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THE REPRODUCTIVE BIOLOGY OF *RUELLIA SUCCULENTA* (ACANTHACEAE) AND THE EFFECTS OF HABITAT FRAGMENTATION

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To: Dean Arthur W. Herriott
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This thesis, written by John H. Geiger, and entitled The Reproductive Biology of *Ruellia Succulenta* (Acanthaceae) and the Effects of Habitat Fragmentation, having been approved in respect to style and intellectual content, is referred to you for judgment.

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

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ABSTRACT OF THE THESIS

THE REPRODUCTIVE BIOLOGY OF *RUELLIA SUCCULENTA* (ACANTHACEAE) AND THE EFFECTS OF HABITAT FRAGMENTATION

by

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The purpose of this study was to determine the reproductive biology of *Ruellia succulenta* and to relate this to the effects of habitat fragmentation. Plants occurring in the intact habitat in Everglades National Park were compared to plants occurring in three different size classes of habitat fragments.

The results of the breeding system experiment show *R. succulenta* to be fully self-compatible and capable of autofertility via corolla abscission. Results of the inbreeding depression study supported theoretical expectations for selfing species of reduced inbreeding depression and its expression late in the life cycle. The most significant effect of habitat fragmentation, in terms of pollination ecology, was a highly significant difference in the proportion of Hymenopteran and Lepidopteran floral visitors by the size class of habitat. Time since last fire was the most important factor positively affecting reproduction in the different size classes of fragments.

TABLE OF CONTENTS

CHAPTER PAGE

I. BREEDING SYSTEM OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

II. EFFECTS OF SELF- AND CROSS-FERTILIZATION ON PROGENY FITNESS OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

III. EFFECTS OF HABITAT FRAGMENTATION ON THE POLLINATION ECOLOGY OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

LIST OF TABLES

TABLE PAGE

Chapter 1.

Chapter 2.

Chapter 3.

Chapter 4.

LIST OF FIGURES

FIGURE PAGE

Chapter 1.

Chapter 2.

Chapter 3.

Chapter 4.

BREEDING SYSTEM OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

ABSTRACT

This study examines the breeding system of *Ruellia succulenta* Small (Acanthaceae), an herbaceous perennial found in the pine rockland habitat of southern Florida. Experimental plants were obtained from stem tip cuttings of plants located in three sites in Long Pine Key, Everglades National Park. Hand pollination treatments were performed in the Florida International University greenhouse on 75 plants, 25 from each of the three sites. The treatments were: no treatment (control), anthers removed, self-pollinated, and cross-pollinated. The pollination protocol investigated facultative autogamy, apomixis, self-compatibility, and self-incompatibility. Treatments were monitored and fruit set and seed number per fruit from the hand pollinations were recorded. In addition to determining breeding system, the data also were used to evaluate inbreeding depression at the earliest life history stages (i.e. fruit and seed set), and the mechanism of self-pollination. Results showed *R. succulenta* to be fully self-compatible and facultatively autogamous. Plants were unable to set fruit without pollen deposition on the stigmas, indicating the lack of apomixis. There was no evidence of inbreeding depression in fruit set or seed set for the self- versus cross-pollination treatments. The mechanism of autofertility appeared to be delayed self-pollination as the corolla abscised and the anthers were dragged past the persistent stigma.

INTRODUCTION

A breeding system includes all facets of sex expression in a species that sets the pattern for the transmission of genes from one generation to the next (Wyatt 1983). In general, selfing restricts gene flow and may lead to inbreeding, which results in reduced genetic variation within populations and increased genetic variation among populations. Outcrossing enhances gene flow and may lead to the reduction of microhabitat divergence and genetic substructuring of populations (Hamrick and Godt 1990). The five basic types of plant breeding/mating systems are: 1) predominantly selfing, 2) predominantly outcrossing, 3) mixed mating (both selfing and outcrossing occurring), 4) partial apomixis, and 5) partial selfing of gametophytes as in ferns (Brown 1990).

