Development of Microsatellite Markers for Population Genetic Studies of *Harrisia portoricensis* and Related Species

Introduction

Harrisia portoricensis (fig.1-2) is a columnar cactus endemic to Puerto Rico. It was first collected and described from southern Puerto Rico, but urban and agricultural development extirpated these populations. At present, the species is restricted to the islands of Mona, Monito and Desecheo. In addition to its limited distribution, *H. portoricensis* is threatened by the activity of feral goats and pigs on two of these islands. These and other considerations motivated its inclusion in the U.S. Fish and Wildlife Service threatened and endangered species list (U.S. Fish and Wildlife Service 1990).

Microsatellites or Simple Sequence Repeat (SSR) markers are being widely used in plants for a variety of applications including the assessment of the genetic diversity of natural populations. Microsatellites are typically highly polymorphic and robust, and also have a high level of transferability to related species (Varshney et al. 2005). The use of these markers could prove very useful for the study of genetic structure, gene flow and the degree of inbreeding in populations of *Harrisia portoricensis.* These analyses could in turn provide insights into the factors and processes that affect the survival of the species, information that will be valuable for formulation of effective management and conservation strategies.

Figure 1. *Harrisia portoricensis.*

Objectives

1) To develop microsatellite markers potentially useful to assess various population genetics parameters in *H. portoricensis*.
2) To test the developed primers in other species of *Harrisia* in order to examine their transferability.

Figure 2. *Harrisia portoricensis* in Mona Island.

Materials and Methods

- A genomic library was developed following a modified protocol by T. Hrbek et al. (UPR-Rio Piedras) and Glenn & Schable (2005). 31 out of 96 sequenced clones contained microsatellite regions, and primer pairs were designed for 10 of these loci.
- Designed primer sets were tested in samples from 15 individuals of *H. portoricensis* collected from three subpopulations in Mona Island ("airport", "bajura indio" and "frontón"). Additionally, primer pairs were tested in the following species of *Harrisia* (one individual each): *H. aboriginum* (Florida), *H. eriophora* (Cuba), *H. fragrans* (Florida), *H. martinii* (Argentina), *H. regelii* (Argentina), *H. simpsonii* (Florida), *H. taetra* (Cuba), *H. taylorii* (Cuba) and *H. tetracantha* (Bolivia).
- DNA was extracted using Qiagen DNeasy Plant Tissue Kit and amplified by PCR according to a standard laboratory protocol.
- Amplified loci were genotyped on the ABI 3130xl genetic analyzer and examined with GeneMapper Software, version 4.0.

Results

- Seven primer pairs successfully amplified microsatellite loci in all samples of *H. portoricensis* and the nine additional species.
- Six loci were monomorphic and just one polymorphic (with two alleles) for *H. portoricensis* (Fig. 3).
- Allelic variation was found among the different species of *Harrisia* (Fig. 4). Upto four loci were polymorphic for some of these species.

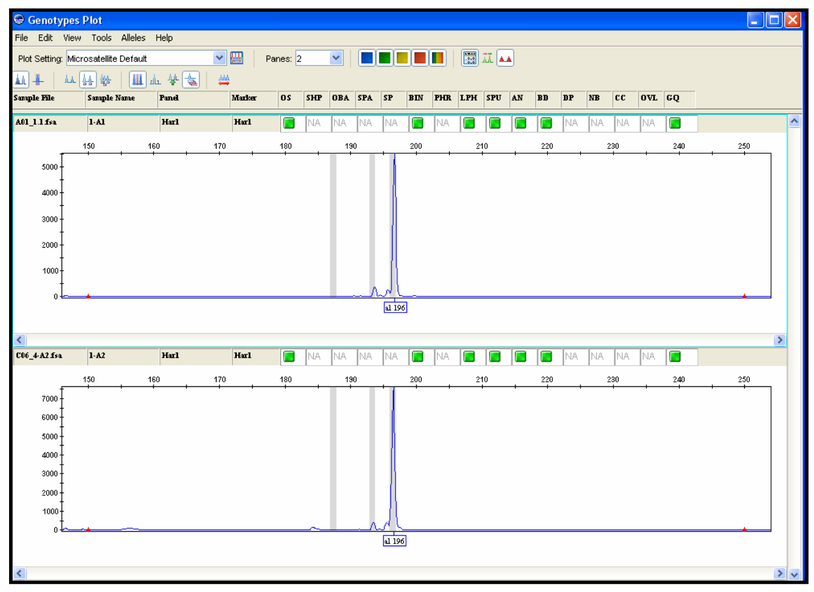

Figure 3. An example of two homozygous individuals of *H. portoricensis* with the same allele for locus 1.

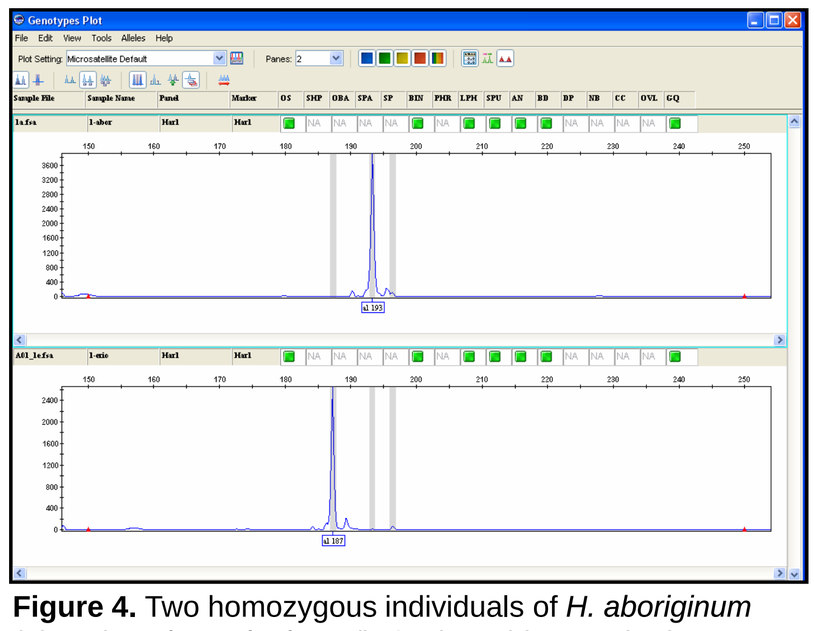

Figure 4. Two homozygous individuals of *H. aboriginum* (above) *and H. eriophora* (below). In this case both species exhibit different alleles for locus 1.

Conclusions and Future directions

The microsatellite markers developed in this study are the first markers for the genus *Harrisia*. Their transferability seems high since they easily amplify across a wide range of species. The markers appear to be much less variable in *H. portoricensis* than in other *Harrisia* species. More extensive sampling from different subpopulations and populations of *H. portoricensis* as well as the other species is needed in order to accurately characterize these markers and their conservation genetic value.

Literature Cited

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