Galápagos’ *Opuntia* (prickly pear) cacti: extensive morphological diversity, low genetic variability

PHILIPPE HELSEN¹*, ROBERT A. BROWNE², DAVID J. ANDERSON², PETER VERDYCK¹ and STEFAN VAN DONGEN¹

¹Evolutionary Ecology Group, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
²Department of Biology, Wake Forest University, Winston-Salem, NC 27109, USA

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Due to the pronounced morphological variation and geographical distribution of Galápagos’ *Opuntia* cacti, numerous hypotheses have been advanced regarding their radiation, diversification, and classification. The currently accepted classification is based on morphology and recognizes six species and fourteen varieties, but the plasticity of many of the characteristics renders any morphological taxonomy problematic. Our analysis of previously published morphological data agrees only partially with the current classification. We present the first molecular phylogeny of these plants. Multiple DNA sequences indicate little genetic distinction among the currently identified species, despite restricted gene flow and limited long distance dispersal within the archipelago. No clear relationship exists between morphological and genetic differences. These results suggest that both molecular and morphological data should be used in conservation planning. © 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 96, 451–461.


INTRODUCTION

Studies on evolution and conservation rely on appropriate estimates of both the phylogeny and the genetic structure of the species of interest. These topics have been studied extensively in the Galápagos Islands (Ecuador), an isolated archipelago exploited as a living laboratory of evolution. Isolated from the South American continent by 1000 km of open ocean, Galápagos comprises thirteen large islands (> 10 km²) and more than 130 smaller islands and rocks (Fig. 1). Island ages range from 0.7–9 Mya along a west–east gradient (Christie et al., 1992). On this archipelago *Opuntia* cacti provide food, shade, water, and nesting places to a variety of organisms and therefore are indicated as keystone species of the arid ecosystem (Hicks & Mauchamp, 1996). Despite their significance from an evolutionary and conservation point of view, their genetic structure and phylogeny is unresolved.

Although not an example of adaptive radiation in the classical sense (Anderson & Walkington, 1971), Galápagos *Opuntia* harbour extensive morphological variability, ranging from shrub-like to 12-m high arborescent plants, from trunkless species to those with trunks up to 1.25 m in diameter, from long bristly spines to short hair-like curly spines. Some of this morphological variation has been attributed to adaptive processes. Treelike forms are thought to have evolved as a response to herbivory by giant tortoises (Stewart, 1911). On islands lacking certain insects, bristly spines may be an adaptation to bird pollination (Grant & Grant, 1979).

To date, taxonomic treatments of Galápagos *Opuntia* have followed a ‘Linnean’ approach, using perceived discontinuities in morphology (Anderson & Walkington, 1971). Six species, further divided into 14 varieties, have been described this way (Fig. 1). Although mainland *Opuntia* species have large

*Corresponding author. E-mail: philippe.helsen@ua.ac.be

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distributional ranges and weak species barriers (Goettsch & Hernandez, 2006), Galápagos species distributions are limited, with every large island having its own taxon (Hicks & Mauchamp, 1996). A drawback to a morphology-based classification, especially in the Cactaceae, is the possible plasticity of morphological characteristics (Nobel, 1983; Gibson & Nobel, 1986; Hicks & Mauchamp, 1996; Hicks & Mauchamp, 2000). In addition, the continuous nature of many of the morphological characters limits their usefulness for classification (Labra et al., 2003). Consequently, workers in the field often prefer to use locality rather than morphology as the major factor for taxonomic identification.

Thus, a clear need exists for a genetic appreciation of the morphology-based taxonomy. Browne et al. (2003), who were the first to study the genetics of these plants, found no variability at eight allozyme loci. Helsen, Verdyck, Tye & Van Dongen (in press) used microsatellite markers to study population genetics and detected high intraspecific variability, but no differentiation between two taxa on one island (Santa Cruz). They also were the first to report the hexaploid nature of these plants. In the present study, we sequenced DNA from four different genomic regions, including the rapidly evolving nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences (Griffith, 2004), the chloroplastic trnT-trnL intergenic spacer (IGS) (Taberlet et al., 1991), and the low copy nuclear genes PepC and PhyC. We combined our genetic data with the previously described morphological data to clarify taxonomic status, determined which characters have high descriptive value, and evaluated concordance between morphology and genetics.