The relationship between pollination and mating system recently has become an area of active research (see review, Barrett and Harder 1996). Previously, a majority of pollination studies tended to be strictly ecological whereas the mating system studies tended to be mostly theoretical, concerning population genetics (Barrett and Harder 1996). Much new research has focused on the interaction between the ecological aspects of plant mating and the different mating patterns' fitness consequences (Gregorius et al. 1987; Holsinger 1991; Lloyd 1992; Kohn and Barrett 1994). For pollination studies experimental evidence of the type of breeding system is necessary to evaluate the link between pollination and seed production (Wyatt 1983; Barrett and Eckert 1990).

In this paper I describe the results of an investigation of the breeding system of *Ruellia succulenta* Small, testing for self-compatibility vs. self-incompatibility. Data also are presented on this species' ability to self-pollinate. This report is part of a larger study evaluating the effects of habitat fragmentation on the reproduction of *R. succulenta*. Knowledge of the species' breeding system is essential in looking at habitat fragmentation effects (Spears 1987; Jennersten 1988; Lamont et al. 1993; Aizen and Feinsinger 1994; Oostermeijer et al. 1998; Morgan 1999; Cunningham 2000).

METHODS

Study Species

The "pineland petunia", *Ruellia succulenta,* is an herbaceous, long-lived perennial endemic to southern Florida (Avery and Loope 1980). The plant most commonly occurs in pine rockland (Snyder et al. 1990) but also is found in ecotones between short hydroperiod sawgrass and muhley grass marshes. Plants are trailing to ascending, usually less than 50 cm tall with one (most often) to more than 30 (rare) stems. I observed tagged buds on plants in the field and in the greenhouse to determine the sequence of maturation, flower opening, and floral longevity. I took several measurements on the greenhouse plants' flowers. The parameters were: overall corolla length, corolla tube length (sensu Barrow and Pickard 1985), flower face diameter, stigma exsertion, nectar volume, nectar sugar concentration, ovule number per ovary, pollen grain number per flower. Nectar volume was measured with calibrated microcapillary pipettes and the percent sugar concentration (on a weight per total weight basis) using a hand-held refractometer (Bellingham and Stanley © brand). The number of pollen grains per flower was estimated using the protocol of Kearns and Inouye (1993). All anthers from a flower were removed and added to a vial of alcohol. An aliquot from this vial was added to a hemacytometer and the number of pollen grains was counted. The number of ovules per ovary was obtained by dissecting ovaries from newly opened flowers and counting the number of ovules under a dissecting microscope.

Breeding System

Plants for the breeding system experiment were obtained from stem tip cuttings of adult plants. Stem tip cuttings *ca.* 10 cm in length were collected on 15 July 1999 from plants at three sites in Long Pine Key, Everglades National Park. This collection date occurs during the time of year when the stems are rapidly elongating. The three sites were each separated by several kilometers. One stem tip cutting was removed from 25 individual plants at each of the three sites and brought to the Florida International University greenhouse where they were potted in 6-celled plastic pots with soil-less potting mix. Initially, all plants were kept under a shadecloth-covered misting table for 2 weeks to maintain a high moisture level. All 75 cuttings rooted within two weeks and were transferred to 20 cm diameter plastic pots using the same soil less potting mix. The plants were grown under ambient light in the greenhouse and were given half-strength liquid fertilizer (Miracle-Gro 30-15-30 h) additions on 22 October 1999 and 29 November 1999 to stimulate growth and flowering.

The abscission of the corolla resulted in the anthers being dragged past the persistent stigma, which often remained attached to the ovary for over a week. To consider the amount of pollen deposited on the stigma of unpollinated plants in this manner, a sample of stigmas from two untreated flower groups was collected. The first group contained stigmas removed shortly before the corollas abscised and the second group contained stigmas from flowers that had already shed their corollas, referred to as corolla on and corolla off groups, respectively. Stigmas from both groups then were mounted in Fuchsin gel (Kearns and Inouye 1993) and examined under a light microscope to determine the number of pollen grains per stigma.

