EVIDENCE FOR LOW GENETIC DIVERGENCE AMONG GALÁPAGOS *OPUNTIA* CACTUS SPECIES

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INTRODUCTION

Islands are often showplaces of dramatic examples of evolutionary divergence and adaptive radiation. A combination of factors, including reproductive isolation from mainland populations, founder effect, limited competition, and freedom from predation can result in rapid evolution. Among the best-known examples of morphological divergence in the Galápagos are tortoises, finches, and prickly pear cacti (*Opuntia*) with markedly different forms on different islands.

Cacti are a prominent, and often dominant, component of the Galápagos flora. During the driest period of the year in the lowlands, they are virtually the only green vegetation to be found. Of the three cactus genera in the islands, *Brachycereus* and *Jasminocereus* are each represented by a single species (Dawson 1962a, 1962b). In contrast, prickly pear (*Opuntia*) are both widespread and highly diverse, growing on all of the major islands and most islets. Six species are currently recognized, with three of these further divided into varieties, yielding a total of fourteen taxa (Anderson and Walkington 1971). A long-standing debate has revolved around how many taxa deserve species status versus recognition as subspecies or varieties. Pronounced morphological variation in such traits as degree of spines, seed size, and height are the basis for taxonomic designation. *Opuntia* in the Galapagos can occur as large arboreal (tree-like) forms up to 12 m in height and as low-growing, shrubby forms. This variation could result from various genetic factors such as genetic drift and selection, but could also be due to environmental influences.

The origins of *Opuntia* in the Galápagos are not well understood. Two methods of colonization have been proposed. One theory suggests that parts of cacti may have rafted to the islands on the Humboldt Current which flows northward along the west coast of South America, turning west as it nears the equator, and proceeding to the Galápagos Islands (Dawson 1962b). Alternatively, cactus seeds may have been carried by birds flying across the ocean from the mainland (Porter 1983).

Cacti are an important resource in the Galápagos because of the extensive use of pads, flowers, and fleshy fruits as a food source by reptiles, birds, and insects. Tortoises on some islands appear to be highly dependent upon *Opuntia* during the dry season for both food and water. This resource may be in jeopardy since populations of *Opuntia* have been greatly reduced or eliminated on a number of islands. Photographs from the early twentieth century show areas occupied by numerous large cacti where none exist today. The most apparent reason for this reduction is damage inflicted upon the cacti by goats which were introduced to the islands (Grant and Grant 1989). Goat populations have been eradicated or reduced on several islands, hopefully allowing for the recovery of *Opuntia* populations, but are at high densities and even expanding on other islands. It appears that even in areas with large numbers of fruits and seeds, germination frequency is low, perhaps due in part to the low density or absence of tortoises. Tortoises may aid seed

germination by dissolving some of the tough seed coat in their digestive track, thereby helping to disperse and speed germination of the seeds. However, it is important to note that *Opuntia* occur on several islands and islets where no tortoises occurred historically.

Galápagos *Opuntia* have been hypothesized to be an outstanding example of the evolutionary divergence for which these islands are well known (Dawson 1962a, 1962b, Anderson and Walkington 1971). However, in contrast to some other Galápagos organisms, such as finches, the evolutionary and genetic relationships of the cacti have not been studied. This study utilizes allozyme electrophoresis 1) to assess levels of genetic variability and to characterize the population genetic structure of Galápagos *Opuntia*; and 2) to estimate the degree of genetic divergence between Galápagos *Opuntia* and the two species of *Opuntia* found in coastal Ecuador which could possibly be ancestral species. This knowledge will help illuminate the evolutionary processes at work in the islands and the genetic implications for insular populations. It could also aid in the conservation of *Opuntia* since the genetic data may clarify taxonomic status and help set resource priorities and management decisions.

MATERIALS AND METHODS

Cactus tissue samples were collected from 15 islands and islets of the Galápagos Islands from 1995-1998 (Appendix 1). Wherever possible, 25 samples were collected at each location. In order to minimize the possibility of collecting individuals from only a single clone, the samples were collected along a 1000 m transect if space and cactus distribution allowed. A single cladode (pad) was collected from the five cacti closest to each of five "sites" along the transect (0 m, 250 m, 500 m, 750 m, and 1000 m). Plants consisting of only a single cladode were excluded.

All cladodes were taken to the Charles Darwin Research Station (CDRS), and within seven days, sections of approximately 20 cc were cut out of each cladode and placed in individual plastic vials or bags. Within three days, samples were transported at ambient temperature to the Department of Biology at Wake Forest University. Upon arrival, samples were placed in storage at –70°C. Remnants of the cladodes were destroyed by burning.

In July 2000, 26 *O. melanosperma* samples were collected from a 30 km section of the Ecuadorian coast centered on the Puerto Lopez area. In addition, 30 *O. macbridei* samples were collected from a single location (~ 1 km in diameter) 1.5 km west of Huayaquilles, Ecuador, located ~ 2 km north of the Peruvian border. In December 2000, five individuals of *Opuntia dillenii* were collected from Isla Monito, Puerto Rico (18° 3.8'N, 67° 51.7W) and were analyzed for comparative purposes.

