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Conservation implications of the reproductive biology of the endangered vine *Ipomoea microdactyla* Griseb. (Convolvulaceae)

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

CONSERVATION IMPLICATIONS OF THE REPRODUCTIVE BIOLOGY OF
THE ENDANGERED VINE *IPOMOEA MICRODACTYLA* GRISEB.
(CONVOLVULACEAE)

A dissertation submitted in partial fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

John H. Geiger

2007

To: Interim Dean Mark Szuchman
College of Arts and Sciences

This dissertation, written by John H. Geiger, and entitled Conservation Implications of the Reproductive Biology of the Endangered Vine *Ipomoea microdactyla* Griseb. (Convolvulaceae), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Tim Collins

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Eric Menges

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Date of Defense: November 9, 2007

The dissertation of John H. Geiger is approved.

Interim Dean Mark Szuchman
College of Arts and Sciences

Dean George Walker
University Graduate School

Florida International University, 2007

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DEDICATION

I dedicate this dissertation to my parents, Drs. Ted and Jane Geiger, and my sisters and brother: Carol, Susan, Karen, Ted, and Kathryn. They have been the most significant influences on me personally and have fostered my professional development through their constant support and encouragement. I owe them all a large debt of gratitude for helping me to realize my goals. Thank you, Geiger gang.

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ABSTRACT OF THE DISSERTATION
CONSERVATION IMPLICATIONS OF THE REPRODUCTIVE BIOLOGY OF
THE ENDANGERED VINE *IPOMOEA MICRODACTYLA* GRISEB.

(CONVOLVULACEAE)

by

John H. Geiger

Florida International University, 2007

Miami, Florida

Professor Suzanne Koptur, Major Professor

A plant's reproductive biology exerts a significant influence on both population persistence within changing environments and successful establishment of new populations. However, the interaction between extrinsic (i.e. ecological) and intrinsic (i.e. genetic) factors also is an important driver of demographic performance for plant populations. It is light of this that I performed a multidisciplinary investigation of the breeding system, seed and seedling establishment dynamics, and population genetic structure of the endangered Caribbean vine *Ipomoea microdactyla* Griseb. (Convolvulaceae). The results from the breeding system study show individuals from Florida, USA and Andros Island, Bahamas to be self-incompatible. Plants from the two regions are cross-compatible but there is evidence for outbreeding depression in their progeny. Significant regional differences were found in floral traits and progeny traits that suggests incipient speciation for the Florida populations. The results from the seed and seedling establishment dynamics experiment demonstrate that the restoration of small populations in Florida via seed and seedling augmentation is a successful strategy. The

demographic performance of the outplanted individuals was driven significantly by ecological factors (e.g. herbivory) rather than by genetic factors which emphasizes that the ecological context is very important for successful restoration attempts. The results from the population genetic study using an analysis of molecular variation (AMOVA) reveal significant differences in genetic variation among individuals from Florida, Andros, and Cuba. A Bayesian analysis of population genetic structuring coincided with the previous AMOVA results among the three regions. The Mantel test indicated significant 'isolation by distance' for these regional populations implying restricted gene flow over relatively short distances. Overall, the Florida populations had the lowest measures of genetic diversity which is most likely due to the effects of both colonization founder events and habitat fragmentation. The results of my study highlight the value of performing multidisciplinary studies in relation to species conservation as knowledge of both extrinsic and intrinsic factors can best guide decisions for species preservation.

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CHAPTER 1. REGIONAL COMPARISON OF FLORAL TRAITS, BREEDING SYSTEM, AND PROGENY OF AN ENDANGERED CARIBBEAN VINE

A plant's reproductive biology exerts a significant influence on both population persistence within changing environments, successful establishment of new populations, and colonization of new areas. Island theory proposes that certain mating system characteristics theoretically should favor the chances for successful colonization of new areas (e.g. mainland to island). One of these advantageous properties is the ability to self-pollinate, which ensures reproduction in the absence of animal pollen-vectors and in the case of limitations to outcrossing as a result of small population size (Baker, 1955; Carlquist, 1974). Studies of plant breeding systems on both oceanic (McMullen, 1987; Anderson et al., 2001; Bernardello et al., 2001) and offshore islands (Spears, 1987; Inoue, 1990; Schueller, 2004) have validated this trend of a higher incidence of self-compatibility and self-pollination in island versus mainland floras. Further, insect-pollinated plants found on islands generally have unspecialized, smaller, and less brightly colored flowers than those on the mainland (Carlquist, 1974). Several authors have suggested that the differences between island and mainland flower traits may be due to the often depauperate pollinator guilds occurring on islands (Baker, 1955; Carlquist, 1974).

Two processes have been put forward to explain how the dearth of island pollinators may shape differences between island and mainland floras: 1) by affecting natural selection on established plant populations towards floral traits and mating systems that do not require specialized animal pollen-vectors, and 2) by favoring the colonization of plant species that already have floral traits and mating systems that are independent

from specialized animal pollen-vectors (Spears, 1987). The issue of floral evolution via pollinator-mediated pressure has been reviewed by several authors (Feinsinger, 1983; Waser, 1983), and research in this area has grown greatly (Fenster, 1991; Campbell et al., 1996; Alexandersson and Johnson, 2002; Schueller, 2004; Lobo et al., 2005; Rivera-Marchand and Ackerman, 2006). Differences in island-mainland floras also may be caused by random genetic processes (e.g. genetic drift, bottlenecks from small founder events), rather than by pollinator-mediated natural selection (Eckert and Barrett, 1992; Barrett and Harder, 1996; Barton, 1996).

While island systems have inspired many insights into fundamental biological questions from biodiversity/area relationships (MacArthur and Wilson, 1967) to species evolution (Grant, 1986), few studies have investigated the role of pollinators in shaping floral traits of island versus mainland plants (but see, Inoue, 1990; Kobayashi et al., 1999; Schueller, 2007). One reason offered for the lack of these types of investigations is that island-mainland populations are too divergent due to a long period of isolation to allow accurate intraspecific assessment (Schueller, 2007). To correctly test the hypothesis of pollinator-mediated selection, several researchers have outlined a daunting list of requisite criteria (Robertson and Wyatt, 1990). The criteria include: 1) differences in plant morphology within the species geographic range, 2) differences in the pollinator guild across the plant species' distribution, 3) both the plant and pollinator need to have evolved in the region under investigation, 4) explicit processes through which the pollinators can affect natural selection have to be presented, and 5) the morphological plant traits shaped via the pollinators need to be heritable. Given this stringent list of requirements, it is not surprising that relatively few researchers have chosen to test the

role pollinators may play in molding intraspecific floral trait differences between island and mainland plants.

The Caribbean region, specifically the Bahamian archipelago and extreme southeastern Florida (USA), provides an ideal system to pursue such investigations. The tropical portion of the flora found in extreme southeastern Florida is derived mostly from within the Caribbean region to the south and is a subset of the more diverse flora found in the Bahamian archipelago. The upland habitat in this part of Florida is geologically quite young, being continuously above sea level for ca. 125,000 years (Lidz and Shinn, 1991), and few species of the tropically-derived flora have become endemic to this area over this time period (Snyder et al., 1990). The nearby islands of the Bahamas (e.g. Andros, Grand Bahamas, and Abaco islands) have a longer geological history, with areas continuously above sea level throughout the Pleistocene (Haq et al., 1987). The Bahamian archipelago is home to many endemic plant species (Correll and Correll, 1982). These two regions host different pollinator guilds, especially vertebrates. Hummingbird pollinators are represented by a single migratory species in extreme southeastern Florida, while Andros Island (Bahamas) has two resident hummingbird species. *Ipomoea microdactyla* is a plant species native to these regions with showy magenta-red flowers that are pollinated by all three hummingbird species. Also, there are both floral and vegetative character differences in plants from these two regions that have a genetic basis.

It is in this context that I performed an intraspecific study to compare the reproductive traits of *Ipomoea microdactyla* individuals from populations in Miami-Dade County, Florida and Andros Island, Bahamas. I specifically addressed the following

questions: 1) Are there regional differences in floral traits?, 2) Are there regional differences in breeding system?, 3) Are there regional differences in cross-compatibility?, and 4) Are there regional differences in progeny traits?

In a novel reversal of the island-mainland paradigm, I chose to recognize extreme southeastern Florida as the ‘island’ and Andros as the ‘mainland’. Extreme southeastern Florida has been likened to an island surrounded by water on three sides with frost on the north (Tomlinson, 1980; Kress and Horvitz, 2005), and has been included as part of the Caribbean region biodiversity ‘hotspot’ (Myers et al., 2000). Historically, and even more so now because of severe habitat loss, Miami-Dade County harbors a smaller area of suitable habitat for *Ipomoea microdactyla*. Also, the habitat is geologically younger (Lidz and Shinn, 1991).

MATERIALS AND METHODS

STUDY ORGANISM

Ipomoea microdactyla Griseb. has a Caribbean distribution and is found throughout Cuba, across the Bahamian archipelago and only in Miami-Dade County, Florida within the USA. *I. microdactyla* occurs primarily in pine forests growing on limestone rock across its range. The plant is abundant in the continuous pine forest on Andros Island in the Bahamas, which is the largest continuous expanse of pine forest in the Caribbean Basin. Outside the natural habitat preserved in Everglades National Park, nearly 99% of the pine forests in Miami-Dade County have been destroyed in the last 120 years. *I. microdactyla* is infrequent in the highly fragmented pine rockland habitat of Miami-Dade County, Florida where it is state-listed as endangered (Coile and Garland,

2002). Population size at most Miami-Dade County fragments is less than 50 individuals (Geiger, 2004).

Ipomoea microdactyla is a perennial vine with twining woody stems, rarely over 10 m in length. Plants are long-lived and have underground tubers much like the congeneric sweet potato (*Ipomoea batatas*). Leaves of Florida plants are described as entire to lobed (Wunderlin, 1998), while those of Bahamas plants can be entire to palmately compound (Correll and Correll, 1982). Individuals produce several to hundreds of hermaphroditic magenta-red flowers in discrete bouts during the blooming season from May to December. Flowers have a single pistil topped by a capitate stigma and five anthers. The anthers and stigma are exerted beyond the flower face with most anthers slightly below the level of the stigma. Flowers last for one day only, after which the corollas abscise. The funnel-shaped flowers are open to visitors from early morning to early/late afternoon and are visited by a diverse group of insects including Hymenoptera and Lepidoptera, but the predominant flower visitors are hummingbirds (Trochiliformes). The capsular fruit contain a maximum of 4 seeds which are covered with ca. 1 cm long copper colored hairs.

PLANT PROVENANCE

To make regional comparisons, I collected individuals from Miami-Dade County, Florida, and from Andros Island, the Bahamas. Florida individuals were propagated from stem-tip cuttings of plants from four sites, and Bahamas individuals were grown from seeds of plants from four sites (Table 1.1). Sampling was conducted on populations covering the extent of *Ipomoea microdactyla*'s range in both areas. I sampled from 10%–30% of all individuals at each of the four discrete (i.e. isolated, habitat fragments)

Florida populations. Sampling intensity was lower on Andros due to the higher densities of individuals and to the continuous nature of the habitat.

FLORAL TRAITS

To gauge differences in floral traits between the two regions, I took measures on flower opening/closing times, and percent sugar concentration of nectar. Flower opening/closing times were determined over two weeks on ten Florida and ten Bahamas individuals planted in a common garden. Buds were marked with numbered jeweler's tags the day before measures were made. Observations began at 0500 and flowers were scored as fully open/fully closed in discrete 15 minute time intervals (e.g. 0530, 0545, and 0600) as both anthesis and closure occurred over this time frame. Opening/closing times were pooled across days and among individuals between the two regions for statistical analysis.

I measured the pollinator's reward by quantifying the sugar concentration of nectar. Sugar concentrations were determined over two weeks on ten Florida and ten Bahamas individuals planted in a common garden. Flowers were covered in fine mesh cloth bags to exclude floral visitors the day before samples were collected. Cloth bags were removed during the hand-pollination protocol and then flowers were covered once again with the cloth bags. Cloth bags were removed permanently the next day. Using a glass microcapillary tube (i.e. micropipette), I withdrew nectar from the base of the corolla and assessed sugar concentration in percent sucrose equivalents with a Bellingham® hand-held refractometer. Data are therefore one-time samples per flower. Sugar concentrations were pooled across days and among individuals between the two regions for statistical analysis.

BREEDING SYSTEM

I performed hand pollination experiments (*sensu* Kearns and Inouye, 1993) to investigate regional differences in the breeding system of *Ipomoea microdactyla* on plants both in the greenhouse and in a common garden using three flower treatments: autogamy (autonomous self-pollination), self-pollination, and cross-pollination (Table 1.2). Bagging of flowers within fine mesh cloth was unnecessary in the insect-free greenhouse experiment as there were no pollinators present. The greenhouse experiment was performed only with Florida individuals. For the common garden experiment with both Florida and Bahamas plants, it was necessary to enclose buds in fine mesh cloth bags the day prior to flower treatments to preclude interference from floral visitors. Bags remained on flowers until dusk when they were removed. I began hand pollinations at 0900 when most flowers had fully opened. The autogamy treatment involved no hand manipulation. The self-pollination treatment involved the complete coating of the stigma with pollen from a single anther removed from the same flower. The cross-pollination treatment was as per the self-pollination treatment; except that the anther used was taken from a separate, single pollen-donor individual (i.e. all seeds from a cross-pollination treatment fruit had a known, single sire). For the cross-pollination treatments, there were four possible mating-pair combinations: Florida dam/Florida sire, Florida dam/Bahamas sire, Bahamas dam/Florida sire, and Bahamas dam/Bahamas sire.

Anthers used for the self- and cross-pollination treatments were excised with fine forceps that were cleaned thoroughly with alcohol-saturated wipes and air-dried after each use. A small numbered jeweler's tag was attached to the pedicel of each flower which detailed the treatment date, flower number, identity of dam/sire, and treatment. To

minimize the risk of fruit abortion due to maternal plant resource limitation rather than cross-incompatibility, less than 20% of the open flowers on a plant were chosen each day for manipulation. Most plants produced tens to hundreds of flowers during a typical month for both the greenhouse and common garden experiments (Figure 1.1). Also, the number of fruits that experimental plants matured was equivalent to observations of natural fruit production (J. Geiger, unpublished data). Unsuccessful pollinations resulted in the abscission of the pedicel within two weeks, while for successful pollinations, fruit development took up to two months. Fruit were removed from plants when the capsule changed from green to brown, but before they had dehisced. Seeds from each fruit were extracted from the capsules, counted, and stored in small coin envelopes (identified with the specific flower number) in an air-conditioned laboratory for future germination trials. The greenhouse experiment on Florida plants was conducted from January-December 2003 and the common garden experiment on Florida and Bahamas plants from October-January 2006.