Hand pollination treatments were performed on plants in the insect free FIU greenhouse from 7 December 1999 through 29 February 1999. The four hand pollination treatments were: no treatment (control), anthers-removed (emasculation), self, and cross (Dafni 1992; Kearns and Inouye 1993). No open pollinated treatments, i.e. flowers in their natural habitat, were performed because of a lack of sufficient numbers of flowers and poor field conditions. The four treatments investigated facultative autogamy, apomixis, self-compatibility, and cross-incompatibility. The control procedure involved no floral manipulation. For the emasculation treatment, I removed all four anthers from the flower with tweezers. I used clean, wooden toothpicks to collect and deposit a large quantity of self and cross pollen on the stigma for the self and cross treatment, respectively. The number of pollen grains placed on the stigma greatly exceeded the number of ovules per ovary. Cross pollen was obtained from flowers of plants from one of the other populations to avoid crossing genetically similar individuals. For identification of all the treatments, I attached a small jeweler's tag around the pedicel of each treated flower. Fruit set and seed set were recorded for all the hand pollinations. To consider genotypic and/or site differences in breeding system, an attempt was made to perform at least two replicates of all four floral treatments on each of the 75 maternal plants.

Data Analysis

Statistical analyses were conducted using the GLM procedure of SPSS© version 10.0 (SPSS Inc. 1999). To test for differences in mean percent fruit set and mean number of seeds per fruit among the four hand pollination treatments, I performed 2-way Analysis of Variance tests. The two main effects were pollination treatment and site. I also tested the interaction between these two main effects. The percent fruit set data were arcsine square root transformed, where p = proportion of hand pollination treatments setting fruit per plant (Sokal and Rohlf 1981). Post hoc analyses were conducted using Bonferroni procedure if the Levene's test was not significant, while Dunnett C procedure was used if the Levene's test was significant. The data for mean number of seeds per fruit were normally distributed. A t-test was performed on the data for the two flower groups, corolla on and corolla off.

RESULTS

Flower Measures

All *Ruellia succulenta* plants observed were monostylous hermaphrodites, with no evidence the distyly reported by Long and Uttal in their 1962 study of *Ruellia caroliniensis* (a synonym of *Ruellia succulenta*) and three other congeners. In addition, all plants had only chasmogamous flowers with no cleistogamy as Long and Uttal (1962) described. Flowers, that last only one day, occur in subsessile clusters found in the leaf axils (Wunderlin 1998). The five-petaled lavender (rarely white or pink) flowers have a funnelform corolla *ca.* 4 cm long, the tube 2.49 ± 0.04 cm long with a flower face diameter 4.79 ± 0.06 cm (Table 1). The flowers are herkogamous (Faegri and Van der Pijl 1977; Lloyd and Webb 1986) with the bilobed stigma being exserted 0.38 ± 0.02 cm above the flower face (Table 1). The filaments of the four individual anthers are adnate halfway down the corolla tube and the anthers occur just below the rim of the corolla tube. In general, flowers open at sunrise and the corollas abscise in the early afternoon (flowers last one day), so they are visited by diurnal insects only. Floral rewards are pollen and nectar that is secreted at the proximal end of the corolla tube surrounding the base of the ovary. The percent sugar concentration of the nectar was 19.6 ± 0.20 and the nectar volume was 1.09 ± 0.06 μl (Table 1). There were no significant differences among flowers from the three sites in either percent sugar concentration or volume of nectar. The insect pollinators of *R. succulenta* include: butterflies, skippers, bombyliid flies, wasps, honeybees, and solitary bees (J. Geiger, personal observation). The number of pollen grains per flower was 4255 ± 343, and the number of ovules per ovary was 10.77 ± 0.03 (Table 1). There were most often an equal number of ovules in each of the two locules of the ovary. Carpels mature into glabrous capsules within one to three weeks containing up to 13 seeds (J. Geiger, personal observation). The fruit is explosively dehiscent, and the seeds are dispersed several meters by the aid of retinacula.

Flowers of *Ruellia succulenta* generally opened just after sunrise on clear days, but on cloudy/overcast days, flower opening was delayed several hours. While still in bud, the stigma was already fully extended above the plane of the anthers and so, upon anthesis, the stigma was pollen free. Anthers tended to split open *ca.* one hour after the petals unfurled; the split was longitudinal and faced toward the center of the corolla tube.

Pollen was sticky and formed clumps containing many individual pollen grains. Hand pollinations were performed between 8:00 and 10:00 am. By 1:00 pm, most of the corollas had abscised from the base of the ovary.

