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IDENTIFICATION OF NUCLEAR DNA MICROSATELLITES FROM RED MANGROVE (RHIZOPHORA MANGLE) POPULATIONS ON SAN SALVADOR

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ABSTRACT

Nuclear DNA was obtained from two separate populations of red mangroves (*Rhizophora mangle*) on San Salvador. One population is located inland on the southern shore of Stout Lake and the other population is located along the north shore of Pigeon Creek. The Stout Lake population is isolated from gene flow due to its inland location and topographical barriers to coastal hydrodynamics. The Pigeon Creek population is open to gene flow because of its estuarine location and connection with the open ocean. In an attempt to determine the degree of gene flow in the two populations, polymorphism of DNA sequences will be examined within and between the two populations. Preliminary results indicate the feasibility of using microsatellites as DNA markers for population studies on San Salvador. Primer sequences previously used to amplify microsatellites in red mangroves from the Pacific coast successfully amplified DNA extracted from leaves collected from red mangroves on San Salvador. The DNA sequences amplified seem to correspond to microsatellite loci: RM7, RM11, RM19, RM21, and RM36. Polymorphism at these loci will be used as a starting point to analyze the degree of genetic isolation and gene flow in these populations.

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 is 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 Rickleffs 2001) and DNA analysis has provided important information on genetic diversity in Pacific red mangrove populations (Arbelaez-Cortes 2007). DNA analyses also provide important management information for restored or reforested mangrove populations (Salas-Leiva et al. 2009). The long-term health and vigor of mangrove communities may depend on gene flow into the 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 initial efforts to obtain and amplify nuclear DNA, and to identify microsatellite sequences from two populations of red mangroves on San Salvador. Identification of polymorphic microsatellites will allow further analysis of gene flow and population dynamics of two separate populations on San Salvador.

METHODS AND MATERIALS

Field Collection

Leaves were collected from red mangroves at the south end of Stout Lake (isolated population; Figure 1–site A) and the north side of Pigeon Creek (open population; Figure 1–site B) in June, 2008. Care was taken to avoid collecting leaves from consanguineous individuals by collecting from spatially separated large trees that were generally a few 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 (Site A – isolated population; Site B – open population).

DNA extraction and amplification

Approximately 0.1 g of leaf material from individual mangrove trees was ground in a mortar and pestle and the powder transferred to microcentrifuge tubes. DNA was extracted from leaf tissue using the DNeasy Mini Kit (Qiagen) following manufacturer's instructions.

Extracted DNA was subjected to PCR amplification as described by Rosaro-Galindo et al. (2002), using 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. Amplified DNA fragments were analyzed by agarose gel electrophoresis.

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 (Figure 2). No amplification was observed with the RM6F/R primers. 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).

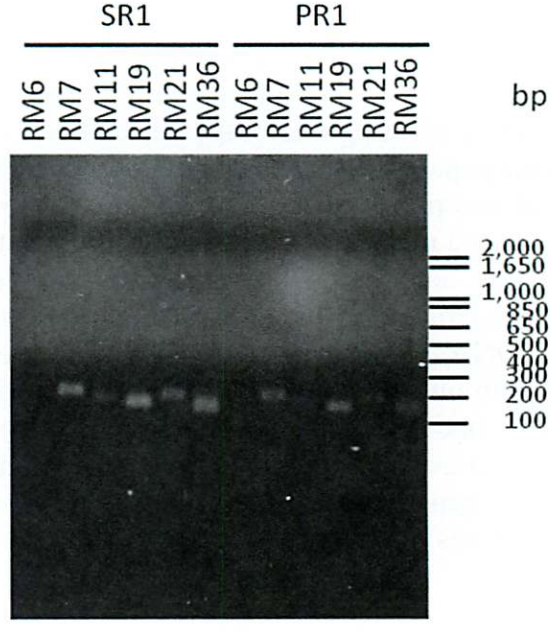

Figure 2. Results of amplification of DNA samples from a mangrove from the Stout Lake population (SR) and a mangrove from the Pigeon Creek population (PR) using primers corresponding to the loci indicated above each lane. The sizes of bands of a molecular weight marker are indicated on the right margin in base pairs.

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*. Future molecular analysis will utilize these microsatellite sequences to study mangrove populations on San Salvador. The information gathered will provide insight as to the degree of isolation of populations and gene flow.

Future molecular research on San Salvador red mangrove populations will also examine expression of stress genes in both the isolated population which is subject to hypersaline conditions and the open population found in a normal salinity environment in Pigeon Creek.

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