PROGENY TRAITS

To explore regional differences in progeny traits, a random subsample of the seeds generated from the breeding system experiments was chosen for further study. A total of 200 seeds, 50 from each regional mating group (i.e. Florida/Florida, Florida/Bahamas, Bahamas/Florida, and Bahamas/Bahamas), were individually weighed on a Mettler balance. Seeds were nicked and planted in 50-cell pack planting trays using Metro-Mix® soilless potting mix in the greenhouse. Germination was assessed when cotyledons emerged from the soil surface. The color of the cotyledons/leaves and the degree of leaf-lobing were recorded for all seedlings. To test for differences in seed

weight and germination among the four mating-pair groups, individuals were pooled within each of the four groups.

STATISTICAL ANALYSIS

I used t-tests to gauge differences between Florida and Bahamas plants for the variables: flower opening/closing time and sugar concentration of nectar. Chi-square tests were utilized to assess the differences in fruit set (i.e. percentage of flowers developing into fruits) between regions and among mating-pair groups. Data on fruit and seed set for the individual plants were pooled by flower treatment, region, and mating-pair groups because the unequal number of flower treatments per plant prevented statistical testing on a per individual basis. ANOVA was used to gauge differences in seed set (i.e. number of seeds per fruit) for the mating-pair groups. Data on seed set violated the assumption of equality of variances (Levene's test, $F_{3, 2759} = 4.08$, $P = 0.007$), therefore, the results were validated using equivalent nonparametric tests (Kruskal-Wallis and Mann-Whitney U). Mann-Whitney U tests also were used to evaluate differences in seed set for the autogamy and self-pollination treatments between Florida and Bahamas plants. Chi-square tests were utilized to assess the differences in cross-compatibility (percentage compatible mating pairs for the three mating classes) among three mating-pair groups (i.e. Florida/Florida, Florida/Bahamas plus Bahamas/Florida, and Bahamas/Bahamas). ANOVA was used to test for differences in seed weight among the regional mating-pair groups (i.e. Florida/Florida, Florida/Bahamas, Bahamas/Florida, and Bahamas/Bahamas). I employed chi-square tests to gauge differences in percentage seed germination among the regional mating-pair groups. Statistical tests were performed with SPSS 15.0 (SPSS, Chicago, Illinois, USA).

RESULTS

FLORAL TRAITS

There were significant differences in flower opening times between Florida and Bahamas plants (Table 1.3). Flowers on Florida plants opened substantially later than those on Bahamas plants (Table 1.3, $P < 0.001$), but there was overlap for opening times (Figure 1.2). Flowers on Florida plants closed significantly later than those on Bahamas plants ($P < 0.001$), and there was no overlap for closing times (Figure 1.3). All Bahamas flowers were fully closed by 1315, while Florida flowers began closing at 1415, and all were fully closed by 1700.

There were significant differences in the sugar concentration of nectar for Florida and Bahamas plants (Figure 1.4). The Bahamas flowers had significantly higher sugar concentrations than Florida flowers, 27.9% and 23.9%, respectively.

FRUIT SET

Fruit set percentages for the three flower treatments ranged from 0.0% – 71.3 % among the four regional mating groups (Table 1.4). Very few of the autogamy treatments produced fruit in the greenhouse experiment, and they occurred at the start of the greenhouse experiment when techniques were being optimized. I believe these early cases of autogamous fruit set may have been a result of inadvertent self-pollination. Fruit set from the self-pollination treatment was not significantly different for Florida and Bahamas plants (Pearson $\chi^2 = 2.073$, $df = 1$, $P = 0.150$). Over all, most plants showed no evidence of self-compatibility, while a small proportion showed low levels of self-compatibility (Figure 1.5). Fruit set percentages for the cross-pollination treatments among the four regional mating groups ranged from 58% – 71.3% (Figure 1.6, Table

1.4), and there were significant differences in fruit set among the four regional mating groups (Table 1.5).

SEED SET

Seed set for the three flower treatments ranged from 0.0 – 3.28 seeds per fruit among the four regional mating groups (Table 1.4). As mentioned above, very few autogamy treatments produced fruit in the greenhouse experiment, and seed set was not significantly different from the self-pollination treatment (Mann-Whitney U test, $Z = -0.711$, $P = 0.477$). Seed set from the self-pollination treatment was not significantly different for Florida and Bahamas plants (Mann-Whitney U test, $Z = -0.471$, $P = 0.637$). There were significant differences in seed set among the cross-pollinations for the regional mating groups (Table 1.5). Seed set was significantly greater for the Bahamas/Bahamas crosses than for the Florida/Florida group, and the crosses between regions (Florida/ Bahamas and Bahamas/ Florida groups) were not significantly different from each other or the other two groups (Figure 1.7).

CROSS-COMPATIBILITY

The proportions of mating-pairs for the three mating classes were significantly different among the three regional mating groups (Pearson $\chi^2 = 16.816$, $df = 4$, $P = 0.002$). For the three mating classes, the range of proportions was: 1) bilaterally compatible, 63.8% – 85.7%, 2) unilaterally compatible, 9.4% – 29.0%, and 3) bilaterally incompatible, 5.7% – 6.8% (Figure 1.8, Table 1.6). There were significant differences in the proportions of compatible mating pairs between the Florida/Florida and Florida/Bahamas regional mating groups (Pearson $\chi^2 = 16.031$, $df = 2$, $P < 0.001$). The proportions of compatible mating pairs for the Bahamas/Bahamas regional mating group

did not differ significantly from either the Florida/Florida (Pearson $\chi^2 = 2.254$, $df = 2$, $P = 0.324$), or the Florida/Bahamas (Pearson $\chi^2 = 3.514$, $df = 2$, $P = 0.173$) regional mating groups.

PROGENY TRAITS

Seed weights differed substantially among the four regional mating groups (Table 1.7), and post-hoc tests indicated that all four groups were significantly different from one another (Figure 1.9). The correlation between seed weight and the number of seeds per fruit was not significant (Pearson's $R = -0.108$, $N = 200$, $P = 0.188$). There were significant differences in seed germination proportions among the four regional mating groups (Table 1.7). Seed germination percentages ranged from 82% – 100% (Figure 1.10). For pair-wise comparisons between regional mating groups, the Florida/Bahamas group had significantly lower seed germination than the Florida/Florida (Pearson $\chi^2 = 5.005$, $df = 1$, $P = 0.025$), and Bahamas/Bahamas (Pearson $\chi^2 = 9.890$, $df = 1$, $P = 0.002$) groups. Also, the Bahamas/Florida group had significantly lower seed germination than the Bahamas/Bahamas group (Pearson $\chi^2 = 6.383$, $df = 1$, $P = 0.012$).

All seedlings from the Bahamas/Bahamas mating group had purple-red cotyledons and true leaves. The degree of leaf lobing was from simple entire to deeply divided five-lobed leaves; there were no instances of palmately compound leaves. Most individuals in the Bahamas/Bahamas group produced leaves of varying morphology at each node. Seedlings from the remaining three regional mating groups (i.e. Florida/Florida, Florida/Bahamas, and Bahamas/Florida) all had green cotyledons and true leaves. Only three of these individuals from the Bahamas/Florida group had leaves with a single shallow lobe, while the remainder all possessed simple entire leaves.

DISCUSSION

FLORAL TRAITS

The genus *Ipomoea* exhibits a wide variety of flower types that are pollinated by a diverse group of animal pollinators. Many studies have explored aspects of the mating system for these species (Schoen and Clegg, 1985; Rausher et al., 1993; Chang and Rausher, 1998; Hull-Sanders et al., 2005; Wolfe and Sowell, 2006). Overall, my results for sugar concentration of nectar and flower opening/closing times are similar to published articles for other *Ipomoea* species. Wolfe and Sowell (2006) reported sugar nectar concentrations for two red-flowered, hummingbird pollinated species of 25% and 26%, which is within the range of values for *I. microdactyla* (24% and 28%). Flower opening/closing times for *I. microdactyla* were similar to those of *I. quamoclit* in their study. While there were statistically significant differences in flower opening times between the two regions, a difference of 30 minutes at this time of day may not be biologically significant in terms of pollinator attraction and reproductive fitness. In subtropical areas close to the equator, sunset does not vary more than an hour over the year. However, the significant difference in flower closing times between the two regions may have important biological effects. These results demonstrate a genetic basis, rather than phenotypic/environmental variation, as the experiment was performed in a common garden. Indeed, flowers on Florida plants stayed open nearly four hours longer than those on Bahamas plants. This shift increases the time that flowers are open to pollinator visitation and possibly, increases reproductive success. In light of the lower diversity of hummingbird pollinators in Florida, this change may be adaptive by allowing greater chances for reproduction in a depauperate pollinator environment.

REGIONAL BREEDING SYSTEM

Ipomoea microdactyla individuals from both regions are self-incompatible (*sensu*, Dafni, 1992), as less than 3% of self-pollinated flowers set fruit. Published studies have found that species within the genus *Ipomoea* fall roughly into two clades: one group is self-compatible, and the other is self-incompatible (Austin and Huaman, 1996; Diaz et al., 1996). *I. microdactyla* clearly requires animal-pollen vectors as less than 1% of the autogamy flower treatments set fruit.

There was no significant difference in fruit set or seed set for self-pollination treatments between the Florida and Bahamas individuals. However, the percentage of Florida plants that showed some degree of self-compatibility was greater than that of Bahamas plants, 33% and 10% respectively. This may represent a historical genetic signature from small founder events in the Florida populations or may be due to natural selection for those individuals capable of some degree of self-compatibility. Seeds produced from the self-pollination treatments showed no significant differences in early fitness traits (i.e. seed weight, seed germination, and seedling survival), but inbreeding depression effects could be manifested in later life fitness measures (Barrett and Harder, 1996).

For the cross-pollination treatments, there were significant differences in both fruit and seed set among the regional mating groups. These results may be explained through effects of genetic variation, as the experimental design minimized environmental effects. Florida individuals have significantly lower levels of genetic diversity compared to Bahamas individuals (J. Geiger, unpublished data). For fruit set percentage, the Florida/Bahamas regional group was the highest and the Bahamas/Florida group was the

lowest, 71% and 58% respectively. This pattern could arise from the higher quality of Bahamas pollen compared to Florida pollen via heterotic effects on fruit set. The results for seed set are in accord with this hypothesis as Florida/Florida seed set was significantly lower than Bahamas/Bahamas seed set, while the other two regional mating groups were not significantly different from one another or the single region groups.

REGIONAL CROSS-COMPATIBILITY

Evidence reveals that the degree of cross-compatibility is lower for inter-region mating groups than for intra-region mating groups. This appears to have a genetic basis as the common garden experiment lessened the chances for environmental effects. The results are somewhat unexpected as plants with this type of self-incompatibility system (i.e. multiallelic, sporophytic) generally show a higher degree of compatibility among geographically isolated populations (Castric and Vekemans, 2004). One explanation may be that Florida and Bahamas populations are diverging to the point that barriers to reproduction are now present.

INCIPIENT SPECIATION?

The strongest genetic evidence of divergence between Florida and Bahamas individuals was expressed in leaf color and morphology. Recent reviews have shown that often there is decoupling of floral and vegetative traits, so that each component is free to vary independently (Ashman and Majetic, 2006). While floral traits proved to be significantly different, the striking regional color difference in the leaves is especially important. Taxonomists working in this group have stated that cotyledon color and morphology are excellent characters to use in species delimitation as they are highly informative and conservative (Sampathkumar, 1982; Das and Mukherjee, 1997;

Ogunwenmo, 2003). Given this, it suggests that Florida populations may be in the process of incipient speciation, especially as the distance between regions is most likely a significant barrier to gene flow (i.e. via pollen) from nearby islands of the Bahamas. The ‘target’ of gene flow also has been severely reduced in Florida due to the loss of ca. 98% of the suitable habitat for *Ipomoea microdactyla* in the last 120 years. Future work on the fitness of the inter-regional progeny grown under field conditions in Florida may offer additional insight into the prognosis of incipient speciation for this species.

Table 1.1. Site provenances for experimental plants of *Ipomoea microdactyla* and sample sizes.

Site	Region	Latitude ^a	Longitude ^a	Sample size
1. Bill Sadowski	Florida	25.518194	80.501056	4
2. Campbell Dr.	Florida	25.475778	80.437444	9
3. Larry and Penny	Florida	25.631750	80.322278	6
4. Turnpike 152	Florida	25.622528	80.378250	2
5. Blanket Sound	Bahamas	24.752944	77.841389	5
6. Forfar Station	Bahamas	24.893861	77.932083	6
7. Stafford Creek	Bahamas	24.910056	77.985278	5
8. Thrinax Ridge	Bahamas	24.928833	78.026389	5

^aCoordinates in decimal degrees.

Table 1.2. Protocol for hand pollination experiments of *Ipomoea microdactyla* utilized to determine the breeding system, including the number of flowers and plants per treatment. Summaries given for separate greenhouse and field experiments.

Flower treatment	Greenhouse experiment		
	Bagging	Hand pollination	No. flower/no plant
Autogamy	No	No	316 / 21
Self-pollination	No	Yes	331 / 21
Cross-pollination	No	Yes	3574 / 21

Flower treatment	Field experiment		
	Bagging	Hand pollination	No. flower/no plant
Autogamy	Yes	No	105 / 21
Self-pollination	Yes	Yes	213 / 21
Cross-pollination	Yes	Yes	1040 / 21

Table 1.3. Results of t-tests performed on flower opening and closing for *Ipomoea microdactyla* plants from Florida and Andros Island.

Variable	Florida mean	Andros mean	t-statistic	P value
Flower opening time	0656	0622	- 3.828	< 0.001
Flower closing time	1529	1136	- 23.518	< 0.001

Table 1.4. Summary of regional hand pollination experiments for *Ipomoea microdactyla* in terms of fruit and seed set. Note: maximum number of seeds/fruit = 4).

Regional mating group ^a		Flower treatment (fruit set percentage) ^b		
Female	Male	Autogamy	Self-pollination	Cross-pollination
Florida	Florida	0.9	3.0	61.9
Bahamas	Bahamas	0.0	0.9	68.1
Florida	Bahamas			71.3
Bahamas	Florida			58.0

Regional mating group ^a		Flower treatment (mean no. seeds per fruit) ^b		
Female	Male	Autogamy	Self-pollination	Cross-pollination
Florida	Florida	1.33	1.22	3.02
Bahamas	Bahamas	0.00	1.00	3.28
Florida	Bahamas			3.05
Bahamas	Florida			3.11

^a Female/male designation relevant for cross-pollination treatment.

^b Only cross-pollination treatment performed for regional mating groups Florida/Bahamas and Bahamas/Florida.

Table 1.5. Results of statistical testing among regional mating groups for percentage fruit set and number of seeds per fruit from cross-pollinations.