There were highly significant differences in the mean percent fruit set/treatment/plant for the four hand pollination treatments but not among sites (Table 2). The assumption of equality of variances for the ANOVA test was not met but because of the large sample sizes, the test was still robust. The cross- and self-pollination treatments resulted in the proportionately highest fruit set followed by the control, then anthers-removed, respectively (Figure 1). There was no significant interaction between floral treatment and site for the variable seed set/treatment/plant (Table 3). The post hoc test, using Dunnett C procedure, showed cross and self as the only two treatment pairs that did not significantly differ from one another (Figure 1).

There were also highly significant differences in the mean seed set/treatment/plant for the control, self, and cross hand pollination treatments (Table 2). The anthers-removed treatment was excluded from the ANOVA test because very few of these treatments set fruit. Only two out of the 80 minus-anther flower treatments resulted in fruit. The two minus-anther flower treatments that did set fruit contained 1 and 2 seeds, respectively. The ANOVA assumption of equality of variances was not met but the test was robust due to the large sample sizes. The mean number of seeds per fruit produced from the cross- and self-pollination treatments was nearly identical and both of these treatments were significantly larger than the control treatment (Figure 2). There were significant site differences for this variable when data for the three hand pollination treatments were pooled by site. However, the interaction between floral treatment and site was not significant (Table 3). The post hoc test, using Dunnett C procedure, showed that the cross and self treatments did not differ significantly from one another; both the cross and self treatments were significantly different from the control (Figure 2). Data from the two flower groups show an average of less than one pollen grain per stigma for the corolla "on" group and an average of *ca.* 27 pollen grains per stigma for the corolla "off" group (Figure 5). Using a t-test, significantly more pollen grains were deposited on stigmas that abscised corollas with adnate stamens than on stigmas with intact corollas ($t = 7.48$, $P < 0.001$).

DISCUSSION

Ruellia succulenta is self-compatible by the criteria of Bawa (1974) and highly self-compatible by those of Dafni (1992). The percent fruit set following self-pollination was 97%, perhaps not surprising for a monostylous hermaphroditic herb. Plants with this habit tend to show a high degree of self-compatibility (Bullock 1985). There was no significant difference in fruit set between the self- and cross-pollination treatments while both were significantly different from the control treatment. The percent fruit set of the anthers- removed treatment, at 2.1 %, may be due to experimental error. Included in the ANOVA calculations were several anthers removed treatments performed very early during the breeding system experiment that produced fruit. I do not know if this fruit set was the product of inexperience in removing the anthers, which may have resulted in pollen deposition on the stigma, or actual cases of apomixis. I believe the former is the more likely explanation as after the first week of hand pollination treatments, there were no more instances of anthers -removed treatments setting fruit. The percent fruit set following the control treatment, with no floral manipulation, was 67%. As these tests were performed in an insect free greenhouse, the results show that *R. succulenta* is facultatively autogamous, because it is able to set fruit in the absence of pollinators. The method of pollination seems to be the deposition of self pollen as the corolla abscises and drags the adnate anthers past the stigma, i.e. delayed self pollination (Dole 1990). The data on pollen grain number on stigmas before and after abscission support this hypothesis.

The similar seed set in the self- and cross-pollination treatments, and the significant difference between these treatments and the control further support the fruit set results indicating that *Ruellia succulenta* is fully self-compatible. Additionally, it offers proof of a lack of inbreeding depression at these initial life history stages (i.e. fruit set and seed set) for the populations sampled.

Fruits from the control treatment had on average *ca* 30% fewer seeds than fruits from the self and cross treatments. This reduced seed set may be due to pollination intensity, which is the number of pollen grains deposited on the stigma, and the relationship intensity has to seed set. Several studies have shown low levels of pollination intensity in the field for different species (Snow 1982; McDade 1983). While my study did not explicitly explore the link between pollination intensity and seed set, the data from pollen grain number on the two flower groups does point to pollination intensity limiting seed set. For the self and cross treatments, the entire stigmatic surface was covered with pollen grains, which probably numbered in the hundreds. It appears that enough pollen is deposited on the stigma by the abscission of the corolla to have some seed set, but not enough to result in near complete (*ca.* 9 seeds) seed set, as with the self and cross treatments. These findings support the idea that low pollination intensity from delayed autogamy results in reduced seed set for this species in the field (J. Geiger, personal observation).