Knowledge on the taxonomic status of Galápagos’ Opuntia species has implications for conservation and management. Due to the introduction of feral animals
and rodents, Galápagos Opuntias are now considered seriously threatened (Tye, 2005). One species is on the red list of the International Union for Conservation of Nature, with a second species considered critically endangered and all other species listed endangered or vulnerable. As a keystone species, threats to the cacti consequently threaten organisms which depend on them. For example, the Floreana mockingbird is thought to have been eliminated from Floreana due to the loss of Opuntia (Grant, Curry & Grant, 2000). An accurate phylogenetic treatment is therefore crucial for an effective conservation management of both Opuntia and the ecosystems in which they are a major component.

MATERIAL AND METHODS

MOdERNAL DATA

Over 75 morphological characters (including plant form, height, trunk, cladodes, flowers, seeds, spines, areoles, glochids, and fruits) described by Anderson & Walkington (1971) were used in a principal coordinate analysis (PCoA) executed in R (Ihaka & Gentleman, 1996). Continuous characters were described as minimum and maximum values, whereas discrete variables were coded numerically. Biplots were used to determine the most important morphological descriptors and to evaluate the position of the different taxa.

GENETIC SAMPLES

From 1995 until 1998, Browne et al. (2003) collected 240 Opuntia tissue samples on 15 different islands and islets (Fig 1), representing 12 of the 14 previously described taxa. Whenever possible, 25 samples were collected per location. Chance of collecting clonal ramets was minimized by sampling along 1000 m transects. Samples were transported as whole pads in paper bags to the Charles Darwin Research Station (CDRS), where four replica samples of approximately 25 cm³ each were cut out and stored at −80 °C. Subsequently, they were sent in a cooled container to the University of Antwerp where they were stored at −20 °C. Samples of two mainland South American Opuntia species were included in the analysis as outgroups. Twenty six specimens of Opuntia dillenii (Madsen, 1989) were collected from a 30 km section of the northern Ecuadorian coast centred on the Puerto Lopez area and 30 Opuntia quitensis (Madsen, 1989) individuals were sampled 1.5 km west of Huayaquil, Ecuador.

Total genomic DNA was isolated (Helsen et al., 2007) and four distinct gene regions were amplified and sequenced. Universal primers were used to amplify the ribosomal nuclear ITS regions (White et al., 1990) and the nuclear gene encoding phosphoenolpyruvate carboxylase (PepC) (Lohmann, 2006). New, specific primers were developed for the amplification of trnT-trnL IGS and PhyC (the exon 1 region of the low copy nuclear photoreceptor gene), namely trnT-Lbf (5′-ACCTTAGCTAGGCTTAGCTATTTAA-3′), trnT-Lbr (5′-TGCTAATCCCTTTGATCTCTG-3′), PhyF (5′-AGCTGGGGTTTCAAATCTT-3′) and PhyR (5′-TCCTCAGTGGACCACCT-3′). Because PepC sequences displayed no variability, they were omitted from further analysis.

The trnT-trnL IGS was amplified in a 30 μl polymerase chain reaction (PCR) mixture containing: 1 × REDTaq Thermophylic Reaction Buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.01% gelatine), 3 mM MgCl₂, 3 μL of template genomic DNA, 1.5 Unit REDTaq DNA polymerase (Sigma-Aldrich), 0.25 μM of each primer, and 0.167 mM of each dNTP. To improve amplification of ITS and PhyC, primer concentrations were changed to 0.11 and 0.17 μM, respectively and dNTP concentration was adjusted to 0.209 mM for PhyC. Amplifications were carried out on a TGradient instrument (Biometa) using cycling protocols as described by Small et al. (1998) (trnT-trnL IGS) and Columbus et al. (1998) (ITS). The amplification protocol for PhyC started with an initial denaturation of 5 min (94 °C), after which 35 cycles were performed each consisting of 1 min of denaturation (94 °C), 90 s of annealing (55 °C), and 2 min of extension (72 °C), and ended with a final extension of 9 min (72 °C).