Variable	Regional mating group ^a				Test statistic	P value
	Fl / Fl	Bh / Bh	Fl / Bh	Bh / Fl		
Fruit set percentage	61.9	68.1	71.3	58.0	$\chi^2 = 18.730$	$P < 0.001$
Mean number seeds per fruit	3.02	3.28	3.05	3.11	$F = 4.974$	$P = 0.002$

^a Regional mating groups denote 'female/male' pairings where Fl = Florida, and Bh = Bahamas.

Table 1.6. Regional summary for the three mating classes from the hand pollinations of *Ipomoea microdactyla*.

Regional mating group ^a (female / male)	Mating classes ^b		
	Bilaterally compatible	Unilaterally compatible	Bilaterally incompatible
Florida / Florida	161 (83.8%)	18 (9.4%)	13 (6.8%)
Florida / Bahamas	44 (63.8%)	20 (29.0%)	5 (6.8%)
Bahamas / Bahamas	30 (85.7%)	6 (11.4%)	1 (5.7%)

^a Fl/Bh and Bh/Fl regional mating groups were pooled.

^b Counts of the number of mating pairs per mating class are presented along with percentage values in parentheses.

Table 1.7. Results of statistical tests among regional hand pollinations of *Ipomoea microdactyla* for seed weight and germination.

Variable	Regional mating group (female / male)				Test statistic	P value
	Fl / Fl	Fl / Bh	Bh / Fl	Bh / Bh		
Mean seed weight (g)	0.0117	0.0132	0.0148	0.0167	F = 32.8	P < 0.001
Germination percent	96	82	88	100	$\chi^2 = 12.7$	P = 0.005

Figure 1.1. One-month sum of open flowers on 21 Florida individuals of *Ipomoea microdactyla* used in the greenhouse breeding system experiment.

Figure 1.2. Regional comparison of flower opening times for *Ipomoea microdactyla*.

Figure 1.3. Regional comparison of flower closing times for *Ipomoea microdactyla*.

Figure 1.4. Regional comparison of percent sugar concentration (± 1 SE) of flower nectar for *Ipomoea microdactyla*. Note that origin begins above zero. Significant statistical differences ($P < 0.05$) between regions denoted by differing letters. Sample sizes were 50 flowers per group.

Figure 1.5. Comparison of fruit set for self-pollinations of *Ipomoea microdactyla* individuals from a) Florida and b) Bahamas.

Figure 1.6. Percentage fruit set for hand pollinations of *Ipomoea microdactyla* by regional mating group. Groups denote regional 'female/male' pairings where FI = Florida, and Bh = Bahamas.

Figure 1.7. Mean seed set per fruit (± 1 SE) for hand pollinations of *Ipomoea microdactyla* by regional mating group. Note that origin begins above zero and groups denote regional 'female/male' pairings where FI = Florida, and Bh = Bahamas. Significant statistical differences ($P < 0.05$) among groups indicated by differing letters.

Figure 1.8. Regional summary of the counts of mating pairs within the three mating classes. Mating class symbols denote: ++ = bilaterally cross-compatible, + - / - + = unilaterally cross-compatible, and -- = bilaterally cross-incompatible.

Figure 1.9. Mean seed weight (g) $\pm$ 1 SE for hand pollinations of *Ipomoea microdactyla* by regional mating group. Note that origin begins above zero and groups denote regional 'female/male' pairings where FI = Florida, and Bh = Bahamas. Significant statistical differences ($P < 0.05$) among groups denoted by differing letters.

Figure 1.10. Germination percentage of *Ipomoea microdactyla* seeds by regional mating group. Groups denote regional 'female/male' pairings where FI = Florida, and Bh = Bahamas.

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CHAPTER 2. EXPERIMENTAL SEED AND SEEDLING PERFORMANCE OF THE ENDANGERED VINE *IPOMOEA MICRODACTYLA* GRISEB. (CONVOLVULACEAE)

Habitat loss, fragmentation and degradation are the main threats to global plant species persistence (Saunders et al., 1991). These processes lead to decreased population sizes and increased isolation of remaining populations. As populations become smaller they are at greater risk of genetic, demographic and environmental stochasticity potentially leading to extinction (Lande, 1988; Newman and Pilson, 1997; Oostermeijer et al., 2003). Negative genetic consequences include lowered genetic variation through inbreeding and loss of alleles through genetic drift (Ellstrand and Elam, 1993; Young et al., 1996; Dudash and Fenster, 2001). Demographic stochasticity detrimentally effects small populations ($N < 50$) by increasing the variability of vital rates (e.g. fecundity) separately applied to each individual (Menges, 1992; Lande, 1993). The interaction between demographic stochasticity and genetic stochasticity can be especially severe for self-incompatible plants in fragmented habitats, leading to inbreeding and lowered reproduction or complete reproductive failure (Demauro, 1993; Luijten et al., 2000; Young et al., 2000; Brys et al., 2004). Environmental stochasticity causes temporal variation in vital rates due to environmental changes such as fire frequency, hurricanes, pollinator availability and herbivory (Menges, 2000). There is growing empirical evidence herbivory has a significant effect on establishment, growth and persistence of plant populations (Parmesan, 2000; Groom, 2001; Ehrlén, 2003; Kauffman and Maron, 2006; Lopez and Terborgh, 2007).

Outside of habitat protection, conservation biologists have developed tools to combat the extinction of threatened species. Increasingly they are using reintroductions

as a means of preserving populations (Maunder, 1992; see review, Seddon et al., 2007). These authors suggest the best reintroduction protocol is an experimental multidisciplinary approach. However, few studies incorporate explicit ecological/environmental criteria in their design and fewer include genetic issues regarding selection of plant material for reintroduction (Husband and Campbell, 2004; but see, Vergeer et al., 2004; Lofflin and Kephart, 2005). The choice of outplanting material from remaining populations may introduce lingering genetic consequences of inbreeding or outbreeding depression. Genetic considerations are vitally important as the majority of species in need of management occur as isolated small populations. These populations are prone to inbreeding depression (i.e. production of less fit offspring by selfing or matings between close relatives) which can increase extinction risk (Newman and Pilson, 1997; Luijten et al., 2002). Gene flow between inbred populations can restore fitness through heterosis (Ingvarsson, 2001; Tallmon et al., 2004). Conversely, gene exchange between populations adapted to different environmental conditions can lead to the production of less fit offspring due to the breakdown of co-evolved gene complexes or outbreeding depression (Lynch, 1991; Waser and Price, 1994).

While genetic concerns are important for the long term persistence of small isolated populations, ecological site conditions such as pollinator limitation, herbivory, fire suppression, and nitrogen enrichment have been shown to negatively influence demographic performance in the short term for natural plant populations (Groom, 1998; Helenurm, 1998; Menges and Dolan, 1998; Brys et al., 2005). Nonetheless, very few reintroduction studies have explored the effect of environmental variation by following plants at more than one outplanting site or in different habitats (but see, Helenurm, 1998;

Maschinski et al., 2004; Maschinski and Duquesnel, 2006). Threatened plants occurring in the fragmented pine rockland habitat of extreme southeastern Florida (USA) offer excellent models for studying the interaction of genetic and environmental effects on the demographic performance of reintroduced versus natural populations. *Ipomoea microdactyla* is a long-lived, self-incompatible perennial vine which in the USA is found only in the fire-maintained pine rockland habitat of Miami-Dade County, Florida. Of the 46 habitat fragment populations only 15% have greater than 50 individuals and 65% contain less than 25 which is the theoretical population threshold to contain a sufficient number of compatible mating types for self-incompatible plant species (Byers and Meagher, 1992). Management of pine rockland habitat includes prescribed fires and clearing of both native and exotic invasive plant species but most sites receive little management.

To explore the suitability of reintroduction as a tool for species restoration of *I. microdactyla* I performed a seed and seedling outplanting experiment. I intentionally addressed the following questions: (1) Are there performance differences via genetic mechanisms for experimentally reintroduced seeds and seedlings derived from small vs. large populations and from within-site vs. between-site cross-pollinations? In other words I was investigating heterosis and outbreeding depression with these contrasts. I had seed from a previous breeding system study to explore these questions in detail. (2) Are there fitness differences operating through environmental site variation for experimentally reintroduced seeds and seedlings in terms of growth, survival, and reproduction? I chose five experimental outplanting sites, covering a range of biotic and abiotic factors to realistically gauge responses of the replicated seed and seedling

outplanting experiments. (3) Are there fitness estimate differences (i.e. maladaptation) between experimentally reintroduced and natural populations of *I. microdactyla*? (4) Is reintroduction a successful strategy for restoring fragmented *I. microdactyla* populations, and, by extension, other threatened plant populations as well? I recorded seed germination, seedling establishment, characters shaping plant performance, and plant status/herbivory in experimental vs. natural populations. To compare variation in plant fitness in natural populations versus reintroduced populations I used a modification of the maladaptation testing procedure (sensu Gomulkiewicz and Holt, 1995; Husband and Campbell, 2004; Lofflin and Kephart, 2005). This allowed me to explore the degree to which unique site conditions affected the success or failure of my reintroduction attempt and how other plant reintroductions may benefit as well.

MATERIALS AND METHODS

STUDY TAXON AND NATURAL/EXPERIMENTAL POPULATIONS

I studied the seed germination and seedling establishment dynamics of the vine *Ipomoea microdactyla* Griseb. (Convolvulaceae) at six sites in Miami-Dade County, Florida, USA (Table 2.1). *I. microdactyla* plants are hermaphroditic with showy scarlet flowers; they are self-incompatible and require pollen vectors (e.g. bees, butterflies, hummingbirds) to produce fruit and seeds (J Geiger, unpublished data). Individuals are long-lived and have underground storage organs much like the congeneric sweetpotato (*Ipomoea batatas*). In the US, this species is found only in the highly fragmented pine rockland habitat of Miami-Dade County, Florida and is state listed endangered (Coile and Garland, 2002); the species also occurs in Cuba and the Bahamas. Outside of the intact pine rockland within Everglades National Park, there presently exists less than 2 % of the

original habitat in Miami-Dade County (Bradley, 2005). *I. microdactyla* is known to occur in 46 of the remaining ca. 400 habitat fragments as well as in Everglades National Park (Gann et al., 2002). Most fragments (ca. 95 %) are less than ten ha in area with few greater than 20 ha; these isolated remnants are embedded within the urban, suburban, agricultural and wildland matrix of populous Miami-Dade County (O'Brien, 1998). Population size at most fragments is less than 50 individuals (Geiger, 2004).

To experimentally gauge seed germination and establishment dynamics of *I. microdactyla* across a range of biotic and abiotic gradients I chose four fragment sites with natural populations and one reintroduction site without a natural population to use for the seed and seedling outplantings. The four sites with natural populations represent a range of fragments in terms of geographic location (i.e. North, South, East and West), area, population size, herbivore guild and management history (Tables 2.1–2.2). Additionally these four sites had very few (< 5) to no naturally produced seedlings to measure. I chose one reintroduction site without any *I. microdactyla* plants as a ‘novel’ environment for reintroduction. The site was formerly a short hydroperiod sawgrass prairie (P. Ruiz, pers. comm.), but is now an environmental preserve on the campus of Florida International University (hereafter called FIU), Miami, Florida (USA) with a vegetative mix typical of small pine rockland remnants. This site receives no management from the present land stewards in the forms of clearing of invading hardwood species or prescribed fire. To evaluate natural seed germination and seedling establishment dynamics of *I. microdactyla*, and to have a comparison for the outplanting experiment, I followed naturally germinated seeds and seedlings at Coral Reef Park in Miami-Dade County, Florida, USA (Table 1).

REINTRODUCTION SITES

I chose five sites to perform both seed and seedling outplantings to assess the dynamics of germination and establishment for experimentally generated progeny of *I. microdactyla* (Table 2.1). I produced the seeds used in the reintroductions from hand pollinations of a total of 21 source plants collected from four natural populations (Table 2.2). I conducted the hand pollinations in the FIU greenhouse. Every seed within each fruit produced was uniquely identified; I recorded the single pollen donor used on each flower (i.e. dam and sire were known for every seed generated). I grouped the resulting seeds in several ways: 1) origin, 2) origin of dam, 3) original population size, 4) crossing type, 5) original population size by crossing type and 6) seed type (Table 2.2). For the ‘origin of dam’ grouping, I combined seeds derived from Bill Sadowski and Turnpike plants into a single category due to small sample sizes. I compared seeds from within and between population-cross treatments for outbreeding depression/heterosis (Lynch, 1991; Waser and Price, 1994). I was unable to consider inbreeding depression sensu stricto (i.e. seeds produced from self-pollination treatments) as most plants showed complete self-incompatibility (J. Geiger, unpublished data). The other grouping variables (Table 2.2) were used to differentially assess the fitness of resulting plants.

NATURAL SEED AND SEEDLING DYNAMICS

SEED GERMINATION AND ESTABLISHMENT

For the naturally generated individuals at Coral Reef I categorized plants as germinated seeds if the ephemeral cotyledons were present at any of the surveys. I included these germinated seeds in the same database with experimental seeds. To avoid injuring the high density delicate seedlings I ‘tagged’ individuals with two unique

measures: 1) a distance to one of three marked galvanized aluminum nails driven into the limestone rock substrate and 2) a compass direction from the chosen nail. I first surveyed seedlings on 7 August 2004 and I subsequently censused them every month for 2 years; plants were added as they appeared.

SEEDLING PERFORMANCE

I followed the same tagging and survey protocol outlined above for those individuals lacking cotyledons; I considered them seedlings. I was able to clearly differentiate these seedlings from the seven adult vines at the Coral Reef site by their much smaller size and lack of flowering. To allow for comparisons, I included these natural seedlings in the same database with experimental seedlings.

EXPERIMENTAL SEED AND SEEDLING DYNAMICS

SEED GERMINATION AND ESTABLISHMENT

I introduced the experimental seeds during the first week of October 2004 at the five outplanting sites. To gauge the fate of each uniquely identified seed, I planted and tracked the seeds individually. At each site I haphazardly established ten replicate seed plots. Using the same standard gridded frame, I planted 24 seeds in each 32 X 30 cm replicate seed plot; seeds were the experimental unit. Eight seeds, one from each seed type were planted 4 cm apart in each of the three rows separated by 10 cm. The position of each seed type within each of the three rows of a replicate plot was randomized. I uniquely identified each seed by the combination of replicate number (1–10) and replicate position (row A 1–8, row B 1–8 and row C 1–8). Seeds were not scarified before planting. I initially cleared preexisting vegetation from all the replicate seed plots. I made an informal survey of the 1 200 seeds (240 seeds X five sites) two weeks after

planting and conducted monthly censuses for the next 2 years from their respective planting dates.