In conclusion the *Ruellia succulenta* populations studied are fully self-compatible and show no signs of inbreeding depression in the earliest life history stages. Plants exhibited a high capacity for autofertility and the mechanism appeared to be the direct contact of the adnate anthers with the persistent stigma as the corolla abscises. This reproductive assurance mechanism may be especially important to the persistence of R. succulenta in habitat fragments where pollinators may be scarce. Further research could be conducted to determine the relationship between pollination intensity and seed set. Another interesting avenue of research would be to investigate the mating system of this species to establish whether primarily selfing, primarily outcrossing, or a mixed mating system occurs for these populations.

Table 1. Summary of floral measurements.

Flower component	# of flowers	# of genotypes	Mean ± SD
Corolla tube length	60	53	2.49 ± 0.29
Flower face diameter	78	62	4.79 ± 0.44
Stigma exsertion	60	53	3.83 ± 1.9
Nectar concentration	103	47	19.6 ± 0.20
Nectar volume	86	47	1.09 ± 0.06
Pollen grains per flower	15	15	4255 ± 343
Ovules per ovary	40	24	10.77 ± 0.97

Table 2. Two-way ANOVA testing the main effects of pollination treatment, site, and their interaction on percent fruit set.

Source of variation	df	SS (type III)	F	P
Treatment	3	68.97	248.59	< 0.001
Site	2	0.05	0.29	0.75
Treatment x site	6	0.31	0.55	0.767
Error	235	21.74		

Table 3. Two-way ANOVA testing main effects of pollination treatment, site, and their interaction on seed set per fruit.

Source of variation	df	SS (type III)	F	P
Treatment	2	250.97	27.09	< 0.001
Site	2	29.68	3.2	0.043
Treatment x site	4	7.17	0.39	0.818
Error	176	815.18		

Figure 1. Mean percentage of flowers producing fruits following four controlled pollination treatments. Treatments with different uppercase letters were found to be significantly different ($P < 0.05$) in a multiple-comparisons test. Percent treatment means shown below uppercase letters. N = number of plants and (flowers).

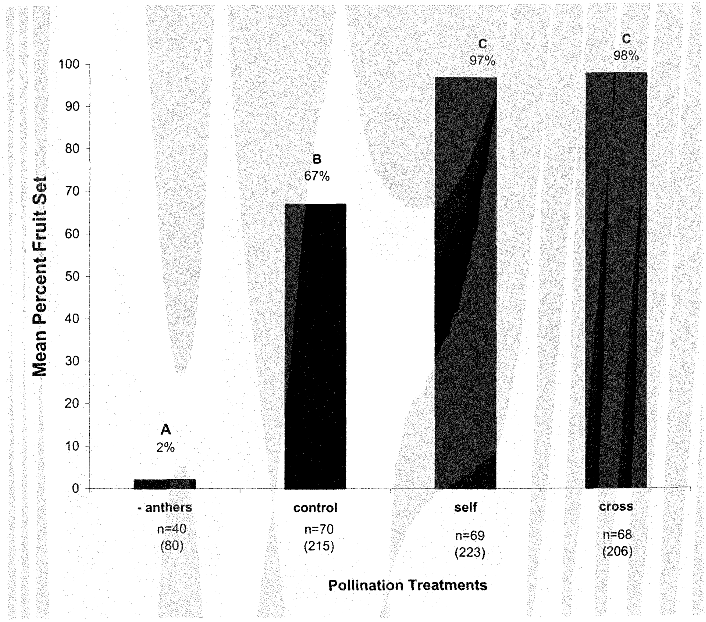


Figure 2. Mean number of seeds per fruit following four controlled pollination treatments. Treatments with different uppercase letters were found to be significantly different. N = number of plants and (flowers).