Purified PCR products (QIAquick PCR purification kit; Qiagen) were redissolved in 20 μL of sterilized water and directly sequenced on a capillary Applied Biosystems 3730 DNA Analyzer using initial primers and ABI PRISM BigDye Terminal cycle sequencing kits. Despite the hexaploid nature of the study species no base ambiguities were found in any of the sequenced PCR products. Consequently, no subcloning steps were undertaken.

SEQUENCE ALIGNMENT AND MODEL SELECTION

Forward and reverse DNA sequences were combined in GeneDoc (http://www.psc.edu/biomed/genedoc) and aligned using ClustalX, version 1.83 (Thompson, Gibson & Plewniak, 1997) with standard gap opening/extension costs (10/0.2). Inconsistencies were visually checked on chromatograms. We tested for the presence of substitution saturation using the test of Xia et al. (2003) implemented in DAMBE, version 4.2.0.13 (Xia & Xie, 2001), and found no indications for saturation. Because genes and gene regions can evolve under different evolutionary models, partition homogeneity tests (Farris et al., 1995) using 200 replicates were run within PAUP 4b10 (Swofford, 2003). Tests indicated that the phylogenetic signals of PhyC and
ITS sequences differ significantly \((P = 0.010)\) and consequently only \(ITS + trnT\) and \(PhyC + trnT\) combined sequences \((P = 0.16\) and \(0.27\) respectively) could be used in further analysis.

**PHYLOGENETIC ANALYSIS**

**Tree-based methods**

To unravel phylogenetic relationships, maximum parsimony (MP), maximum likelihood (ML), Neighbour-joining (NJ), and Bayesian inference methods were used on single loci and combinations of loci which proved to generate nonconflicting phylogenetic signals. Gaps were treated both as missing data and fifth characters. *Opuntia dillenii* and *Opuntia macbridei* were designated outgroups. ML, MP, and NJ methods were run in PAUP* 4.0b10. ML and NJ models and parameters were determined by a hierarchical likelihood ratio test (HLRT) as implemented in Modeltest, version 3.7 (Posada & Crandall, 1998) for individual loci and subsequently for combined sequences (Table 1). The estimated models were used in a ML heuristic tree search with ten random addition sequence replicates, and tree bisection and reconnection (TBR) branch swapping. MP analysis was performed using heuristic searches with TBR branch swapping, stepwise addition starting tree, and random addition sequence using ten replicates.

Branch robustness was estimated by nonparametric bootstrapping (Felsenstein, 1985) with 2000 replicates for MP and 500 replicates for ML and NJ, with a single random addition sequence replicate per bootstrap replicate. Only bootstrap values over 50 are visualized in the resulting trees.

Bayesian analyses, using the Markov Chain Monte Carlo method (MCMC), were performed within MrBayes, version 3.1.1 (Huelsenbeck & Ronquist, 2001). MrModeltest, version 2.2 (Nylander, 2004) determined the best-fit model of DNA sequence (Table 1). Two simultaneous independent searches were run for \(2 \times 10^6\) generations, with trees saved every 1000 generations, and the first \(5 \times 10^6\) sampled trees of each search discarded as ‘burn-in’. Stationary of the Markov chain was determined as the point when sampled log-likelihood scores plotted against generation time reached equilibrium.