SEEDLING PERFORMANCE

I introduced experimental seedlings during the last week in June 2004 at the five outplanting sites. At the time of planting the seedlings were 3-months-old. They were grown in the greenhouse with ample water and were given half-strength applications of Miracle-Gro® soluble plant fertilizer (30 N – 15 P – 30 K ratio) at two-weeks, one-, and two-months-old prior to outplanting. I haphazardly established nine replicate seedling plots at each site. I planted eight seedlings, one from each seed type, 15 cm apart in a row, for a total of 72 seedlings per site. The position of each seedling type within replicate plots was randomized. I tagged each seedling with a uniquely numbered Aluma Boss® tag driven into the ground with a galvanized aluminum nail. I surveyed the 360 seedlings monthly from their respective planting dates for the next 2 years.

GREENHOUSE SEED GERMINATION TRAILS

To verify seed viability I performed a seed germination trial in the greenhouse on 10 September 2004. I weighed the seeds on a Mettler® balance and scarified each with a scalpel. I planted ca. 30 seeds from each seed type in 50-cell pack trays filled with MetroMix® potting mixture. I scored seeds as germinated when cotyledons appeared above ground.

COMPARISON OF EXPERIMENTAL AND NATURAL POPULATIONS

PERFORMANCE MEASURES AND MALADAPTATION

To gauge the performance of both experimental and natural seeds and seedlings at the six sites, I took several monthly measures. These included the number of cotyledons,

leaves, and stem length. I also recorded flowering and assigned a status/herbivory code. From a separate demographic study, I knew dipteran, hymenopteran, and molluscan herbivores were important biotic factors affecting the fitness of *I. microdactyla* individuals (Table 2.3). To gauge their impact, I designated the following status/herbivory codes to score each individual plant: 1) no above ground parts, 2) any herbivore damage to cotyledons/leaves or stem, 3) 'stasis' (i.e. same number of leaves and stem length as the last monthly survey) and 4) new basal resprout. From the counts of these four codes over the 24 surveys I calculated a proportion value for each the four codes (e.g. % code 1 = # of surveys code 1/24) separately for each plant.

I measured survival at both the 1-year and 2-year survey points for germinated seeds and seedlings at all six sites. Due to plant dormancy, I scored seedlings as alive at the 1-year point if they possessed cotyledons and leaves in any of the previous four monthly surveys or if they subsequently resprouted. Otherwise, I scored them as dead. For survivorship at the 2 year point I recorded plants as alive if leaves were present on the final survey or by gentle excavation to verify a healthy underground tuber; otherwise plants were scored as dead.

To compare the performance of the plants reintroduced at the five outplanting sites with the naturally occurring plants at Coral Reef, I utilized a modification of Lofflin and Kephart's (2005) maladaptation formula:

$$M = [(H - A) \times 100]/A$$

where M = % maladaptation, H = trait averages derived from natural plants in their native habitat ('home'), and A = trait averages derived from reintroduced plants ('alien'). This value is analogous to quantitative genetics approximations of the selection difference

between the original population and one under natural selection pressure (Husband and Campbell, 2004). To allow for negative maladaptation values (i.e. reintroduced plants outperforming natural plants) I removed the absolute value symbols from the original formula. I selected the traits maximum number of leaves, maximum stem length, and the four status/herbivory code proportions to use as estimated fitness values for growth.

UNPLANNED FIRES

An unplanned fire burned most tagged plants at Coral Reef on 4 February 2006 and a prescribed fire burned all experimental plants at Glancy on 15 December 2005. For these plants I also scored survival post-fire as both fires happened several months after the 1 year survival tally. Survival at the 2 year point was assessed using the previously outlined protocol.

STATISTICAL ANALYSIS

I used SPSS 15.0 statistical software packages (SPSS, Inc., Chicago, IL, USA) for all analyses. Chi-square (χ^2) tests were used to consider statistical differences among seed origin, origin of dam, original population size, crossing type, original population size by crossing type, seed type, and study site for the binary variables of germination and survival. ANOVAs and t-tests were used to evaluate differences among seed origin, origin of dam, original population size, crossing type, original population size by crossing type, seed type, and study sites for the continuous variables of seed weight, greatest leaf number, longest stem, and the four status/herbivory code proportions. Proportion data were arcsine-square-root transformed to achieve homoscedasticity. If the Levene's test for equality of variances was still significant for the transformed data, I chose to perform nonparametric equivalents (e.g., Kruskal-Wallis test, Mann-Whitney U test). Tukey's

HSD multiple comparisons test or Mann-Whitney U tests were chosen to detect significant pairwise combinations a posteriori to significant ANOVA's.

RESULTS

SEED GERMINATION AND ESTABLISHMENT

GREENHOUSE SEED GERMINATION

Greenhouse germination of seed types ranged from 88–100% ($N = 233$) and there were no significant differences in germination for the Chi-Square tests on origin ($df = 2$, $\chi^2 = 2.0$, $P = 0.36$), origin of dam ($df = 2$, $\chi^2 = 5.2$, $P = 0.08$), original population size ($df = 1$, $\chi^2 = 0.01$, $P = 0.94$), crossing type ($df = 1$, $\chi^2 = 1.8$, $P = 0.18$), original population size by crossing type ($df = 4$, $\chi^2 = 4.3$, $P > 0.36$) and seed type ($df = 7$, $\chi^2 = 12.0$, $P = 0.10$). However, there were significant differences in seed weight by origin, origin of dam (Figure 2.1), original population size by crossing type, seed type (Figure 2.2) and original population size; seed weight was not significantly different by crossing type (Table 2.4).

FIELD SEED GERMINATION AND ESTABLISHMENT

The phenology of seed germination was similar for both naturally occurring and experimentally outplanted seeds; most seed germination occurred in the months of November–January (Figure 2.3). Of the total number of germinated seeds at the five outplanting sites ($N = 460$), I recorded 58.7% during the first monthly survey (November 2004) and 81.3% cumulatively by the third monthly survey (January 2005). The germination rate at the five outplanting sites ranged from 31.6–50.8% or 76–122 germinated seeds, and I observed 142 'natural' germinated seeds at Coral Reef (Table 2.5). Seed germination at the five outplanting sites as a function of origin, origin of dam,

original population size by crossing type, seed type, site, original population size and crossing type was statistically significant (Table 2.5; χ^2 tests, $P < 0.05$).

SURVIVAL

One- and two- year survival of seedlings were significantly different by site (Table 2.6). Seedling survival at the six sites ranged from 31.1–64.4% (Table 2.5) and 9.2–32.6% (Table 2.5) at the one- and two-year points, respectively which overall represents a 27.8% decline from year 1 to year 2. Testing also established significant differences in two year survival when I excluded the two burned sites (Chi square test, $\chi^2 = 22.58$, $P < 0.001$). Seedling survival at the one- and two-year points grouped by origin, origin of dam, original population size by crossing type, seed type, original population size and crossing type revealed no significant differences (Table 2.6). At the two burned sites, I found no significant differences in post-fire seedling survival, 15.2% and 19.6% respectively at the Glancy and Coral Reef sites (Table 2.6). There were significant differences in post-fire survival at Glancy as a function of crossing type; all the other factors were statistically similar (Table 2.6). The post-fire survival rate was 25.0% for ‘between site’ seedlings and 4.5% for ‘within site’ seedlings.

GROWTH

I found significant differences in the maximum number of leaves and maximum stem length for the germinated seeds at the six sites (Table 2.6). The naturally generated Coral Reef seedlings had the highest values for leaves and stem length (Figure 2.4). As a function of the grouping factors of origin, origin of dam, original population size by crossing type, seed type, original population size, and crossing type, the results showed no significant differences (Table 2.6).

SEEDLING PERFORMANCE

SURVIVAL

There were significant differences in survival for seedlings among the six sites at both the one- and two-year points (Table 2.7). One-year survival ranged from 50.0–85.5% (Table 2.8) and two-year survival ranged from 25.0–66.7% (Table 2.8); overall this represents a 25.3% decline from year 1 to year 2. Testing also identified significant differences in two-year survival when I excluded the two burned sites (Chi square test, $\chi^2 = 25.51$, $P < 0.001$). I found significant differences for first-year survival by crossing type and none of the other grouping factors showed any differences (Table 2.7). Comparisons between the two sites that were burned failed to reveal any differences in survival for the seedlings (Table 2.7).

REPRODUCTION

I found significant differences among the six sites for seedlings that flowered (Table 2.7). The proportion of seedlings that survived up to the two-year point and flowered ranged from 0.0–26.1% (Table 2.8); few plants flowered at the six sites with the exception of those planted at the ‘novel’ habitat at FIU. None of the natural seedlings at Coral Reef had flowered by the two year mark. Statistical testing failed to confirm any differences in the number of flowering plants among the sites in terms of the other grouping factors (Table 2.7). The small number of flowering plants in each group affected the power to discern important differences.

GROWTH AND HERBIVORY

For the variables maximum number of leaves and maximum stem length there were significant differences among the six sites (Table 2.9). Both the mean maximum

number of leaves and mean maximum stem length were the highest at the ‘novel’ habitat at FIU while values at the other five sites were statistically similar (Figure 2.5). The average maximum number of leaves ranged from 4.9–43.4 with plants at the ‘novel’ habitat at FIU having nearly ten times as many leaves on average as plants at Campbell. Mean maximum stem length ranged from 2.4–69.4cm. Again the average stem length was highest for plants at the ‘novel’ habitat at FIU, nearly 30 times longer on average than at Coral Reef. I found no differences for these two variables as a function of origin, origin of dam, original population size, crossing type, original population size by crossing type, and seed type (Table 2.9).

There were significant differences for all four status/herbivory codes among the six sites (Figure 2.6, Table 2.9). The range of values for the four status/herbivory codes at the six sites was: code 1) 25–58.5%, code 2) 4.8–21.2%, code 3) 8.1–42.6% and code 4) 17.5–32.6% (Table 2.10). Plants at Campbell and Glancy had the highest combined totals for codes 1 and 2, 72.1% and 75.4% respectively, and the lowest combined totals for codes 3 and 4, 27.5% and 25.3% respectively (Table 2.10). Plants at FIU, Moon, Turnpike, and Coral Reef tended to have higher proportions for codes 3 and 4 than Campbell or Glancy. Code 2, which scored herbivory, was lowest at Coral Reef (Table 2.10; 4.8%). I found significant differences in codes 1, 3, and 4 as a function of crossing type (Figure 2.7). The mean percentage for code 1 was higher for within site crosses while between site crosses had higher mean percentages for codes 3 and 4 (Figure 2.7). There were no significant differences in the four status/herbivory codes for grouping factors origin, origin of dam, original population size, original population size by crossing type, and seed type (Table 2.9).

COMPARISON OF EXPERIMENTAL AND NATURAL POPULATIONS

PERFORMANCE MEASURES AND MALADAPTATION

Naturally germinated seeds displayed larger fitness approximations than experimentally outplanted seeds at most reintroduction sites for both growth traits I measured (Table 2.11). The maladaptation values for maximum number of leaves and maximum stem length ranged from -9.5% to 217.5%. The single negative value represented a non-significant pairwise comparison between the mean maximum number of leaves for experimental plants at Turnpike relative to the natural plants at Coral Reef (Table 2.11). This was the only pairwise comparison that was not significantly different from the mean of the natural plants ($P > 0.05$, Mann-Whitney U test); for all other contrasts natural seeds had higher fitness estimates than outplanted seeds.

Seedlings at Campbell and Glancy had significantly lower, and those at FIU had significantly higher, mean maximum number of leaves than seedlings at Coral Reef (Table 2.12, $P < 0.001$, Mann-Whitney U test). The other two reintroduction sites were not significantly different than the natural site. Maladaptation values for this variable ranged from -84.8% to 34.3%. Mean maximum stem length was significantly greater for plants at Campbell, FIU, Glancy, and Turnpike sites than for plants at the natural Coral Reef site (Table 2.12, $P < 0.05$, Mann-Whitney U test). Maladaptation values ranged from -2.7% to -96.5%, indicating superior performance of seedlings at all five outplanting sites compared to seedlings at the natural Coral Reef site.

The mean values for status/herbivory code 1 (no above ground parts) were significantly greater than the mean at the natural site for Campbell and Glancy (Table 2.12, $P < 0.05$, Mann-Whitney U test) and maladaptation values were from -1.2% to

-57.4%. No above ground plant parts seen during the census could have been due to herbivory, dormancy, and possibly death. The natural site had the lowest mean fitness value for status/herbivory code 2 (herbivory evident) and all five outplanting sites had significantly higher mean fitness values (Table 2.12, $P < 0.001$, Mann-Whitney U test). The maladaptation values ranging from -52.1% to -77.1% show that plants at all reintroduction sites suffered greater herbivory than those at Coral Reef. In contrast to plants at the natural site, the outplanted seedlings at all five sites showed significantly lower mean fitness values for code 3 (Table 2.12, $P < 0.01$, Mann-Whitney U test). Code 3 scored 'stasis', or no change in leaf number and stem length from the previous survey (i.e. no herbivory). The maladaptation estimates from 32.9%-429.1% revealed this measure had the strongest performance differential. I detected significantly lower mean values for code 4 (new basal resprout) on plants at Campbell and Glancy, and significantly higher mean values for code 4 on plants at FIU than those at Coral Reef (Table 2.12, $P < 0.05$, Mann-Whitney U test). Code 4 marked a new basal resprout (i.e. previous herbivory or dormancy but alive). I calculated maladaptation values from -15.3% to 57.9%, with seedlings resprouting significantly more often at FIU and less often at Campbell and Glancy than seedlings at the natural Coral Reef site.

DISCUSSION

The restoration, via reintroduction, of endangered plant populations reduced in size is a powerful tool increasingly used by conservation biologists. The efficacy of this approach rarely has been gauged through an experimental multi-site/multi-year protocol exploring the interaction between genetic and ecological factors in shaping demographic performance. My study confirms ecological site variation as an important driver of

demographic performance for reintroduced populations, specifically herbivory.

Surprisingly, seedlings introduced into a 'novel' habitat had the highest fitness estimates; 26.1% of surviving transplants had reached sexual maturity in less than 2 years for this long-lived perennial. Significant genetic effects were found in favor of progeny resulting from between site vs. within site cross-pollinations. This study emphasizes the critical value of understanding the ecological context for successful restoration of *I. microdactyla* populations as well as other threatened plant taxa.

GENETIC EFFECTS IN EXPERIMENTAL SEEDLING ESTABLISHMENT

My first objective was to assess fitness differences for reintroduced seeds and seedlings derived from small vs. large populations and from within-site vs. between-site cross-pollinations. Possibly, this would expose genetic effects of heterosis or outbreeding depression. The pre-outplanting seed germination trial showed very high germination (88% – 100%) rates for all seed types even though there were significant differences in seed weight for all grouping factors except crossing type. Seeds derived from the largest population were 22% heavier than those from the smallest populations, but again, these differences did not affect germination. Maternal effects manifested through higher seed weight generally result in better performing offspring (Roach and Wulff, 1987). Other studies have failed to find significant germination effects between cross-pollination treatments and concluded benign greenhouse conditions inadequate for expression of fitness-related differences (Charlesworth and Charlesworth, 1987; Dudash, 1990).