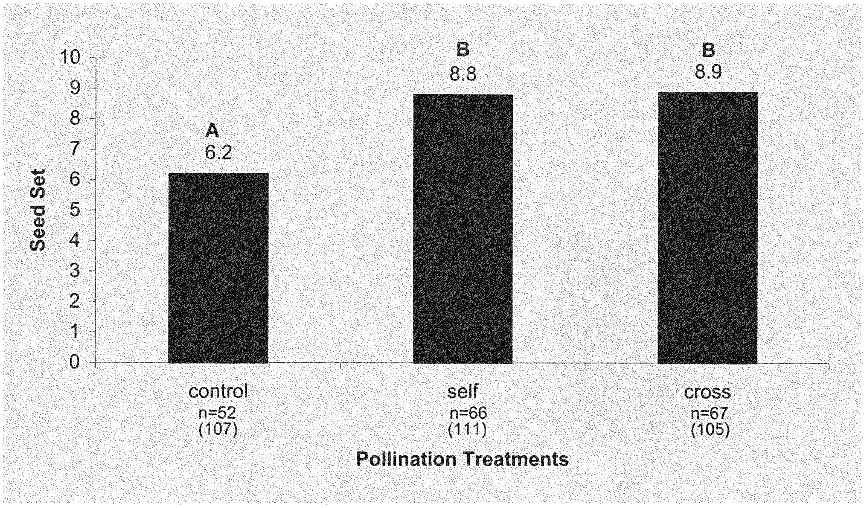
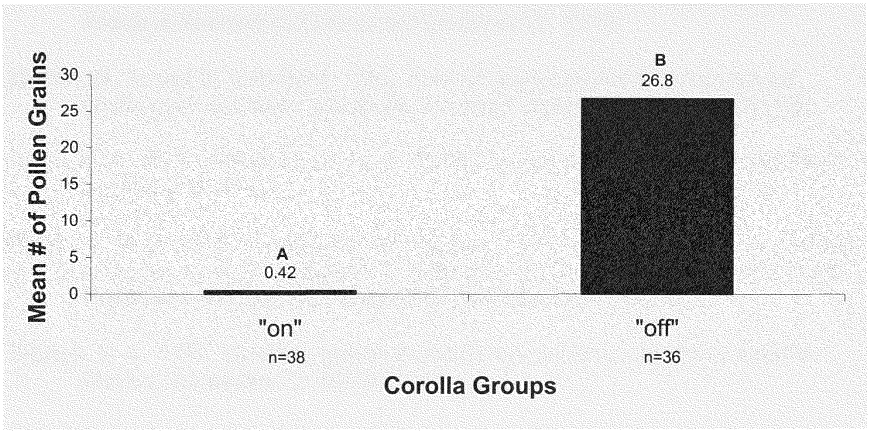


Figure 3. Mean number of pollen grains on stigmas for the two flower groups, corolla on and corolla off. The two corolla groups were found to be significantly different ($P < 0.001$) using a t-test; n = number of flowers.



LITERATURE CITED

Aizen, M. A., and P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine "chaco Serrano." Ecological Applications 4: 387-392.

Avery, G. N., and L. L. Loope. 1980. Endemic taxa in the flora of South Florida. Report T-558, Everglades Park South Florida Research Center. 39 pages. Available from: Everglades National Park, Homestead, FL.

Barrett, S. C. H., and C. G. Eckert. 1990. Variation and evolution of mating systems in seed plants. pp. 229-254 *in* S. Kawano, editor. Biological Approaches and Evolutionary Trends in Plants. Academic Press.

Barrett, S. C. H., and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Research in Ecology and Evolution 11: 73-79.

Barrow, D. A., and R. S. Pickard. 1985. Estimating corolla length in the study of bumble bees and their food plants. Journal of Apicultural Research 24: 3-6.

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85-92.

Brown, A. H. D. 1990. Genetic characterizations of plant mating systems. pp. 145-162 *in* Brown, A. H. D., Clegg, M. T., Kahler, A. L., and B. S. Weir, editors. Plant Population Genetics, Breeding and Genetic Resources. Sinauer.

Bullock, S. H. 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. Biotropica 17: 287-301.

Cunningham, S. A. 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in Mallee woodland. Conservation Biology 14: 758-768.

Dafni, A. 1992. Pollination Ecology, A Practical Approach. Oxford University Press, New York.

Dole, J. A. 1990. Role of corolla abscission in delayed self-pollination of *Mimulus guttatus*. American Journal of Botany 77: 1505-1507.

Faegri, K., and L. Van Der Pijl. 1979. The principles of pollination ecology. Pergamon Press, New York.

Gregorius, H-R., Ziehe, M., and M. D. Ross. 1987. Selection caused by self-fertilization I. Four measures of self-fertilization and their effects on fitness. Theoretical Population Biology 31: 91-115.