**Networks**

Constructing phylogenetic trees for closely-related species, such as the *Opuntia* species under study (Browne et al., 2003), can be misleading or even inappropriate. Micro-evolutionary processes can, for example, produce patterns that are incongruent with the underlying bifurcating pattern of species diversification (Hegarty & Hiscock, 2005). Therefore, we performed nested clade phylogeographical analysis (NCPA), that uses a coalescent-based approach to extract information from haplotype trees to infer the qualitative nature of past population structure and historical demographic events (Templeton, 2007). Here, loci were not pooled, but cross validated to minimize potential problems with lineage sorting and limited introgression (Templeton, 2005). Analyses were run in NCPA, version 1.0 (Panchal & Beaumont, 2007), a platform heavily dependent on TCS, version 1.2.1 (Clement, Posada & Crandall, 2000) and GeoDis software (Posada, Crandall & Templeton, 2000). The haplotype network was estimated using the 95% reconnection limit between haplotypes, although several other limits (between 90% and 99%) were examined to test networks. Clades were tested against the coordinates of their sample locations through a 10^6 permutational contingency analysis (Templeton & Sing, 1993) and the results interpreted using the inference key.

**RESULTS**

**MORPHOLOGY**

In the data analysed by Anderson & Walkington (1971), flower characteristics were documented in

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Table 1. Summary of the sequence characteristics of each partition and the combined variable sequence data

<table>
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<th>Characteristics/data</th>
<th>trnT-trnL</th>
<th>IGS</th>
<th>trnT-trnL</th>
<th>IGS</th>
<th>PhyC</th>
<th>trnT-trnL</th>
<th>IGS</th>
<th>PhyC</th>
<th>All</th>
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<td>HKY + I</td>
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<td>HKY</td>
<td>F81 + I + G</td>
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</table>

Model selection determines the model used for the construction of ML, NJ, and Bayesian trees resulting from (Mr) Modeltest outcomes.

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detailed for most species except for \textit{Opuntia insularis}. Because PCoA is unable to incorporate missing data, we excluded \textit{O. insularis} from our morphological analysis in order to maximize the number of data points. For this smaller dataset, the first three principal components (PCs) described respectively 42.4%, 26.1%, and 16.7% (cumulatively 85.2%) of the total variance. Plots of pairwise combinations of these three first PCs indicated that only PC1 and PC2 were able to distinguish between earlier described species (Fig. 2). PC1, mainly based on fruit, flower, and seed characteristics, shows a clear differentiation between \textit{Opuntia galapageia} varieties and all other taxa. PC2, based on flower, spines, and trunk characteristics, generally separates all groups, with \textit{O. galapageia} placed in the center; whereas \textit{Opuntia echios} and \textit{Opuntia megasperma} have more marginal distributions. However, the varieties \textit{O. echios} var \textit{gigantea} and \textit{O. megasperma} var \textit{mesophytica} do not follow this trend, especially the former, which is situated more closely to the \textit{megasperma} varieties than to the other \textit{echios} taxa.

\section*{Sequence Variation and Phylogenetic Trees}

Phylogenetically informative characters were only found for \textit{trnT-trnL} IGS, \textit{ITS}, and \textit{PhyC} sequences. Sequence information for individual and combined loci containing nonconflicting phylogenetic signals is given in Table 1.

The distribution of haplotypes appeared to be linked to geographical features (Fig. 3). For example, \textit{Opuntias} on satellite islands shared their haplotype with those inhabiting the nearest large island (e.g. Champion and Floreana). Some islands harboured more than one haplotype, but differences between these haplotypes were small. (Statistical tests on the distribution of genetic variance are included in the NCPA results.)