Field seed germination was lower than the greenhouse but at 38.3% across outplanting sites was much greater than recorded for other perennials (Drayton and

Primack, 2000; Lofflin and Kephart, 2005). Germination was affected significantly by original population size. Seeds derived from the smallest populations had the greatest germination rates which may imply individuals have a high degree of genetic variation. These long-lived plants may not be affected by genetic erosion in the small isolated populations as the habitat only recently has become fragmented (Young et al., 1993) or the remaining individuals may represent the most fit (i.e. heterozygous) genotypes. I found little evidence of outbreeding depression in germination, as seeds derived from between- vs. within-site cross-pollinations had significantly higher germination rates.

I found no significant effects of heterosis and outbreeding depression on both one- and two-year survival of germinated seeds. However, outplanted seedlings from between-site crosses had significantly higher one-year survival than those from within-site crosses (72.1% vs. 61.3%, respectively); this difference was no longer present for two-year survival. Two-year survival rates for germinated seeds averaged 18.8% and for seedlings it averaged 47.5%. Post-fire survival was significantly greater for germinated seeds derived from between-site vs. within-site crosses, while post-fire seedling survival was nonsignificant for this factor. I found no evidence of outbreeding depression and heterosis for seeds and seedlings for the growth measures. For those variables where significant differences were detected by crossing type, seeds and seedlings derived from between-site cross-pollinations had superior performance. The pattern I observed is consistent with expectations for predominantly outcrossing species, i.e., inbreeding depression (*sensu lato*, biparental inbreeding for this self-incompatible species) is expressed throughout the plant's life cycle encompassing germination, survival, growth and reproduction (Barrett and Harder, 1996; Husband and Schemske, 1996).

ENVIRONMENTAL EFFECTS IN EXPERIMENTAL SEEDLING ESTABLISHMENT

The second objective of my study was to explore the effect of different ecological conditions on demographic performance of experimental plants. For all variables I tested, except post-fire survival, there were highly significant differences among the sites. This result emphasizes the importance of using several outplanting sites to be able to measure variance in the response of the reintroduced plants. The few studies utilizing a multi-site approach have found significant differences in reintroduction success among sites (Helenurm, 1998; Maschinski and Duquesnel, 2006). Overall there were no clear trends in performance of experimental germinated seeds in relation to habitat management. Germination rate was highest at the 'novel' site that had neither been burned nor cleared. At this site dessication of newly sprouted seeds was the major cause of death which has been reported for other studies (Helenurm, 1998). Growth of germinated seeds showed no pattern with habitat management while germinated seeds seemed to have higher survival in the more 'open' (i.e. sunny with sparse vegetation) sites managed with fire or clearing which agrees with evidence from other studies of plants in successional habitats (Menges, 1990; Oostermeijer et al., 1994; Menges, 1995; Menges and Dolan, 1998; Liu et al., 2005). Growth of seedlings was significantly greater at the 'novel' site than at the other four reintroduction sites where seedling performance was indistinguishable. Survival of outplanted seedlings was remarkably high. One-year survival averaged across all five outplanting sites was 70.5% and two-year survival averaged 46.4%. Even though seedlings were only 3-months-old at the time of transplanting they already had developed swollen storage roots with carbohydrate reserves much like the congeneric *Ipomoea batatas* (sweetpotato) and possibly could

survive lower light in ‘closed’ (i.e. shady with dense vegetation) sites or greater herbivory by utilizing stored carbon resources. Myers and Kitajima (2007) have reported the first experimental evidence of tropical seedling use of stored carbohydrates in recovering from negative carbon balance due to occasional light reduction by canopy growth and from leaf area removal by herbivores.

I recorded herbivory on seedlings at all outplanting sites by a guild of four consumers, the most damaging of which were caterpillars of the native moth *Syntomeida ipomoeae* and the exotic snail *Zachrysia provisoria*. *Z. provisoria* damage was especially severe at the Campbell and Glancy sites. The Campbell site was the most ‘closed’ outplanting site (i.e. overgrown, long time since burn) also hosting the highest densities of this introduced snail in addition to being the only site with the native moth. The Glancy site was adjacent to a plant nursery, and likewise had abundant snails. It appears that mortality of germinated seeds at these two sites was associated with the presence of *Z. provisoria*. Seedlings also experienced high rates of herbivory similar to germinated seeds, and two-year seedling survival was significantly lower at the Campbell site which contained the complete herbivore guild than at the other four outplanting sites. Intense herbivory of seedlings has been implicated in recruitment failure and in negative population dynamics (Kauffman and Maron, 2006; Lopez and Terborgh, 2007).

COMPARISON OF NATURAL AND REINTRODUCED POPULATIONS

The third goal of my study was to compute fitness estimates (i.e. maladaptation) between the experimentally reintroduced plants and the naturally occurring plants. This comparison validated all the previously discussed significant site differences and reinforced the striking conclusion that performance was generally the best for plants at

the ‘novel’ site where adults are not present. To my knowledge this is the first study to report negative maladaptation values for a plant species implying greater fitness estimates for plants in ‘novel’ habitat than for plants in their natural habitat (see review, Husband and Campbell, 2004; Lofflin and Kephart, 2005). The maladaptation protocol allowed me to quantify the effect of reintroduction against the performance of natural plants. The natural Coral Reef site is the only fragmented population in which the production of seeds and seedlings was large enough for meaningful comparisons. This site was both very small in area and population size, yet plants responded vigorously to an unplanned fire with prodigious flowering followed by fruit and seed set. Most of the native understory pine rockland plant species at this site also responded with vigorous growth and profuse flowering followed by abundant fruit and seed production as did *I. microdactyla*. Episodic recruitment of seedlings following fire is common for long-lived species in fire-maintained habitats; this appears to be part of *I. microdactyla*’s life history strategy. In addition, the fire seems to have extirpated the exotic snail herbivore from the site (i.e. only empty charred snail shells seen) which released seedlings from this negative pressure. Nearly 50% of the seedlings at this site were free from herbivory for two years. Likewise, seedlings at the ‘novel’ site where *Z. provisoria* was absent were released from this species’ herbivory and at this isolated site, 25% of seedlings showed no sign of herbivory over my two year study. The data I collected support ecological, rather than genetic, mechanisms as responsible for the superior performance of reintroduced seedlings. In the sense that all fragmented populations are subjected to new biotic and abiotic stresses, my study reiterates that fragment populations may be released from negative population pressures as well (Groom, 2001).

WAS THIS REINTRODUCTION A SUCCESS?

The fourth goal of my study was to determine if reintroduction can successfully restore small threatened plant populations. Field seed germination rates for *I. microdactyla* were much higher than has been reported for other perennial species. Survival rates for both germinated seeds and transplanted seedlings were much higher than reported for other reintroduction attempts. The post-fire survival of seedlings at the reintroduced and natural sites was statistically similar, an important finding, as their habitat (pine rockland) is fire-maintained. For the four reintroduction sites with *in situ* populations, reintroduced plants added from 28–68 individuals after two years. These additions bolstered the population sizes 23–380%. Population size at the ‘novel’ site went from 0 to 59 individuals after two years and this total includes 12 plants that flowered and produced seeds, a very unexpected and positive result given *I. microdactyla* adults have very low mortality rates (J. Geiger, unpublished data). While it may be too early to gauge whether these results match the stringent requirements for a viable population (Pavlik, 1996), these results are promising.

This research describes the effects of both genetic and ecological factors in shaping the demographic performance of a rare plant reintroduction, and offers quantitative estimates of fitness to explore maladaptation of transplanted versus natural individuals. Environmental factors (specifically herbivory) were shown to have important demographic effects for transplanted as well as natural populations. Additional multi-site/multi-year studies can confirm the significance of these effects for other endangered plant taxa. Testing for genetic effects, also, should be a requisite component of similar studies as the long-term viability and ability of the populations to track future

environmental changes is best achieved by a large reservoir of genetic variation. In this light, the creation of source propagules from several populations may be one reason for the initial restoration success of *I. microdactyla*. Successful establishment of reintroduced populations in ‘novel’ habitats is possible for this species, and perhaps, other threatened species as well. Continued experiments may further elucidate the conditions under which this unexpected but welcome event occurs.

Table 2.1. Location and site variables for study populations of *Ipomoea microdactyla* in Miami-Dade County, Florida (USA).

Study site (site abbr.)	Natural or outplanted	In situ adult population size	Habitat area (ha)	Habitat mgmt. ^a	Latitude- longitude
Campbell (CMD)	Outplanted	56	4.1	Cleared	25°28'33" N 80°26'15" W
Florida International University (FIU)	Outplanted	0	2.8		25°45'18" N 80°22'44" W
Glancy (GLC)	Outplanted	68	6.1	Fire (2)	25°29'16" N 80°31'52" W
Moon (MON)	Outplanted	280	2.0	Cleared	25°31'05" N 80°31'02" W
Turnpike (TPK)	Outplanted	18	0.12	Cleared; Fire (1)	25°37'21" N 80°22'42" W
Coral Reef (CRF)	Natural	7	2.43	Cleared; Fire (2)	25°37'54" N 80°19'20" W

^aCleared signifies partial removal of hardwood saplings by county land managers; fire indicates partial to complete site fire in the last 10 years and number of fires within 10 years in parentheses.

Table 2.2. Location and site variables of source plant populations of *Ipomoea microdactyla* in Miami-Dade County, Florida (USA) used to generate seeds for the reintroductions including code delineations for the grouping categories.

Study site (site abbr.)	Habitat area (ha)	Source plants	In situ adult population size	Origin code ^a	Population size code ^b	Cross code ^c	Population size by cross code ^d	Seed code ^e	Latitude- longitude
Bill Sadowski Park (BLW)	1.8	4	12	1	2	1,2	1,2,3	7,8	25°31'06" N 80°30'04" W
Campbell (CMD)	4.1	9	56	2	1	1,2	3,4,5	1,2,3	25°28'33" N 80°26'15" W
Larry and Penny Thompson Park (LYT)	58.8	6	81	3	1	1,2	3,4,5	4,5,6	25°35'54" N 80°23'57" W
Turnpike (TPK)	0.3	2	18	1	2	1,2	1,2,3	7,8	25°37'21" N 80°22'42" W

^aOrigin and origin of dam codes: 1 = BLW and TPK, 2 = CMD, 3 = LYT.

^bPopulation size code: 1 = adult population > 50, 2 = adult population < 50.

^cCross codes: 1 = within site (w/in), 2 = between site (btw)

^dPopulation size by cross codes: 1 = < 50 w/in, 2 = < 50 btw, 3 = > 50 < 50 btw, 4 = > 50 w/in, 5 = > 50 btw.

^eSeed codes: 1 = CMD dam and CMD sire (CMD-w/in), 2 = CMD dam and LYT sire (CMD-LYT), 3 = CMD dam and TPK or BLW sire (CMD-TPK/BLW), 4 = LYT dam and LYT sire (LYT-w/in), 5 = LYT dam and CMD sire (LYT-CMD), 6 = LYT dam and TPK or BLW sire (LYT-TPK/BLW), 7 = TPK dam and TPK sire or BLW dam and BLW sire (TPK/BLW-w/in), 8 = TPK dam and BLW sire or BLW dam and TPK sire (TPK/BLW-btw).

Table 2.3. List of herbivores of *Ipomoea microdactyla* at the six study sites in Miami-Dade County, Florida (USA).^a

Site (site abbr.)	Dipteran	Lepidopteran	Molluscan		Total herbivore spp. ^c
	Unknown Caddis-fly larvae	<i>Syntomeida ipomoeae</i>	Unknown snail	<i>Zachrysia provisoria</i> ^b	
Campbell (CMD) Florida	Present	Present	Present	Present	4
International University (FIU)	Present	Absent	Present	Absent	2
Glancy (GLC)	Present	Absent	Present	Present	3
Moon (MON)	Present	Absent	Present	Present	3
Turnpike (TPK)	Present	Absent	Present	Present	3
Coral Reef (CRF)	Present	Absent	Absent	Absent	1

^aPresence or absence denoted.

^bExotic snail introduced from Cuba.

^cTotal number of herbivore species present at site.

Table 2.4. Results of Kruskal-Wallis^a and Mann-Whitney U^b test on seed weight as a function of origin, origin of dam, original population size, crossing type, original population size by crossing type and seed type.

Source of variation	df	χ^2	<i>U</i>	<i>P</i>
		Seed weight		
Origin	2	15.51		< 0.001
Origin of dam	2	45.96		< 0.001
Original population size by crossing type	4	26.13		< 0.001
Seed type	7	52.03		< 0.001
Original population size	1		1816	< 0.001
Crossing type	1		6168	0.59

^a χ^2 test statistic.

^b*U* test statistic.

Table 2.5. Summary of germination, one-, and two-year survival for experimental and natural seeds of *Ipomoea microdactyla* at the six sites. At each of the five experimental outplanting sites, a total of 240 seeds were planted. The total number of naturally produced seeds present at the Coral Reef site was unknown and 142 germinated seeds were recorded over the two years.

Population (site abbr)	% germinated seeds	% one-year survival	% two-year survival
Natural (CRF)		56.3	23.9
Outplanted 1 (CMD)	31.7	42.5	9.2
Outplanted 2 (FIU)	50.8	31.1	10.7
Outplanted 3 (GLC)	38.3	40.0	13.0
Outplanted 4 (MON)	32.5	46.7	23.1
Outplanted 5 (TPK)	38.3	64.4	32.6

Table 2.6. Results of Chi-square (χ^2 test statistic), ANOVA (F test statistic), and Mann-Whitney U tests^a for natural and experimental seeds of *Ipomoea microdactyla* at the six sites.^b

Source of variation	df	χ^2	P	df	χ^2	P	df	χ^2	P
	Germination ^c			Alive year 1			Alive year 2		
Origin	2	7.16	0.03	2	1.23	0.54	2	1.32	0.52
Origin of dam	2	26.84	<0.001	2	0.56	0.76	2	0.59	0.74
Original population size by crossing type	4	40.96	<0.001	4	6.57	0.16	4	4.72	0.32
Seed type	7	42.50	<0.001	7	7.18	0.41	7	7.12	0.42
Site	4	23.83	<0.001	5	25.88	<0.001	5	26.61	<0.001
Original population size	1	17.13	<0.001	1	0.19	0.67	1	0.04	0.85
Crossing type	1	20.70	<0.001	1	1.97	0.16	1	2.59	0.11
				2	1.23	0.54	2	1.32	0.52
Source of variation	df	χ^2	P	df	F/U	P	df	F/U	P
	Alive post-fire ^d			Maximum leaf no.			Maximum stem length		
Origin	2	1.03	0.59	2	0.69	0.50	2	0.77	0.47
Origin of dam	2	0.81	0.67	2	0.88	0.41	2	0.38	0.69
Original population size by crossing type	4	9.27	0.06	4	0.80	0.52	4	1.09	0.36
Seed type	7	11.52	0.12	7	0.63	0.74	7	0.75	0.63
Site	1	0.73	0.39	5	3.45	<0.001	5	6.63	<0.001
Original population size	1	0.06	0.79	1	15618	0.34	1	1065	0.63
Crossing type	1	7.45	0.006	1	24972	0.36	1	1952	0.96

^aMann-Whitney U tests performed on maximum leaf no. and maximum stem length for original population size and crossing type.