Hamrick, J. L., and M. J. Godt. 1990. Allozyme diversity in plant species. pp. 43-63 *in* Brown, A. H. D., Clegg, M. T., Kahler, A. L., and B. S. Weir, editors. Plant Population Genetics, Breeding and Genetic Resources. Sinauer.

Holsinger, K. E. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. The American Naturalist 138: 606-622.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado. Colorado.

Kohn, J. R., and S. C. H. Barrett. 1994. Pollen discounting and the spread of a selfing variant in tristylous *Eichornia paniculata*: evidence from experimental populations. Evolution 48: 1576-1594.

Lamont, B. B., P. G. L. Klinkhamer, and E. T. F. Witkowski. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* – a demonstration of the Allee effect. Oecologia 94: 446-450.

Lloyd, D. G. 1992. Self- and cross- fertilization in plants. II. The selection of self-fertilization. International Journal of Plant Science 153: 370-380.

Lloyd, D. G., and C. J. Webb. 1986. The avoidance of interference between the Presentation of pollen and stigmas in angiosperms, I Dichogamy. New Zealand Journal of Botany 24: 135-162.

Long, R. W., and L. J. Uttal. 1962. Some observations on flowering in *Ruellia* (Acanthaceae). Rhodora 64: 200-206.

McDade, L. A. 1983. Pollination intensity and seed set in *Tricanthera gigantea* (Acanthaceae). Biotropica 15: 122-124.

Morgan, J. W. 1999. Effects of population size on seed production in an endangered, fragmented grassland plant. Conservation Biology 13: 266-273.

Oostermeijer, J. G. B., S. H. Luijten, Z. V. Krenova, and H. C. M. Nijs. 1998. Relationships between population and habitat characteristics of the rare *Gentiana pneumonanthe* L. Conservation Biology 12: 1042-1053.

Snow, A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia.* Oecologia 55: 231-237.

Snyder, J. R., A. Herndon, and W. B. Robertson. 1990. South Florida rockland. Pages 230-277, in R. L. Myers, and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, University Presses of Florida, Orlando.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, San Francisco.

Spears, E. E. 1987. Island and mainland pollination ecology of *Centrosema virginianum* and *Opuntia stricta*. Journal of Ecology 75: 351-362.

SPSS Inc. 1999. SPSS Base 10.0 User's Guide. SPSS Inc., Chicago.

Wunderlin, R. P. 1998. Guide to the vascular plants of Florida. University Press of Florida, Gainesville, FL.

Wyatt, R. 1983. Pollinator-plant interactions and the evolution of breeding systems. pp. 51-95 *in* L. Real, editor. Pollination biology. Academic Press, Orlando.

EFFECTS OF SELF- AND CROSS-FERTILIZATION ON PROGENY FITNESS OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

ABSTRACT

A study was conducted to determine both the timing and magnitude of inbreeding depression for *Ruellia succulenta* Small (Acanthaceae). This species is an herbaceous perennial plant common in the pine rockland habitat of southern Florida and has a partial selfing/partial outcrossing breeding system. Measures of inbreeding depression were taken at several stages of the life cycle. Experimental plants were obtained from cuttings, in three sites in Long Pine Key, Everglades National Park. The inbreeding depression study was carried out in the Florida International University greenhouse. Results showed evidence of statistically significant inbreeding depression for only one parameter of the life cycle, seed weight. The cumulative measure of inbreeding depression for three stages of the life cycle agrees with theoretical predictions that selfing species show lower levels of inbreeding depression than outcrossing species. In addition, the majority of inbreeding depression in *R. succulenta* was expressed late in the life cycle at the stages of offspring growth and reproduction, also in agreement with theory. Finally, it appears likely that selfing has evolved in this species primarily as a means of reproductive assurance in the face of pollinator limitation.