Because phylogenetic signals of individual loci are weak, trees constructed with these data were only weakly supported (i.e. below the 50% bootstrap level). Notably, it was impossible to form a monophyletic ingroup for the \textit{trnT-trnL} IGS dataset using the predefined outgroups. One outgroup, \textit{O. dillenii}, was placed as a sister taxon of the monophyletic group consisting of \textit{O. megasperma} and \textit{O. insularis} species, with the remaining samples more closely affiliated with \textit{O. quitensis}. Although consistent with the ‘two colonization’ scenario (Dawson, 1962), it was more likely the result of the low number of parsimony-informative sites for this locus. Similar problems were not encountered when constructing \textit{ITS} (Fig. 3A) and \textit{PhyC} trees (not shown). For \textit{ITS}, three derived mono-
Phyletic groups were found. One (cluster A3) contained all cacti from Pinzon ($N = 9$), a second (cluster A2) was composed of $O. galapageia$ ($N = 28$) and $O. insularis$ ($N = 9$) individuals, and the last (cluster A1) comprised a mixture of $O. megasperma$ ($N = 19$) and one $O. echios$ individual from Santa Fé, situated at the border of the distribution ranges of $O. echios$ and $O. megasperma$. The phylogenetic tree based on the $PhyC$ sequences (not shown) had only two weakly supported monophyletic groups: one consisting of half of the San Cristóbal cacti; the other of one individual each from North Seymour and Isabela.

Based on the results of the partition homogeneity tests, we also constructed trees for the combined $trnT + ITS$ and $trnT + PhyC$ sequences. As for the individual datasets, the $trnT + PhyC$ combined sequences resulted in a weakly supported tree (Fig. 3B). Because of difficulties regarding ingroup monophyly as described above, $O. dillenii$ was used as the sole outgroup. The resulting tree contained one larger monophyletic group (cluster B1) containing all $O. echios$ and most $O. galapageia$ individuals, wherein cacti from Pinzon formed a smaller derived monophyletic cluster (B2). Another group was only treated as monophyletic when analysed by Bayesian interference, which is probably a result of the overestimating nature of Bayesian posterior probabilities (Simmons, Pickett & Miya, 2004).

**Figure 3.** Consensus tree for the (A) $ITS$ and (B) combined $trnT-trnL$ IGS + $ITS$ datasets. Values above and underneath branches indicate bootstrap support according to maximum parsimony/maximum likelihood/Neighbour-joining clade credibility and Bayesian support respectively. Haplotypes are indicated both as their previously described species name and the islands where they were found. Colour codes for species are identical to those used earlier (black, $Opuntia megasperma$; white, $Opuntia insularis$; dashed, $Opuntia galapageia$; grey, $Opuntia echios$).

**NETWORKS**

TCS analysis for $trnT-trnL$ IGS sequences produced two separated 1-step clades (i.e. separated by one missing haplotype) at the 95% reconnection limit (Fig. 4A). The same network was achieved when this reconnection limit was set from 99% to 90%. The first clade (1-1) contains all individuals from Española, Floreana, San Cristóbal, and Isabela. Notably, individuals from Isabela, one of the youngest islands, clustered with those of the oldest islands. Cacti from Floreana and the surrounding satellite islands formed a unique haplotype (T3) and were represented as descendants from the larger T2 haplotype. The 1-1 clade also unexpectedly included one Santiago individual (T4). The second clade (1-2) consisted exclusively of individuals from the central islands (Santa Cruz, Santa Fé, Pinzon, Santiago and their surrounding smaller islands). Statistically significant values for population structure/historical events were only found for clade 1-1. The analysis indicated that gene flow is restricted among haplotypes, with $D_s$ values for tip clades (0 and 2.326) significantly smaller than that of the internal clade (68.6) ($D_{st} < D_{st}$). On the other hand, $D$ showed the opposite pattern (Table 2a) indicating some gene flow due to long distance dispersal. However, there were too few clades to definitively exclude other processes.