^bTest statistics are: χ^2 = Chi-square, F = ANOVA, and U = Mann-Whitney U.

^cGLC and CRF used for site test; only GLC used for all other tests.

Table 2.7. Results of Chi-square (χ^2) tests for natural and experimental seedlings of *Ipomoea microdactyla* at the six sites for one-year survival, two-year survival, post-fire survival and flowering as a function of origin, origin of dam, original population size, crossing type, original population size by crossing type, seed type and site.

Source of variation	df	χ^2	P	df	χ^2	P
	Alive year 1			Alive year 2		
Origin	2	0.25	0.88	2	0.55	0.76
Origin of dam	2	0.66	0.72	2	0.42	0.81
Original population size	1	0.41	0.52	1	0.03	0.86
Crossing type	1	4.50	0.03	1	2.41	0.12
Original population size by crossing type	4	6.42	0.17	4	2.93	0.57
Seed type	7	7.28	0.40	7	4.44	0.73
Site	5	37.62	<0.001	5	44.75	<0.001
Source of variation	df	χ^2	P	df	χ^2	P
	Alive post-fire ^a			Flowering		
Origin	2	1.96	0.38	2	1.05	0.59
Origin of dam	2	1.55	0.85	2	0.32	0.85
Original population size	1	0.05	0.83	1	0.03	0.86
Crossing type	1	1.38	0.24	1	0.30	0.58
Original population size by crossing type	4	2.79	0.59	4	3.06	0.55
Seed type	7	4.50	0.72	7	4.39	0.73
Site	1	1.75	0.19	5	34.45	<0.001

^aGLC and CRF used for site test; only GLC used for all other tests.

Table 2.8. Summary of one-year survival, two-year survival, and flowering for experimental and natural seedlings of *Ipomoea microdactyla* at the six sites. At of the five experimental outplanting sites, a total of 72 seedlings were planted. At the beginning of surveys, there were 76 naturally occurring seedlings present at the Coral Reef site.

Population (site abbr)	% one-year survival	% two-year survival	% plants flowering ^a
Natural (CRF)	85.5	40.8	0.0
Outplanted 1 (CMD)	66.7	29.2	4.8
Outplanted 2 (FIU)	75.0	63.9	26.1
Outplanted 3 (GLC)	50.0	25.0	5.6
Outplanted 4 (MON)	80.6	66.7	2.1
Outplanted 5 (TPK)	65.3	52.8	10.5

^aThe percentage of plants that had flowered by the two-year survey point.

Table 2.9. Results of ANOVA (F test statistic) and t-tests^a(t statistic) for natural and experimental seedlings/juveniles of *Ipomoea microdactyla* at the six study sites for maximum leaf number, maximum stem length, and the four status/herbivory code proportions as a function of site, crossing type, seed type and original population size.^b

Source of variation	df	F/t	P	df	F/t	P	df	F/t	P
	Maximum leaf no.			Maximum stem length			Code 1		
Origin	2	0.25	0.78	2	0.26	0.77	2	0.07	0.93
Origin of dam	2	0.98	0.38	2	0.68	0.51	2	0.57	0.57
Original population size	268	0.12	0.91	268	0.14	0.89	268	-0.54	0.59
Crossing type	358	0.49	0.63	358	0.32	0.75	358	2.28	0.02
Original population size by crossing type	4	0.19	0.95	4	0.34	0.85	4	1.49	0.20
Seed type	7	0.42	0.89	7	0.47	0.86	7	0.95	0.47
Site	5	23.17	< 0.001	5	19.00	< 0.001	5	16.45	< 0.001
Source of variation	df	F/t	P	df	F/t	P	df	F/t	P
	Code 2			Code 3			Code 4		
Origin	2	0.34	0.71	2	0.01	0.99	2	0.04	0.97
Origin of dam	2	0.35	0.71	2	0.92	0.40	2	0.31	0.73
Original population size	268	-0.12	0.90	268	1.06	0.29	268	-0.01	0.99
Crossing type	358	-0.25	0.80	358	-1.96	0.05	358	-1.98	0.05
Original population size by crossing type	4	0.53	0.71	4	1.44	0.22	4	1.01	0.40
Seed type	7	0.51	0.83	7	0.84	0.56	7	0.73	0.65
Site	5	21.60	<0.001	5	52.04	<0.001	5	9.62	<0.001

^at-tests used for factors original population size and crossing type on all dependent variables.

^bStatus/herbivory codes: code 1—no above ground parts, code 2—minor/major herbivory, code 3—stasis and code 4—basal resprout.

Table 2.10. Summary of mean percentage of surveys with status/herbivory codes 1-4^a for *Ipomoea microdactyla* seedlings at the six study sites (Table 1).

Population (site abbr.)	Mean % code 1	Mean % code 2	Mean % code 3	Mean % code 4
Natural (CRF)	24.9	4.9	42.6	27.6
Outplanted 1 (CMD)	50.9	21.2	8.5	19.3
Outplanted 2 (FIU)	25.2	10.1	32.0	32.6
Outplanted 3 (GLC)	58.5	16.0	8.1	17.5
Outplanted 4 (MON)	29.0	13.8	26.4	30.7
Outplanted 5 (TPK)	32.7	17.4	23.3	26.6

^aStatus/herbivory codes: code 1—no above ground parts, code 2—minor/major herbivory, code 3—stasis and code 4—basal sprout.

Table 2.11. Estimated fitness values for plant growth of germinated seeds and maladaptation (%)^a of outplanted populations to the natural population^b. *P* values obtained from Mann-Whitney U tests.

Population (site abbr.)	Maximum no. leaves			Maximum stem length (cm)		
	Mean ± SE	<i>P</i>	(%)	Mean ± SE	<i>P</i>	(%)
Natural (CRF)	2.9 ± 0.19			1.1 ± 0.06		
Outplanted 1 (CMD)	2.0 ± 0.24	0.004	50.0	0.33 ± 0.04	< 0.001	217.5
Outplanted 2 (FIU)	2.8 ± 0.37	0.028	3.5	0.89 ± 0.17	0.006	19.1
Outplanted 3 (GLC)	2.1 ± 0.22	0.002	41.3	0.38 ± 0.03	< 0.001	175.9
Outplanted 4 (MON)	2.1 ± 0.28	0.004	40.7	0.51 ± 0.07	< 0.001	108.7
Outplanted 5 (TPK)	3.2 ± 0.32	0.951	-9.5	0.76 ± 0.13	0.018	38.5

^aMaladaptation %'s presented in columns labeled '%'.
^bNegative maladaptation (%) values indicate superior performance of outplanted versus natural plants.

Table 2.12. Estimated fitness values for plant growth and status/herbivory codes^a of seedlings and maladaptation (%)^b of outplanted populations to the natural population^c. *P* values obtained from Mann-Whitney U tests.

Population (site abbr.)	Maximum no. leaves			Maximum stem length (cm)			Code 1		
	Mean ± SE	<i>P</i>	(%)	Mean ± SE	<i>P</i>	(%)	Mean ± SE	<i>P</i>	(%)
Natural (CRF)	6.6 ± 0.3			2.4 ± 0.2			24.9 ± 0.02		
Outplanted 1 (CMD)	4.9 ± 0.8	< 0.001	34.3	3.4 ± 1.2	0.001	-27.4	50.9 ± 0.03	< 0.001	-51.0
Outplanted 2 (FIU)	43.4 ± 6.9	< 0.001	-84.8	69.4 ± 14.5	< 0.001	-96.5	25.2 ± 0.04	0.118	-1.2
Outplanted 3 (GLC)	5.9 ± 2.1	< 0.001	10.9	2.5 ± 1.3	< 0.001	-2.7	58.5 ± 0.03	< 0.001	-57.4
Outplanted 4 (MON)	8.9 ± 0.9	0.396	-26.1	3.9 ± 1.1	0.211	-36.7	29.0 ± 0.04	0.833	-14.1
Outplanted 5 (TPK)	11.7 ± 1.8	0.235	-43.9	11.8 ± 3.3	0.051	-79.4	32.7 ± 0.04	0.800	-23.8
Population (site abbr.)	Code 2			Code 3			Code 4		
	Mean ± SE	<i>P</i>	(%)	Mean ± SE	<i>P</i>	(%)	Mean ± SE	<i>P</i>	(%)
Natural (CRF)	4.9 ± 0.01			42.6 ± 0.02			27.6 ± 0.01		
Outplanted 1 (CMD)	21.2 ± 0.02	< 0.001	-77.1	8.5 ± 0.01	< 0.001	400.2	19.3 ± 0.02	< 0.001	42.7
Outplanted 2 (FIU)	10.1 ± 0.01	< 0.001	-52.1	32.1 ± 0.02	0.002	32.9	32.6 ± 0.03	0.017	-15.3
Outplanted 3 (GLC)	15.9 ± 0.01	< 0.001	-69.6	8.1 ± 0.01	< 0.001	429.1	17.5 ± 0.02	< 0.001	57.9
Outplanted 4 (MON)	13.8 ± 0.01	< 0.001	-64.9	26.4 ± 0.02	< 0.001	61.3	30.7 ± 0.02	0.109	-10.2
Outplanted 5 (TPK)	17.4 ± 0.01	< 0.001	-72.1	23.2 ± 0.02	< 0.001	83.4	26.6 ± 0.02	0.956	3.7

^aMean status/herbivory code values are percentages.

^bMaladaptation %'s presented in columns labeled '%'.
^cNegative maladaptation (%) values indicate superior performance of outplanted versus natural plants.

Figure 2.1. Mean seed weight (± 1 SE) by origin of dam prior to greenhouse germination trial of *Ipomoea microdactyla* (Table 1). Significant differences are indicated with different letters (Mann-Whitney U tests, $\alpha = 0.05$). Note: Seeds from sites BLW and TPK are combined into a single 'origin of dam' category.

Figure 2.2. Mean seed weight (± 1 SE) prior to greenhouse germination trial of *Ipomoea microdactyla* (Table 1).

Figure 2.3. Sum of germinated seeds of *Ipomoea microdactyla* for the six sites.

Figure 2.4. Mean maximum stem length (± 1 SE) of germinated seeds of *Ipomoea microdactyla* at the six sites (Table 1). Significant differences among sites are indicated with different letters (Mann-Whitney U tests, $\alpha = 0.05$).

Figure 2.5. Mean maximum number of leaves (± 1 SE) and mean maximum stem length (± 1 SE) of *Ipomoea microdactyla* seedlings at the six sites (Table 1). Significant differences among sites are indicated with different letters (Tukey's HSD multiple comparisons test, $\alpha = 0.05$).

Figure 2.6. Mean percentage of surveys with status/herbivory codes 1–4 for *Ipomoea microdactyla* seedlings at the six sites (Table 1). Significant differences among the sites are indicated with different letters (Tukey's test, $\alpha = 0.05$).

Figure 2.7. Mean proportion of surveys with status/herbivory codes 1–4 as a function of crossing type for *Ipomoea microdactyla* seedlings at the five outplanting sites (Table 1). Significant differences among crossing types are indicated with different letters (t-tests, $\alpha = 0.05$).

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CHAPTER 3. RANGE-WIDE POPULATION GENETIC DIVERSITY OF THE ENDANGERED CARIBBEAN VINE *IPOMOEA MICRODACTYLA* GRISEB. (CONVOLVULACEAE)

The ‘conservation genetics’ paradigm broadly frames many investigations into preserving biodiversity by considering the link between the loss of population-level genetic diversity and the increased risk of population extinction (Ouborg *et al.*, 2006). While controversy still exists regarding the relationship between plant population size, fitness, and genetic variation (Oostermeijer *et al.*, 2003), recent reviews have shown the association to be generally positive (Reed and Frankham, 2003; Leimu *et al.*, 2006). From the conservation genetics perspective, threats to biodiversity are perceived via properties of the populations themselves (e.g. reduced size, increased isolation from conspecific populations) rather than abiotic aspects of their habitat (e.g. reduced habitat quality).

A major threat to biodiversity is habitat fragmentation which directly results in the loss of populations, reduces population sizes, and increases the distance among the remaining populations. A suite of evolutionary forces such as genetic drift, gene flow, and inbreeding can be affected indirectly by habitat fragmentation, leading to negative consequences for these populations (Young *et al.*, 1996; Galeuchet *et al.*, 2005). Genetic drift becomes more severe in small populations, leading to a loss of genetic variation over time (Ellstrand and Elam, 1993; Hartl, 2000). Isolated, small populations may be less attractive to pollinators, which may lead to lower levels of gene flow (Agren, 1996; Goodell *et al.*, 1997). With lower levels of gene flow, there is a greater chance of population differentiation via changes in allele frequency among populations (Ellstrand and Elam, 1993). Changes in pollinator behavior may increase within-population

matings, leading to inbreeding depression (Lennartsson, 2002). Inbreeding depression may lower fitness and negatively affect the viability of populations (Charlesworth and Charlesworth, 1987; Young et al., 1996). This, in turn, may lead to population extinction (Mills and Smouse, 1994; Newman and Pilson, 1997).

While the effects of habitat fragmentation on genetic variation have been studied for a wide range of rare and endangered plant species (Mills and Smouse, 1994; Rossetto et al., 1995; Hogbin and Peakall, 1999; Lammi et al., 1999; Fischer et al., 2000; Collevatti et al., 2001; Zawko et al., 2001; Lira et al., 2003), fewer studies have focused on common species (Culley and Grubb, 2003; Vergeer et al., 2003; Hooftman et al., 2004; Rossetto et al., 2004; Mix et al., 2006). Very few inquiries have compared genetic measures between rare and common species (Karron, 1989; Gitzendanner and Soltis, 2000; Van Rossum et al., 2004), yet several authors have emphasized utilizing comparative studies of endangered and common plants to elucidate general concepts of biodiversity maintenance (Ouborg *et al.*, 2006). Common species may be less sensitive to habitat fragmentation as gene flow among populations may not lead to population differentiation. The case may be the opposite for widely dispersed rare plant populations with greater isolation leading to ever increasing population differentiation. The patterns of inbreeding and lowered genetic variation caused by genetic drift also may be different for common and rare species. Ultimately, the loss of genetic diversity may reduce the evolutionary ability of species to adapt to environmental alterations (Soule, 1980; Simberloff, 1988).