Enzymes were extracted from the tissue samples by grinding 0.5 g of frozen tissue in 1 ml of chilled "moderate" extraction buffer modified from Wendel and Weeden (1989). Horizontal starch gel electrophoresis was con-

ducted using standard techniques as described in Murphy *et al.* (1996). Resolution of thirty different enzymes was attempted repeatedly on as many as ten different buffer systems. Buffer and stain recipes were taken from Wendel and Weeden (1989), Soltis and Soltis (1989) and Hillis *et al.* (1996). The remainder of all tissue samples and extracts are stored at –70°C at Wake Forest University.

RESULTS

The majority of buffer/stain combinations did not produce enzyme activity and/or scorable loci, a problem encountered in other cactus studies (Parker and Hamrick 1992). Eight presumptive loci were identified which were consistently expressed and scorable; all were resolved using Poulik's buffer: Malate Dehydrogenase (MDH), EC 1.1.1.37; Phosphogluconate Dehydrogenase (PGDH), EC 1.1.1.44; Glutamate Dehydrogenase (GTDH), EC 1.4.1.2; Glycerol-3-Phosphate Dehydrogenase (G3PDH), EC 1.1.1.8; Phosphoglucomutase (PGM), EC 5.4.2.2; Glucose-6-Phosphate Dehydrogenase (G6PDH), EC 1.1.1.49; Glucose-6-Phosphate Isomerase (GPI), EC 5.3.1.9; Superoxide Dismutase (SOD), EC 1.15.1.1.

Allele frequencies, percent of loci polymorphic, and average individual heterozygosity for the populations are listed in Table 1. All eight loci were found to show no detectable variability across all 240 individuals from the Galápagos and hence the listing in Table 1 is a single entry "*Opuntia* sp. Galápagos n=240". G6PDH, G3PDH, PGDH, PGM and SOD were nonvariable among all 301 samples. While no variability occurred within *O. melanosperma* or within *O. macbridei*, these species were distinguishable from the Galápagos *Opuntia* by the fixed difference in alleles at the GPI locus. Puerto Rican *Opuntia* differed from Galápagos and coastal Ecuadorian *Opuntia* at GTDH, MDH, and GPI loci. Only the Puerto Rican population exhibited heterozygosity. Genetic distance values (Nei 1972) are 0.134 between Galapagos *Opuntia* and both *O. melanosperma* and *O. macbridei* and 0.470 between Puerto Rican *Opuntia* and *O. melanosperma*, *O. macbridei* and Galapagos *Opuntia*.

DISCUSSION

The low number of scorable loci limits confidence in data interpretation within ecological and evolutionary contexts. Nevertheless, the complete absence of genetic variabity among the 240 samples from 15 islands/islets of the Galápagos archipelago, as well as the lack of variability in the two *Opuntia* species from coastal Ecuador, is notable.

There are several explanations for the lack of genetic variability for these species. The first is sampling error of the loci used to estimate genetic diversity. Due to the difficulty of obtaining metabolically active and scorable allozymes from *Opuntia*, data are available from only eight loci, much less than 20+ loci constituting the basis

Table 1. Allele frequencies at eight loci for *Opuntia* species from coastal Ecuador, Galápagos Islands, and Puerto Rico. Percent polymorphism (%P) and average individual heterozygosity values (H) are listed for each population.

Enzyme	Allele	*O. melanosperma* N. coast Ecuador N=26	*O. macbridei* S. coast Ecuador N=30	*Opuntia sp.* Galápagos N=240	*Opuntia dillenii* Puerto Rico N=5
PGDH	100	1.00	1.00	1.00	1.00
G3PDH	100	1.00	1.00	1.00	1.00
SOD	100	1.00	1.00	1.00	1.00
GTDH	95	-	-	-	1.00
	100	1.00	1.00	1.00	-
G6PDH	100	1.00	1.00	1.00	1.00
MDH	95	-	-	-	1.00
	100	1.00	1.00	1.00	-
PGM	100	1.00	1.00	1.00	1.00
GPI	93	1.00	1.00	-	-
	95	-	-	1.00	-
	98	-	-	-	0.90
	100	-	-	-	0.10
%P		0	0	0	12.5
H		0	0	0	0.025

for most allozymic studies. However, since the percent of loci polymorphic averages 26% across plant species (Nevo 1978), the complete absence of polymorphism is markedly different from the two to three loci expected. The fact that the Puerto Rican population of *Opuntia*, even with a sample size of five, was found to be allozymically variable, and that other cactus species are genetically variable (Parker and Hamrick 1992, Dougherty 1996, Austin *et al.* 1999), suggests that monomorphism documented in this study generally reflects low genetic variability of the Galápagos and coastal Ecuadorian species.