Figure 4. Haplotype network for (A) trnT-trnL IGS and (B) ITS sequences in which full and empty circles represent present and extinct or unsampled haplotypes respectively. Circle sizes are proportional to the number of individuals containing that haplotype and coloured pies represent the relative frequency of the earlier described species. Colour codes are identical to those used earlier (black, *Opuntia megasperma*; white, *Opuntia insularis*; dashed, *Opuntia galapageia*; grey, *Opuntia echios*). Lines connecting haplotypes indicate single mutational differences. Thin and thick lined polygons enclose one-and two-step clades respectively and are designated by ‘1-n’ and ‘2-n’. The first letter of haplotypic labels indicate the sequence they are based on (T = trnT-trnL IGS and I = ITS) and there codes specify the locations where they can be found: T1-A = Sa1 Co Ra Pi Ba; T1-B = Ba Da Cru1-2 SF SN; T2-A = Is; T2-B = Ch Cri1-2-3 Esp Ga Os; T3 = Ch FL; T4 = Sa; I1 = Da Cru2 SF; I2-A = Ba1 Cru1-2 Da SF SN; I2-B = Ch Cri1 Fl; I3-A = SF; I3-B = Cri2-3 Esp Ga Os; I4 = Cri2; I5 = Fl; I6-A = Is; I6-B = Bar Co Ra Sa1-2; I7 = Is; I8 = I9 = Pi.

Table 2. Nested Clade distance analysis of (A) trnT-trnL IGS and (B) ITS haplotypes

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<th>(D_n)</th>
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<th>Clade</th>
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Brackets reflect the nesting structure (Fig. 4). \(D_c\) and \(D_n\) are clade and nested clade distances, respectively. I-T represent the interior versus tip contrast in the corresponding clade. Interior clades are indicated in italic and bold. ’s’ and ’l’ indicate that distance measures are significantly smaller and larger, respectively, than expected under random distribution of haplotypes.

NCPA analysis of the ITS region arranged the nine different haplotypes in six, one-step clusters and two, two-step clusters (Fig. 4B). Stepwise increase of the reconnection limit from 90% to 99% had no effect on this network. Individuals from the younger islands (Isabela and Santiago with their surrounding satellite islands) formed two separated clusters (clade 1-4 and 2-2). Opuntia cacti from Pinzon occupied a more derived position (clade 1-5). Another notable feature is the derived position for some of the Floreana individuals. Opuntia from the older islands (clades: 1-1, 1-2, and 1-3) provided evidence for restricted gene flow, as demonstrated by $D_{c,T} < D_{c,I}$, isolation by distance (clade 1-2), and long distance colonization and/or past fragmentation due to opposite trends in $D_c$ and $D_s$ (Table 2b). The two-step clade 2-1 also showed restricted gene flow ($D_{c,T} < D_{c,I}$) but with some long distance dispersal ($D_s$ opposite trends). Because PhyC haplotypes showed no significant geographic relationship or hints for reticulate evolution, the results for this locus are not presented.

DISCUSSION

The results show that Opuntia species differ morphologically but not genetically. Intraspecific varieties clustered morphologically, except for O. echios var gigantea which occurs on the same island as O. echios var echios. A detailed study on the population genetics of these two varieties using microsatellite markers detected no differentiation (Helsen et al., in press), indicating that these two morphological varieties are not genetically different. The discrepancy between morphology and genetics may be due to: (1) the existence of a small set of unsampled loci determining morphology; (2) the fact that adaptive radiation, a process attributed to these cacti, is characterized by rapid phenotypic evolution with comparatively little genetic variation (Givnish & Sytsma, 1997); (3) character plasticity, which is well documented for several Opuntia species; and (4) the use of continuous morphological characters (Labra et al., 2003). Therefore, caution must be exercised when evaluating only morphological or only genetic data.