Unique areas of the world, identified as biodiversity 'hot spots', are especially vulnerable to the loss of great numbers of species in the coming decades due primarily to

anthropogenically-driven habitat loss . The Caribbean region is one of the 25 global biodiversity ‘hot spots’ (Myers et al., 2000). This island system was the setting for important concepts in evolution and island ecology, such as adaptive radiation (Losos et al., 1998) and the species-island area relationship (MacArthur and Wilson, 1967). However, the Caribbean Basin is poised to lose a large amount of its biodiversity (Davis et al., 1997) as conservation endeavors have grown weaker and biological studies fewer across the region in general (Kress and Horvitz, 2005). Researchers have suggested that projects aimed at identifying the factors imperiling the Caribbean’s singular biodiversity, especially investigations of the genetic variation among populations of threatened plant species, are vital for conservation efforts (Kress and Horvitz, 2005). Our study seeks to fill this lacuna.

Ipomoea microdactyla is a common species in the understory of pine forests throughout its range in the Caribbean region where it is found in Cuba, across the Bahamian archipelago, and only in Miami-Dade County, Florida within the USA. However, there is a marked contrast in habitat between populations found in Miami-Dade County, and those on Andros Island in the Bahamas. Outside of the natural habitat preserved in Everglades National Park, nearly 99% of the pine forests in Miami-Dade County have been destroyed in the last 120 years. *I. microdactyla* is restricted to 36 habitat fragments and is a Florida State listed endangered plant. Most of the pine forest fragments are less than 10 ha in size and are embedded within the agricultural, suburban and urban landscape of populous and expanding Miami-Dade County. Conversely, Andros Island supports the largest continuous expanse of pine forest in the Caribbean Basin, is sparsely populated, and presently, is experiencing very little development

pressure. *I. microdactyla* is found in high densities across Andros Island pine forests. We find this species to be a worthwhile candidate to compare the population genetic effects of habitat fragmentation on a simultaneously endangered and common species.

We examined genetic diversity in 212 individuals from 12 populations of *Ipomoea microdactyla* across its entire geographic range using seven polymorphic microsatellite loci. Specifically, we focused on the following questions: (i) What is the population genetic diversity of *I. microdactyla* across its entire geographic range? (ii) Is there significant population genetic structure and differentiation among populations from the three sampled regions? (iii) Do populations within habitat fragments show lower levels of genetic variation than populations within continuous habitat? (iv) What are the conservation implications of these findings for *I. microdactyla* and for other threatened taxa in this biodiversity ‘hot spot’?

MATERIALS AND METHODS

STUDY ORGANISM

Ipomoea microdactyla Griseb. (Convolvulaceae) has a Caribbean distribution and is found throughout Cuba, across the Bahamian archipelago and only in Miami-Dade County, Florida within the USA. *I. microdactyla* occurs primarily in pine forests growing on limestone rock across its range. The plant is abundant in the continuous pine forest on Andros Island in the Bahamas but infrequent in the highly fragmented pine rockland habitat of Miami-Dade County, Florida where it is state-listed as endangered (Coile and Garland, 2002). Outside of the intact pine rockland within Everglades National Park, there presently exists less than 2 % of the original habitat in Miami-Dade County (Bradley, 2005). *I. microdactyla* is known to occur in 36 of the remaining ca.

400 habitat fragments as well as in Everglades National Park (Gann et al., 2002). Most fragments (ca. 95 %) are less than ten ha in area, with few greater than 20 ha; these isolated remnants are embedded within the urban, suburban, agricultural and wildland matrix of populous Miami-Dade County (O'Brien, 1998). Population size at most fragments is less than 50 individuals (Geiger, 2004).

Ipomoea microdactyla is a perennial vine with twining woody stems to over 10 m in length. Plants are long-lived and have underground tubers much like the congeneric sweet potato (*Ipomoea batatas*). Individuals produce several to hundreds of hermaphroditic magenta-red flowers in discrete bouts during the blooming season from May to December. Flowers are visited by a diverse group of insects including Hymenoptera and Lepidoptera, but the predominant flower visitors are hummingbirds (Trochiliformes). As with its congeners, *I. microdactyla* is self-incompatible and requires pollen vectors for fruit/seed production (J. Geiger, unpublished data). Capsular fruit contain a maximum of four seeds covered with 1 cm long hairs. Seeds weigh ca. 1.5 mg and are dispersed by wind, but are capable of floating on water; they generally germinate immediately after release in late autumn or early winter (J. Geiger, unpublished data).

POPULATIONS, SAMPLING AND DNA EXTRACTION

We surveyed 12 populations across the entire geographic range of *Ipomoea microdactyla*, which represented the continuum from intact pine forest through highly fragmented habitat remnants, to gauge genetic diversity and population genetic structure (Table 3.1). This included: six sampling sites in the largest extant continuous pine forest in the Caribbean on Andros Island in the Bahamas; five habitat remnant sites in the hyper-fragmented pine rockland of Miami-Dade County, Florida (USA); and one scrub

forest site in northwestern Cuba (Table 3.1). Fresh or silica-gel dried leaf samples were collected from 13 to 21 adults at each site (Table 3.1). Sampled individuals were chosen haphazardly by following a random walk at each of the field sites using a compass heading and distance derived from a random number table. Overall, 212 individuals were sampled. The entire population was sampled at sites BWS, CRF and TPK in Florida. On Andros Island, plant densities were relatively uniform and the plant sampling areas were roughly 10,000 m² at the six collection sites. DNA was extracted using the FastDNA Spin Kit BIO 101 System with FastPrep instrument (Q-BIOgene).

MICROSATELLITE ANALYSIS

We used seven primers originally developed for the congeneric sweet potato (*Ipomoea batatas*) to measure population genetic structure at microsatellite loci in *I. microdactyla*: bm757, ib286, ib318, s11, ssr01, ssr13, and ssr14 (Buteler *et al.*, 1999; Hu *et al.*, 2004). To genotype individuals, polymerase chain reaction (PCR) amplifications were performed in 10- μ L volumes with 25 ng genomic DNA, 0.5 μ M each of the fluorescent dye-labeled forward primer and of the reverse primer, 200 μ M of each dNTP, 1 $\times$ reaction buffer (10 mM Tris-HCL, pH 8.3, 50 mM KCL, 1.5 mM MgCl₂), and 1 unit GoTaq DNA polymerase (Promega). PCR amplifications were performed in a PT-100 thermal cycler (MJ Research) using the following protocol: 96 °C for 1 min (one cycle), 94 °C for 40 s, 55 °C for 1 min, 72 °C for 1 min (30 cycles); and 72 °C for 5 min (one cycle). We combined 2 μ L of the PCR product with 10 μ L of a 20:1 solution of formamide and GENESCAN-500(ROX) size standard (Applied Biosystems) mix. Samples were run on an ABI Prism 3100 Genetic Analyser and allele sizes were scored using GENESCAN version 3.1.12 (Applied Biosystems).

STATISTICAL ANALYSIS OF MICROSATELLITE VARIATION

We gauged per locus microsatellite genetic diversity by calculating the number of alleles (A), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), and F_{ST} (Weir and Cockerham, 1984). We computed overall and mean per population values of percent of polymorphic loci (P), number of alleles (A), number of private alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficients (F_{IS}) (Weir and Cockerham, 1984) using GENALEX version 6 software (Peakall and Smouse, 2006). Departures from Hardy-Weinberg expectations per locus/population combination were calculated using χ^2 tests as implemented in GENALEX 6. We determined the population genetic variation using F_{ST} which utilizes the infinite allele model (Weir and Cockerham, 1984). MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was used to check for the presence of null alleles at each locus for each population. Several genetic statistics (i.e. A , H_O , H_E , and F_{IS}) for the fragment populations in Florida and the continuous populations on Andros Island were contrasted with nonparametric Mann-Whitney U tests as the data were not normally distributed.

We utilized STRUCTURE to evaluate population structure given the data we collected. This software applies a Bayesian clustering algorithm to distinguish genetically similar groups of individuals (i.e. populations) and to discern cases of individuals ensuing from admixture (Pritchard *et al.*, 2000). K , the number of clusters/populations was fixed from 1 to 12. We did not use any prior knowledge of the individual's true population and ran each K for 10 replications with a burn-in period of 100 000 for 1 000 000 iterations. We used the default settings for the remaining choices. We compared the value of K determined by STRUCTURE with the statistic ΔK which is

derived from the change in the log probability of successive values of K (Evanno *et al.*, 2005). Using POPULATIONS version 1.2.30 software we generated a D_A genetic distance matrix from the frequencies of alleles (Nei *et al.*, 1983). We created a neighbor-joining tree from this genetic distance matrix and substantiated the branching pattern by performing 1000 bootstrap permutations with POPULATIONS version 1.2.30 software (available at <http://bioinformatics.org/~tryphon/populations/>). The resulting trees were visualized using TREEVIEW version 1.6.6 (Page, 1996).

Using F_{ST} we analyzed the hierarchical distribution of genetic variation of the three regions, among populations nested within the regions, and among all populations with an analysis of molecular variation (AMOVA) (Excoffier *et al.*, 1992), as performed in GENALEX version 6, with 10 000 permutations. Separate AMOVAs were conducted on Florida and Andros Island populations as above. To evaluate the relationship between geographic and genetic distances we first constructed both types of distance matrices using GENALEX version 6. The genetic distance matrix contained estimates of linearized pairwise F_{ST} values among all the populations. We then used the Mantel test option in GENALEX version 6 to test isolation by distance with 10 000 permutations. We also ran separate Mantel tests on Florida and Andros Island populations as above.

RESULTS

POPULATION GENETIC DIVERSITY

The estimates of genetic diversity differed among microsatellite loci (Table 3.2) and among populations (Table 3.3). For the 212 plants sampled, we recorded between 5 and 14 alleles per locus for a total of 66 alleles at the seven loci. Average observed and expected heterozygosity for all loci was 0.545 and 0.558, respectively. Three loci were

monomorphic in two of the Florida populations. Average F_{ST} for all loci was 0.149. Polymorphism within populations ranged from 71 to 100%. Over all populations, the average number of alleles was 4.6. We detected 12 private alleles among the populations, nearly half of these (5 alleles) at *Atala* coppice in the Bahamas. Values of F_{IS} were between -0.087 and 0.320, with an average of 0.037 for all populations, suggesting low levels of inbreeding for this self-incompatible species. For the 84 locus/population combinations, 39 showed significant deviation from Hardy-Weinberg expectations with nearly half (16) displaying heterozygosity deficit. We discerned the presence of null alleles in some populations at four loci, but overall we did not detect significant instances of null alleles.

POPULATION GENETIC STRUCTURE AND DIFFERENTIATION

STRUCTURE distinguished four main clusters of populations: a cluster containing all five Florida populations, a cluster containing five of the six Andros Island populations, a cluster containing a single Andros Island population (*Atala* coppice), and a cluster containing the Cuba population (Figure 3.1). There was little evidence of admixture in the Florida populations. However, most Andros Island populations showed evidence of mixed ancestry. The number of groups identified using the ΔK estimation was also four main clusters. The neighbor-joining tree did not reflect completely the clustering obtained by STRUCTURE (Figure 3.2). There was a separate clade of the Florida populations, with the Cuba population nested within this group. Five of the six Andros Island populations formed a group, while a single Andros Island population (*Rhizophora*) came out as a single branch. Overall, there was low support for the branching pattern.

Most of the genetic variation was found within populations (83.09%), as opposed to among regions (10.43%) or among populations within regions (6.49%), for all 12 populations (Table 3.4). AMOVA validated significant population differentiation at all three of these hierarchical levels ($P < 0.01$). From this test, the F_{ST} value among the 12 populations was 0.169. Significant isolation-by-distance was detected by the Mantel test among all the populations in the three regions (Figure 3.3). A separate Mantel test performed excluding the Cuba population also found significant isolation-by-distance (Figure 3.4). A third Mantel test using the Florida populations identified significant isolation-by-distance (Figure 3.5). There was no evidence of isolation-by-distance for the Andros Island populations ($r = -0.035$, $P = 0.230$).

GENETIC VARIATION IN FRAGMENTED VS. CONTINUOUS HABITAT

We found significant differences for most genetic diversity estimates between fragmented populations in Florida and continuous populations on Andros Island (Table 3.5). The average number of alleles for these two groups was significantly different: the mean number of alleles for Florida and Andros Island populations were 3.6 and 5.1, respectively. Observed and expected heterozygosity also were significantly lower for Florida than for Andros Island populations. Although not significant, the mean values for F_{IS} demonstrate the same pattern of lower measures for the Florida than the Andros Island populations as seen in the previous significant comparisons.

DISCUSSION

POPULATION GENETIC DIVERSITY

We detected measures of genetic variation (e.g. total number of alleles, H_O , and H_E) for *Ipomoea microdactyla* that fall within the intervals found in a recent review of

intraspecific genetic diversity in plants (Nybom, 2004). These measures are similar to those found for other species within the family Convolvulaceae (Kim and Chung, 1995; Wolf et al., 2000). Not surprisingly, we found greater numbers of alleles per locus for this wild species than the cultivated *I. batatas* (sweet potato) for which the primers we used were originally developed (Buteler et al., 1999; Hu et al., 2004).

Three loci were monomorphic at the Florida sites (Bill Sadowski and Coral Reef) that have the smallest population sizes. As we genotyped all individuals at these sites, there was no possibility of missing alleles due to statistical sampling (Weir, 1996). Rather, this observation may be due to either the presence of null alleles or to the fixation of alleles via genetic drift. We found no evidence of null alleles at any of the other Florida sites for these three loci. The Bill Sadowski and Coral Reef populations are located the furthest north and east of the remaining populations in Miami-Dade County. They have been isolated for the longest time as the habitat fragmentation in this area proceeded south and westward. Theory predicts the loss of genetic variation over time through genetic drift for small, isolated populations (Ellstrand and Elam, 1993; Young et al., 1996) and empirical support has been discovered for many species (Raijmann et al., 1994; Fischer and Matthies, 1998; Luijten et al., 2000; Galeuchet et al., 2002; Paschke et al., 2002; Hooftman et al., 2003).

We detected private alleles in all three regions, with nearly 50% (5) found in the Atala site on Andros Island and 25% found at the Cuban site. This is the only sampling site in the Bahamas where we found *Ipomoea microdactyla* co-occurring with the congeneric *I. carolina*. All five of these private alleles exist in high allelic proportions among the *I. carolina* individuals that we have genotyped, and in low allelic proportions

among the *I. microdactyla* individuals (J. Geiger, unpublished data). In a separate study, we have shown the two species to be cross-compatible and capable of producing viable hybrid seed (J. Geiger, unpublished data). These two direct lines of proof point to the likelihood of hybrid introgression in our detection of predominately *I. carolina* alleles within *I. microdactyla* individuals at this single site on Andros. Cross-compatibility within other members of this genus has been shown previously (Diaz et al., 1996). The large number of private alleles identified in the Cuban population also may be a result of hybrid introgression with other congeners or simply may be indicative of higher levels of genetic diversity. This latter explanation seems more plausible given the geographic range of this species. With Cuba being much older geologically than the other two regions, *I. microdactyla* very likely speciated here and therefore retains higher levels of genetic variation at its center of distribution.