Low genetic variability has been reported for other Galápagos organisms. No heterozygosity and low polymorphism were found in populations of the Galápagos tomato, *Lycopersicon cheesmani* (Rick and Forbes 1975, Rick 1983). Galápagos petrels (*Pterodoma*) also have no detectable allozymic variability among populations occurring on three islands in the Galápagos, although petrels from Galápagos are allozymically distinguishable from those in Hawaii (Browne *et al.* 1997). Limited genetic differentiation has been reported for populations of marine iguanas (Rassman 1996) and tortoises (Marlow and Patton 1981, J. Palmer, pers. comm.) which have pronounced morphological differentiation.

This study may have bearing on the taxonomic status of Galápagos *Opuntia* which is currently based on morphological traits. The allozymic data suggest that minimal genetic differences exist among the six Galápagos *Opuntia* species. However, a number of authors (Wiggins and Focht 1967a, 1967b, Racine and Downhower 1974, Nobel 1981, Hicks and Mauchamp 2000) have suggested that environmental factors such as rainfall, herbivory, and vegetative/sexual reproduction may profoundly shape *Opuntia* morphology, especially height. Nearly eighty years ago, Britten and Rose (1923) noted that arborescent cactus occur throughout the western hemisphere and that wide variation in height occurs within a species. Anderson and Walkington (1971) noted that both arborescent and sprawling individuals of *O. melanosperma* occurred in coastal Ecuador. Furthermore, Arp (1971) made the observation that in the Galápagos *Opuntia* reach the height of surrounding vegetation of each island, suggesting that competition for light (and perhaps moisture) is a major determinant of *Opuntia* growth form. Alternatively, low genetic variability does not necessarily mean that morphological differences are environmentally determined since changes in only a few regulatory genes, which may not be detected in molecular investigations (Turelli *et al.* 2001), could result in potentially dramatic differences in selected morphological traits such as height. The classical approach for investigating genetic and environmental influences on plant growth and morphology is the "common garden experiment" whereby different taxa are raised from seeds in a common environment. However, this is difficult with *Opuntia* due to the length of time required for them to reach maturity.

This study offers preliminary evidence that few genetic differences exist among the *Opuntia* species or

subspecies of the Galápagos, and from a conservation viewpoint, local extinctions would not necessarily lead to loss of unique genes. However, morphological and functional variation among Galápagos *Opuntia* taxa is extensive. Given what appears to be the long-lived nature of the *Opuntia*, the extensive reliance upon them by large components of the coastal zone communities, and the fact that we are still relatively ignorant of the forces that shape *Opuntia* morphology, it would be prudent to maintain as much taxonomic and morphological variability as possible. As we have stressed, the current study, while strongly suggestive of low genetic variability, is based on a relatively small number of loci. Peter Verdyck and Hilde Dhuyvetter (University of Antwerp and RBINS Belgium) and Alan Tye (Charles Darwin Research Station) have conducted DNA sequence analysis of *Opuntia* from a few islands and have detected little variability (P. Verdyck, pers. com.). An expanded study using DNA sequence and microsatellite analyses of the samples we collected should further help resolve the question of how much genetic divergence has occurred in Galápagos.

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Appendix 1. Location, taxonomic designation, and sample sizes for *Opuntia* used in allozymic analyses.

Galápagos Islands

Opuntia echios echios: Daphne, 2; Baltra, 2;
O. e. barringtonensis; Santa Fé, 2;
O. e. gigantea: Santa Cruz, 25;
O. e. zacana; Seymour, 25;

O. helleri; Genovesa, 22;

O. insularis; Isabela, 25;

O. galapageia galapageia; Bartolomé, 25; Santiago , 25;
O. g. macrocarpa; Pinzón, 25;
O. g. profusa; Rábida, 25;

O. megasperma megasperma; Floreana, 2; Gardner (by Floreana), 2;
O. m. mesophytica; San Cristóbal, 25;
O. m. orientalis; Española, 2;

Mainland Ecuador

O. melanosperma; Central Coast 26;
O. macbridei; South Coast, 30;

U.S.A.

O. dillenii; Puerto Rico, 5

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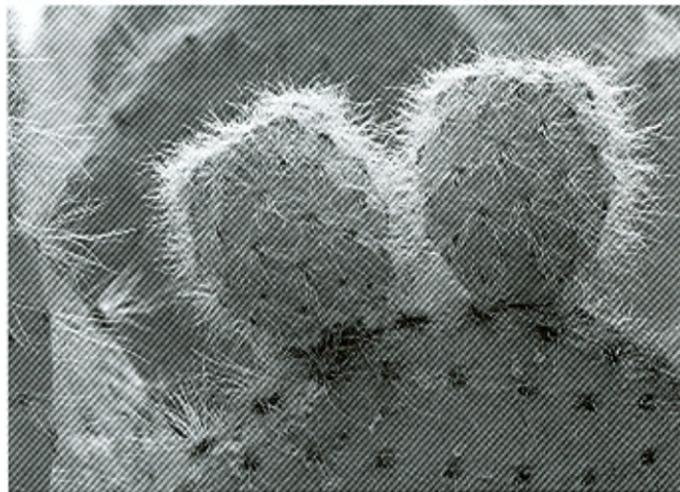
Opuntia megasperma orientalis, Isla Española.