA analysis has provided important information on genetic diversity in Pacific red mangrove populations (Arbelaez-Cortes et al. 2007). Knowledge of genetic variation may be important in the long-term restoration and conservation of mangrove populations (Salas-Leiva et al. 2009) since the health and vigor of mangrove communities may depend on gene flow into these systems. The loss of genetic diversity can affect how populations are able to respond to changes in their environment such as changing salinity regimes or changing water levels due to global climate change.

Here we report on our efforts to understand the genetic diversity present in mangroves by analyzing DNA microsatellites from three populations of red mangroves on San Salvador Island: two inland isolated populations growing in a hypersaline portion of south Stouts Lake and in Mermaid Pond, and an open population subject to gene flow in Pigeon Creek. In our initial efforts, we used specific primers previously identified for red mangrove populations on the Pacific Coast of Colombia (Rosero-Galindo, et al 2002). These microsatellite markers were used to determine variability within each population. Very low ge-

netic variation was expected in the *Rhizophora mangle* populations of South Stouts Lake and Mermaid Pond, due to their isolation and extremely limited opportunities for gene flow into these populations.

MATERIALS AND METHODS

Field Collection

Leaves were collected from red mangroves at the south end of Stouts Lake and Mermaid Pond (Fig. 1 – sites A and B respectively), and the north side of Pigeon Creek (Fig. 1 – site C) in June of 2010. Care was taken to avoid collecting leaves from consanguineous individuals by collecting from spatially separated large trees that were generally more than 3 meters apart. The leaves were placed in plastic bags and transported to the Gerace Lab where they were refrigerated until transport to Florida Gulf Coast University. Leaves were then stored at -80° C until extraction of DNA.

Figure 1. Leaf collection sites on San Salvador (A: Stouts Lake, B: Mermaid Pond, C: Pigeon Creek).

DNA Extraction and Amplification

Approximately 0.1 g of leaf material from individual mangrove trees was ground with a mortar and pestle and the powder transferred to microcentrifuge tubes. DNA was extracted from leaf tissue using the DNeasy Mini Kit (Qiagen, Valencia, CA) following manufacturer's instructions. The quantity and quality of extracted DNA was assessed by agarose gel electrophoresis.

Extracted DNA was subjected to PCR amplification using the primers: RM6 forward and reverse, RM7 forward and reverse, RM11 forward and reverse, RM19 forward and reverse, RM21 forward and reverse, and RM36 forward and reverse. Table I shows the sequences of these primers, the microsatellite sequences, and the approximate expected size of the microsatellites (Rosaro-Galindo et al. 2002). Polymerase chain reactions were performed in a volume of 25 μl, 0.1 μM of each primer, and 10 μl of 2.5x master mix (5 Prime). This corresponds to the following final concentrations in the reaction: 50 mM KCl, 30 mM Tris-HCl pH 8.3, 1.5 mM magnesium acetate, 200 μM of each dNTP, and 0.625 U of Taq Polymerase. The conditions used were those previously described for amplification of the microsatellites with these primers: an initial denaturation step at 96º C for 2 minutes, followed by 35 cycles, consisting of 15 seconds at 94º C, 15 seconds at 51º C and 15 seconds at 72º C, and a final extension step of 5 minutes at 72º C. Amplified fragments were initially analyzed by agarose gel electrophoresis to determine whether amplification of the satellite sequences had occurred.

Analysis of Microsatellites

Amplified fragments were further analyzed by electrophoresis on denaturing 6% polyacrylamide gels containing 7M urea, as described by Creste et al. (2001), in order to be able determine small size difference between different alleles. Gels were silver-stained for DNA detection using the Silver Sequence™ reagents (Promega, Madison, WI) following manufacturer's instructions.

RESULTS

Agarose gel electrophoresis showed amplification of leaf DNA for the following sets of primers: RM7F/R, RM11F/R, RM19F/R, RM21F/R, and RM36F/R. Based on the size of the observed bands, the amplified fragments correspond to the microsatellite loci previously identified in populations of red mangroves in the Colombian Pacific Coast (Rosero-Galindo et al, 2002). Only primers RM6F/R did not result in any amplification of the DNA samples from the mangroves on San Salvador.