Low genetic variability associated with relatively high morphological divergence has been found for a number of other Galápagos organisms. Penguins Spheniscus mendiculus (Bollmer, Vargas & Parker, 2007), storm-petrels Oceanodroma castro (Smith & Friesen, 2007), petrels Pterodroma phaeopygia (Browne et al., 1997; Friesen, Gonzalez & Cruz-Delgado, 2006), hawks Buteo galapagoensis (Bollmer et al., 2006), marine iguanas Amblyrhynchus cristatus (Rassmann et al., 1997), coleopteran beetles (Finston & Peck, 1997), and Darwin’s Geospiza finches (Freeland & Boag, 1999) show the same genetic ‘stability’ as Opuntia, suggesting this may be typical of many Galápagos taxa. This may be due to the remoteness of Galápagos (Finston & Peck, 1997) and its relatively young age (< 9 Myr), hampering colonization resulting in low levels of genetic differentiation. For Opuntia species, the timing of first colonization may even be more recent. Galápagos’ climate shifted during the last million years from hot and wet to dry (Seltzer et al., 2002), making it less likely for cactus species to colonize or survive on these islands earlier than during the last 1 Myr. This scenario is supported by the star like structure of Opuntia gene trees, as would be predicted for haplotypes from young, exponentially growing populations (Sladkin & Hudson, 1991). Opuntia cacti may still be at the start of their allopatric speciation process (Smith & Friesen, 2007), and may not yet have completed reproductive isolation (Coyne & Orr, 2004). The rate of speciation is also positively related to the number of species on an island (Benton & Emerson, 2007). Because Opuntia cacti inhabit the species poor arid zones, they may exhibit slow speciation rates.

The question remains as to why Opuntias didn’t speciate more extensively after their arrival? Our genetic data show restricted gene flow, which is ideal for allopatric speciation, one of the most promising speciation mechanisms on islands. However, we also found indications of some long distance dispersal which counters allopatric speciation. It remains unclear how Opuntias manage to disperse over long distances (e.g. Española and Isabela lie 100 km apart). Indications for such long distance dispersals were found in a maternally inherited locus, which rules out the effect of long distance pollen mediated gene flow (e.g. carpenter bees Xylocopa darwini or Phoebis sennae marcellina; McMullen, 1987). Opuntias most probably spread among islands via seed dispersal by Darwin finches and mocking birds (Petren et al., 2005). However, giant tortoises or land iguanas could also transport seeds over water as well as floating seeds or whole pads could be dispersed among islands. Clonal reproduction (by fallen of pads) is less likely to explain the low differentiation because microsatellite data indicated no clones within a population (Helsen et al., in press).

Speciation can arise from changes at one (Orr, 1991) or very few (Tauber & Tauber, 1977) unsampled loci. Genetic divergence can therefore be underestimated because the loci surveyed are not associated with speciation (Finston & Peck, 1997). Because, at present, we are unable to identify candidate speciation genes, more variable neutral markers such as flanking regions of microsatellite loci (Grimaldi & CrouauRoy, 1997; Chapuis & Estoup, 2007) could define the geographic pattern of diversification better within these Opuntia.
Although intra-individual nrDNA polymorphism generally has been considered to be the exception (Mayol & Rossello, 2001), studies within the Cactaceae report evidence for nonconcerted ITS evolution (Harpke & Peterson, 2006; Ritz et al., 2007). The present observations of single peaks per position within chromatograms provides evidence against this nonconcerted ITS evolution in Cactaceae. However, we believe this finding is more likely to be the result of low genetic variability and/or recent diversification and therefore should not be seen as evidence for or against concerted evolution. The presence of one band per PCR product during gel electrophoresis may, on the other hand, be caused by the absence of pseudogenes within Galápagos Opuntia.

The present study is a first step in a more complete conservation plan for the Galápagos Opuntia species. On Española, for example, the number of adult plants (O. megasperma var. megasperma) decreased drastically over the last century due to herbivory by introduced animals. After the removal of these herbivores, Opuntia failed to regenerate successfully. Ecological studies tried to elucidate why restoration was poor. One study suggested that genetic bottlenecks cause this problem (Tye, 2005), which is in agreement with the low genetic variability found in the present study. Knowing that plants from satellite islands share their haplotype with the neighbouring large island may be of critical use for further conservation planning. Recently, a program was initiated to germinate seeds from Española in the laboratory, aiming to replant them in the field when mature (which is many years for Galápagos Opuntia). Adding seeds from Española’s surrounding satellite islands (e.g. Gardner and Osborne) to this seedling program will not only potentially increase genetic variability for further restoration (which can be tested by microsatellite analyses), but also will act as a crucial common garden experiment testing phenotypic plasticity.

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Non-concerted ITS evolution


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