REPRODUCTION IN THE PALEOENDEMIC ISLAND IRONWOOD, *LYONOTHAMNUS FLORIBUNDUS*

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INTRODUCTION

The ironwood, *Lyonothamnus floribundus*, is a relictual species endemic to four of the eight California Channel Islands. While fossil evidence indicates that the genus was widespread across the western United States during the Miocene (6 to 18 million years ago) the range is now restricted to Santa Cruz, San Clemente, Santa Rosa and Santa Catalina islands where the trees grow in isolated groves of 1 to more than 100 trunks. *L. floribundus* has been placed in the Rosaceae based on morphological and molecular characteristics, although no closely related species have been identified. Two subspecies of *L. floribundus* can be distinguished based on leaf morphology. *L. floribundus* ssp. *floribundus* (Gray) Raven which has single entire leaves is found only on Santa Catalina Island (ca. 150 groves). *L. floribundus* ssp. *aspleniifolius* (Green) Raven has pinnately compound leaves with deeply toothed margins and is found on the islands of Santa Cruz (ca. 1000 groves), San Clemente (ca. 200 groves), and Santa Rosa (ca. 10 groves). Fossils suggest that these distinct leaf morphologies may have existed for millions of years, but it has also been proposed that the ssp. *floribundus* is a recent derivative of the more abundant ssp. *aspleniifolius*. Comparison of DNA sequences of variable regions in the genome may provide information on the length of time these two lineages have been separated.

The mature plants of both subspecies produce many flowers, but few seeds are viable and natural seedlings on the islands are very rare. Reproduction occurs primarily by crown and root sprouting. A study of genetic diversity of the Santa Cruz Island ironwood using random amplified polymorphic DNA (RAPD) analysis has shown that the groves are largely clonal, but each grove is apparently a distinct clone. The clonal nature of the groves may have severe consequences for sexual reproduction in this species. Pollinators may move primarily between flowers of a single individual, and rarely carry pollen from one isolated grove to another. However, paternity analyses of seeds produced by *L. floribundus* on Santa Cruz Island have not yet been carried out.

On the mainland, on the other hand, groups of trees under cultivation produce abundant viable seeds. A simple explanation for this observation might be that these mainland groups are composed of genetically distinct individuals. Pollinators would be able to move frequently among different individuals, thus enhancing the frequency of cross-pollination. RAPD analysis provides an efficient method for determining whether trees in a group are genetic individuals.

Investigations related to reproduction in *L. floribundus* are based on the assumption that sexual reproduction occurs in this species. Intersubspecific hybrids, cultivated in the Santa Barbara Botanic Garden, show a unique leaf morphology which has features of both subspecies. This strongly suggests genetic contributions from both parents. Direct evidence of the recombination of parental loci in the intersubspecific crosses can be obtained via molecular analysis.

MATERIALS AND METHODS

We have extracted genomic DNA from frozen samples of young leaves of *L. floribundus* using a DNeasy Plant kit (Qiagen, Inc.) according to the instruction provided by the manufacturer. DNA concentrations were determined using fluorometer and an average yield of 6 mg was obtained from approximately 200mg (wet weight) of tissue. Primers for RAPD analysis were obtained from Operon Technologies and PCR was performed using Ready to Go RAPD analysis beads (Pharmacia Biotech). Amplification was as follows: 95°C for 5 min followed by 45 cycles of 1 min at 95°C, 1 min at 36°C and 2 min at 72°C. The PCR products were analyzed on agarose gels and stained with ethidium bromide.

The internal transcribed spacers of the ribosomal genes from each of the two subspecies were amplified with ITS1P and ITS4 primers and sequenced using automated sequencing.

Seeds from *L. floribundus* collected from a single tree in a clonal grove on Santa Cruz Island were germinated and the seedlings used for paternity analysis.

RESULTS

We used random amplification of polymorphic DNA (RAPD) markers to investigate genetic diversity and sexual reproduction in *L. floribundus*. Analyses of multiple groves of the two subspecies, *floribundus* and *aspleniifolius*, and sequencing of the internal transcribed spacers in rDNA indicate that both subspecies of *L. floribundus* are closely related. The sequences of the ITS regions are identical. If the subspecies diverged in the Miocene (6-18 mya) as fossil evidence suggests, it is unusual to find no sequence differences in this variable region.

RAPD markers were also used to analyze DNA from an intersubspecific hybrid and the parent trees. Parental loci showed recombination in the offspring of the intersubspecific cross. These results provide direct evidence that sexual reproduction occurs in *L. floribundus*.

Seeds collected from a single tree in a clonal grove on Santa Cruz Island appear to result from self-fertilization. Five seedlings were tested and all the RAPD bands found in the seedlings corresponded to bands present in the mother. A group of trees (ssp. *aspleniifolius*) cultivated on the mainland, which are used as a commercial sources of ironwood seeds, were found to be comprised of genetically distinct individuals. This suggests that these mainland trees are cross pollinated and this may explain the production of abundant viable seeds by these trees. However, we have not yet carried out paternity analyses on the seeds. These studies are currently in progress.

CONCLUSION

The results of these investigations have implications for conservation of *L. floribundus* on the Channel Islands. Our data thus far is consistent with the hypothesis that *L. floribundus* experiences inbreeding depression and that one consequence of clonal growth of the groves is a severe reduction in seed production and viability. Erosion is a threat to native groves and the number of groves may gradually diminish. In order to prevent extinction of this unique species, the condition and number of existing groves should be monitored.

Keywords: Endemic, *Lyonothamnus floribundus*, RAPD, Rosaceae, reproduction, conservation.