The amplified fragments obtained with 10 samples from each population and each of the five different sets of primers were analyzed by electrophoresis on a denaturing polyacrylamide gel. Only the RM19 primer set produced amplified fragments that showed polymorphism among individuals of each population. These results are shown in figure 2. As can be observed in this figure amplification of the DNA from some of the mangroves from Stout's Lake and Pigeon Creek resulted in two bands of different sizes, whereas amplification of the DNA from others resulted in a single band. This indicates that some mangroves are heterozygous, some are homozygous for the RM19 microsatellite.

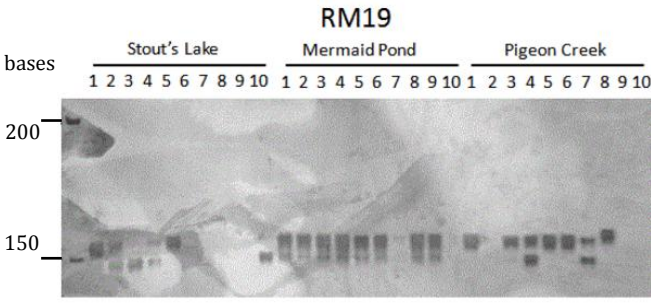

Figure 2. Results of amplification of DNA samples from 10 mangroves each from the Stouts Lake, Mermaid Pond and Pigeon Creek populations using the RM19 primers.

DISCUSSION

Polymorphic microsatellite sequences were previously identified in *Rhizophora mangle* populations on the Pacific Coast of Colombia (Rosero-Galindo, et al. 2002). Our initial efforts were aimed at confirming the presence of these microsatellites in the red mangroves on San Salvador.

The results indicate that at least several of the microsatellite sequences are conserved among geographically distant populations of *R. mangle*, and these markers can be used to analyze the degree of isolation of populations on San Salvador and gene flow.

In our preliminary analysis, the PCR products, using five primers from 10 individuals from each of the three populations, revealed limited polymorphism. Only the microsatellite locus amplified with primer RM19 exhibited variation among mangroves in the Pigeon Creek and Stouts Lake populations. In both of these populations, some individuals were heterozygotes while some were homozygotes for the number of repeats. However, no polymorphism for this primer was detected among the individuals in the Mermaid Pond population where all individuals were heterozygous. No polymorphism was detected in any of the populations for the microsatellites amplified with the other four primers. A possible explanation for the genetic variation found in the Stouts Lake population could be associated with the location and size of the lake. Stouts Lake is much larger than Mermaid Pond and is closer to the island's coastline.

However, due to the low number of individuals analyzed so far from each population, no definite conclusions can be made at this time. More samples need to be analyzed in order to obtain a stronger conclusion regarding genetic variation within and between these "open" and "closed" populations of mangroves.

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Table 1. Microsatellites used for the analysis

Locus	Primer sequences	Microsatellite sequence	Size
RM6	F: GGACAACAATAAAAGAGAGTGGGG R: TTTGTGAAGATGCCAAAGAGGA	$(GA)_{14}$	150
RM7	F:TGTTGTGCTACAGACTTCATGC R:AGAGATACCAATCCAAGAACTGC	$(TA)_4(TGTA)_2(CA)_{11}(TA)_3(GA)_4(GA)_2$	190
RM11	F: GGTGGGATTTGGTCTGGC R:TTGCTTCCACACAGGAAAGAAG	$(CT)_{16(}CA)_3$	180
RM19	F: TGCCCTCTACGTTGTGAATG R: CTGTCTGAGCTTGCATCATTG	$(AG)_{26}$	150
RM21	F: TTGCACTACCACAAACCAACC R: TTGCACAAGCACAAGAGCATAC	$(CT)_{12}$	200
RM36	F:GAGATGGAAATGGTTCTGTTGTTG R: TGTTCCTCGCTGTCTCAAAGTG	(GA)21	210