The deviations from Hardy-Weinberg we detected could be due to several reasons. First, this species has a sporophytic self-incompatibility mating system; therefore, the assumption of random mating is violated (Richards, 1986; deNetancourt, 1997). For this breeding system, successful sexual reproduction only occurs with compatible S-locus mating types, and this generally precludes selfing and matings between close relatives. Second, the Hardy-Weinberg assumption of an infinitely large population able to buffer the effects of genetic drift is not met in Miami-Dade County; four of the five sampled populations have less than 100 individuals, with three of them containing less than 30 individuals. Third, we have evidence that populations on Andros are admixed. Populations that are composed of individuals of mixed ancestry may show the Wahlund effect which results in higher levels of homozygosity than would be

expected for populations not subject to admixture (Hartl, 2000). We have no direct evidence that natural selection is operating or that mutation of the microsatellites has happened. It is likely that the above outlined processes are implicated in the observed Hardy-Weinberg departures.

Overall, mean within-population F_{IS} was 0.037 (range – 0.087 to 0.320) which suggests low levels of inbreeding (*sensu lato*, biparental inbreeding for this self-incompatible species). Low F_{IS} values are expected for sporophytic self-incompatible species such as *Ipomoea microdactyla* that are obligate outcrossers. The highest F_{IS} values were recorded at Bill Sadowski and Coral Reef sites in Miami-Dade County where three of the loci were monomorphic. In Miami-Dade County, the most common floral visitors were native solitary bees (Halictidae) while a single species of migratory hummingbird (ruby-throated, *Archilochus colubris*) was rarely seen visiting flowers. The lowest F_{IS} values were noted at sites on Andros where two species of resident hummingbirds (Bahamas woodstar, *Calliphlox evelynae*, and Cuban emerald, *Chlorostilbon ricordii*) occurred at high densities, and were the most frequent visitors to flowers (J. Geiger, unpublished data). Hummingbirds have been shown to be effective pollinators of many species and are capable of traveling relatively long distances (Singer and Sazima, 2000; Kraemer, 2001). On Andros, they may promote larger effective population sizes in this continuous forest, and therefore we would expect lower levels of biparental inbreeding.

POPULATION GENETIC STRUCTURE AND DIFFERENTIATION

We found population genetic structuring that generally coincides with the three sampled geographic regions (Figure 3.1). STRUCTURE identified four genetic clusters from

the 12 populations that we sampled. The first cluster was composed of the five Miami-Dade County sites where there was little admixture from the other genetic clusters. In contrast, some individuals in the second genetic cluster made up of five of the six Andros populations are admixed with alleles from the fourth genetic cluster (i.e. the Cuban population). As seeds disperse adjacent to the parent plant, this points to the capacity of the resident hummingbirds to effect sufficient gene flow via pollen transfer across populations on Andros. It also raises the possibility of long-distance gene flow from other islands via the Cuban emerald hummingbird which is found throughout the Bahamian archipelago, and Cuba. The third genetic cluster is composed of individuals at Atala, the sixth Andros site. Individuals at this site have five private alleles that are shared with the co-occurring conspecific *Ipomoea carolina*, which we have shown to be cross-compatible with *I. microdactyla*, and the resulting seeds are viable. We believe the unique genetic signature of individuals at this site to be the result of hybrid introgression. The fourth genetic cluster contains individuals from the Cuban population with some admixture of alleles from the second and third genetic cluster (i.e. the Andros populations).

The level of among-population genetic variation for *Ipomoea microdactyla* ($F_{ST} = 0.169$) is larger than the mean value reported by Hamrick and Godt (1996) for other long-lived, outcrossing perennial plants ($G_{ST} = 0.094$). This difference can be explained by the effect the size of the geographic area sampled has on the estimate of F_{ST} , where estimates tend to increase by increasing the geographic extent of sampling (Morjan and Rieseberg, 2004). We obtained much smaller F_{ST} estimates when we tested Florida and Andros populations separately, 0.070 and 0.075, respectively (data not shown).

The results from the AMOVA agree with those from the STRUCTURE analysis, namely, that there are significant differences in genetic variation among the three geographic regions. The effect of past colonization events (i.e. long distance seed dispersal) to these geographically isolated islands is a reasonable explanation for these results. In a separate study covering the same geographic extent, Maskas and Cruzan (2000) concluded that historical dispersal processes were the most likely causative agents for the present day genetic structure found in the *Piriqueta caroliniana* complex. They propose at least three historical cases of long-distance seed dispersal from the northern Bahamas islands of Grand Bahamas and Abaco to the southeastern coast of Florida. *Ipomoea microdactyla* shares several traits with their study species. It is an obligately animal pollinated, self-incompatible, herbaceous perennial that lacks any specialized features for long-distance seed dispersal. Present day gene flow, through either seeds or pollen, among these geographically isolated populations may be small enough to maintain these significant genetic differences. We found statistically significant but weak levels of genetic variation among populations within regions. It appears that if present day rates of gene flow are equal to those of the recent past, then populations within regions will have little opportunity for divergence.

Our data suggest that across the three regions sampled, *Ipomoea microdactyla* populations are distinguished by the process of 'isolation by distance'. Support for this comes from a significant Mantel test. The process of 'isolation by distance' posits that gene flow is restricted to relatively short distances, so that geographically isolated populations begin to diverge over time. Several authors have pointed out that, as microsatellites have high mutation rates, care should be taken when interpreting this

process when utilizing these markers (Slatkin, 1995; Hedrick, 1999). They suggest it is important to consider the time since populations became isolated, as this may be the most significant factor explaining the observed pattern. The habitat of *I. microdactyla* in Miami-Dade County has been above sea level for approximately 125,000 years (Lidz and Shinn, 1991), making it the youngest of the three sampled regions. Most likely, Andros island was inundated several times during sea-level changes over the course of the Pleistocene, but had portions that were high enough to support pine forest vegetation during this time (Haq et al., 1987). Cuba is the oldest of the three regions, with extreme eastern and western Cuba being continuously above sea level for perhaps 30 million years. It is probable that the pattern of ‘isolation by distance’ found for these three regions is a consequence of historical colonization events (i.e. long-distance dispersal of seeds) along with contemporary restricted gene flow (i.e. pollen) as others have inferred from similar results (Rivera-Ocasio et al., 2002; Jump et al., 2003; Van Rossum and Prentice, 2004; Hodgins and Barrett, 2007). Further, this may describe the significant Mantel test when Florida and Andros populations were analyzed separately. On the other hand, the significant ‘isolation by distance’ found in the Miami-Dade County populations may be attributed to the pollinator guild. While hummingbirds can be effective pollinators over relatively long distances, the migratory nature of the species seen in Miami-Dade County may weaken its capacity to link isolated populations via pollen flow. The other main floral visitors (i.e. native Halictid bees) generally remain within a small foraging area therefore excluding them from delivering pollen among isolated populations. On the contrary, there was no evidence of ‘isolation by distance’ for the Andros populations. The high densities of the two resident hummingbird species on

Andros may promote very effective pollen flow across the entire island, hence explaining the lack of significance for the Mantel test.

GENETIC VARIATION IN FRAGMENTED VS. CONTINUOUS HABITAT

We found significantly greater mean numbers of alleles, and higher mean values for both observed and expected heterozygosity in populations from the continuous forest on Andros than those from the habitat fragments in Miami-Dade County. Inbreeding values were marginally larger ($P = 0.065$) for fragment populations than for continuous forest populations. We believe these findings of lower genetic diversity in fragment populations to be an historical artifact of the geologically recent arrival of *Ipomoea microdactyla* to Florida, rather than a case of habitat fragmentation/isolation causing genetic erosion in these populations. Considering the relatively young age of the habitat in Miami-Dade County, we would expect lower levels of genetic variation due to the lingering effects of founder events, especially if there is little gene flow into these isolated populations. Conversely, the higher levels of genetic variation we observed in the Andros populations are not surprising as this island (and neighboring islands within the Bahamian archipelago) has a much older geological age. Populations within Miami-Dade County also constitute the expanding range edge for *I. microdactyla* in the Caribbean region as they occur as an isolated biogeographic unit in the northwestern periphery of the larger complete geographic range of this species within the Caribbean region. Other researchers have found lower levels of genetic diversity for populations at the species range edge, both on islands (Maskas and Cruzan, 2000; Rivera-Ocasio et al., 2002) and in continental systems (Jump et al., 2003; Petit et al., 2003; Van Rossum and Prentice, 2004).

CONSERVATION IMPLICATIONS

The Caribbean region is one of the 25 global biodiversity ‘hotspots’ and its unique biota is endangered by rapid population growth that fuels increasing habitat destruction. *Ipomoea microdactyla* is one of many plant species threatened by these landscape alterations, yet few studies have focused on the implications of biogeographical patterns of species-level genetic variation in guiding conservation efforts.

Significant regional differences in genetic variation highlight the need to sample across the breadth of a species’ range to adequately assess population differentiation and structure. The unique genetic makeup of populations within the three sampled areas, along with low levels of gene flow via seeds and pollen in this island system, suggests the need to maintain the integrity of these populations in future conservation plans. Areas that still retain large expanses of continuous habitat, like on Andros island, should be high priority targets for conservation, as populations there may harbor higher levels of species-wide genetic variation as in *Ipomoea microdactyla*. This does not negate the need to protect small isolated populations in other parts of the species range, as individuals there may still have high levels of heterozygosity and hold unique genetic variation. Additional data, collected in a similar manner from other plant taxa, will aid the creation of larger, regional management strategies to foster the conservation of the Caribbean’s imperiled flora.

Table 3.1 Characteristics of sampled *Ipomoea microdactyla* populations sorted by region.

Site	Latitude	Longitude	Habitat	Sample size	Region
1. Bill Sadowski	25.518194	80.501056	fragment	17	Florida
2. Campbell Dr.	25.475778	80.437444	fragment	18	Florida
3. Coral Reef	25.631750	80.322278	fragment	15	Florida
4. Moon/Schaffer	25.518167	80.517333	fragment	21	Florida
5. Turnpike 152	25.622528	80.378250	fragment	19	Florida
6. Ant Coppice	24.752944	77.841389	continuous	16	Bahamas
7. Atala Coppice	24.928833	78.026389	continuous	18	Bahamas
8. Ecotone	25.102722	78.152083	continuous	17	Bahamas
9. Forfar Station	24.893861	77.932083	continuous	20	Bahamas
10. Coast Ridge	24.856083	77.917417	continuous	17	Bahamas
11. Rhizophora	24.910056	77.985278	continuous	13	Bahamas
12. Cuba	23.131944	82.364167	continuous	21	Cuba

Coordinates in decimal degrees, population's habitat either isolated fragment or continuous forest, sample size used per population and geographical region.

Table 3.2 Statistics of genetic diversity at seven microsatellite loci in *Ipomoea microdactyla*: number of alleles (A); observed heterozygosity (H_O); expected heterozygosity (H_E); among population genetic differentiation (F_{ST}).

Locus	A	H_O	H_E	F_{ST}
bm757	8	0.321	0.506	0.147
ib286	11	0.766	0.686	0.092
ib318	6	0.468	0.499	0.324
s11	9	0.810	0.704	0.135
ssr01	13	0.520	0.561	0.101
ssr13	14	0.623	0.737	0.158
ssr14	5	0.311	0.422	0.087
Mean	9.4	0.545	0.558	0.149

Table 3.3 Statistics of genetic diversity for 12 populations of *Ipomoea microdactyla*: percent of polymorphic loci (P); mean number of alleles per locus (A); number of private alleles (A_{PV}); observed heterozygosity (H_O); expected heterozygosity (H_E); mean inbreeding coefficient per population (F_{IS}).

Population	P	A	A_{PV}	H_O	H_E	F_{IS}
1. Bill Sadowski	71	3.4	1	0.420	0.577	0.318
2. Campbell Dr.	100	4.0	0	0.516	0.599	0.109
3. Coral Reef	86	3.0	1	0.305	0.427	0.320
4. Moon-Schaffer	100	4.3	0	0.456	0.559	0.198
5. Turnpike 152	100	3.3	0	0.489	0.535	0.024
6. Ant Coppice	100	4.9	0	0.598	0.563	-0.054
7. Atala Coppice	100	5.6	5	0.540	0.674	0.0244
8. Ecotone	100	4.1	0	0.597	0.571	0.011
9. Forfar Station	100	6.4	0	0.750	0.705	-0.033
10. Coast Ridge	100	4.9	0	0.681	0.629	-0.087
11. Rhizophora	100	4.7	1	0.637	0.682	0.111
12. Cuba	100	6.3	4	0.558	0.630	0.152
Mean	96.4	4.6		0.559	0.591	0.037

Table 3.4 Analysis of molecular variance (AMOVA) for *Ipomoea microdactyla* based on seven microsatellite loci for 12 populations in three regions. * denotes significant values $P < 0.01$.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among regions	2	81.53	0.26	10.43*
Among populations within regions	9	69.57	0.16	6.49*
Within populations	412	860.11	2.09	83.09*
Total	423	1011.11	2.51	100

Table 3.5 Summary of Mann Whitney U tests performed on the genetic statistics A , H_O , H_E , and F_{IS} for *Ipomoea microdactyla* contrasting fragmented populations in Florida (USA) with continuous populations on Andros Island, the Bahamas.

Genetic statistic	Mean of Florida populations	Mean of Andros Island populations	P - value
A	3.6	5.1	< 0.001
H_O	0.437	0.633	< 0.001
H_E	0.539	0.637	0.006
F_{IS}	0.194	0.032	0.065

Figure 3.1 The population genetic structure of *Ipomoea microdactyla* constructed using seven polymorphic microsatellite loci. A total of 12 sites were chosen with 13 to 21 individuals sampled per population. The four clusters identified from STRUCTURE analysis with no prior knowledge of population classifications. Populations numbered as in Table 3.1.

Figure 3.2 Neighbor-joining tree based on genetic distances (Nei's D_A) between pairs of 12 populations of *Ipomoea microdactyla* from three regions (Florida, Bahamas, and Cuba) with statistical support from 1000 bootstraps (numbers to right of nodes). Populations numbered as in Table 3.1.

Figure 3.3 Association between linearized pairwise F_{ST} and geographical distances in km among 12 populations in three regions of *Ipomoea microdactyla* (Mantel test of correlation, $r = 0.305$, $P < 0.001$).

Figure 3.4 Association between linearized pairwise F_{ST} and geographical distances in km among 11 Florida (USA) and Andros Island (Bahamas) populations of *Ipomoea microdactyla* (Mantel test of correlation, $r = 0.365$, $P < 0.001$).

Figure 3.5 Association between linearized pairwise F_{ST} and geographical distances in km among 5 Florida (USA) populations of *Ipomoea microdactyla* (Mantel test of correlation, $r = 0.133$, $P < 0.001$).

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