INTRODUCTION

In 1876, Charles Darwin described the results of crossing experiments on 57 species of angiosperms: 1) outcrossed progeny tended to be more vigorous than inbred progeny, and 2) crosses between inbred lines resulted in highly vigorous offspring. His were among the first data to show evidence of inbreeding depression and hybrid vigor. High levels of inbreeding depression exist in natural plant populations (Price and Waser 1979; Schemske 1983; Schoen 1983; Waller 1984; Kalisz 1989; Karron 1989; Dudash 1990; Fenster 1991; Johnston 1992; see review, Charlesworth and Charlesworth 1987). Two genetic causes have been offered to explain inbreeding depression. The first hypothesis, overdominance, posits that inbreeding depression is due to heterozygote advantage at viability loci. The second hypothesis, partial dominance, states that inbreeding depression is due to recessive deleterious alleles.

Since inbreeding depression is the most general selective disadvantage of self-fertilization, it is a potentially significant factor in the evolution of plant breeding systems (Charlesworth and Charlesworth 1979). For an initially outcrossing population, an allele that enhances self-fertilization with no subsequent affect on pollen production will become more common because of its transmission advantage (Fisher 1941). Inbreeding depression (δ) is defined as $\delta = 1 - w_s / w_x$, where w_s and w_x denote the two separate mean fitnesses of self- and outcross-progeny, respectively (Charlesworth and Charlesworth 1987). To counter the transmission advantage and for outcrossing to be preserved, many models predict that inbreeding depression has to be over 0.50, with the assumption of a constant value of inbreeding depression (Maynard Smith 1978;

Charlesworth and Charlesworth 1979; Lloyd 1979; Lande and Schemske 1985). The threshold value of 0.50 can be modified by several factors. Holsinger et al. (1984) proposed pollen discounting while others offered "reproductive assurance", i.e. a higher probability of pollination in self-fertilizing than in outcrossing plants, as potential modifiers (Baker 1955; Jain 1976; Lloyd 1979). Most theoretical models for the evolution of mating systems have predicted only two possible outcomes, complete outcrossing or complete selfing (Lloyd 1979; Lande and Schemske 1985; but see refutation, Waller 1986). However, under special conditions, mixed-mating systems combining both selfing and outcrossing have been predicted (Lloyd 1980; Holsinger 1986; Uyenoyama and Waller 1991). While there exists a large body of theoretical works on these topics, fewer studies have considered the fitness effects for progeny created by selfing or outcrossing in the greenhouse or the field (see reviews, Charlesworth and Charlesworth 1987; Husband and Schemske 1996).

In addition, less is known about influences on the timing of inbreeding depression, that is, the stage (e.g. seed, seedling, adult) or aspect (e.g. seed weight, days to germination, fruit set) in the life cycle in which inbreeding depression is expressed. The idea that the effects of inbreeding may not be equivalent at different life-history stages was first shown by Schemske (1983) and Schoen (1983). In a review of published literature, Charlesworth and Charlesworth (1987) concluded that inbreeding depression was different among stages in gymnosperms, but no result could be offered in angiosperms, as published data were insufficient at that time. In a more recent review, Husband and Schemske (1996) found that most selfing species expressed the majority of inbreeding depression late in their life cycles, while outcrossers commonly had substantial inbreeding depression at all life-history stages.

In this paper, I present estimates of inbreeding depression at several life-history stages for three populations of the perennial herb, *Ruellia succulenta* (Acanthaceae), from Long Pine Key, Everglades National Park. This species exhibits characters promoting outcrossing (spatial separation of anthers and stigma, as well as nectar and pollen floral rewards) but self-pollination in the absence of insect pollinators is common via corolla abscission and results in high percent fruit set and viable seeds (Chap. 1). There is also evidence that most flowers visited by insects receive a large amount of pollen on their stigmas and this pollen load most likely contains a mixture of both outcross- and self-pollen (Chap. 3). This species exhibits characteristics of a mixed mating system. The timing and magnitude of inbreeding depression were determined by comparing the fruit set, seed set, seed weight, seed germination, days to germination, and on the 80-day –old offspring: flower production, fruit set, seed set, and final biomass of selfed and outcrossed progeny.

Natural History

Ruellia succulenta Small (Acanthaceae) is an herbaceous perennial common in the pine rockland habitat of southern Florida (Avery and Loope 1980; Snyder et al. 1990). Plants are hermaphroditic and capable of producing flowers year round with a peak during the wet season from May to October. The five petaled lavender flowers, which open at sunrise and last until early afternoon, have a lavender funnelform corolla. The plant is fully self-compatible and capable of autogamous reproduction via delayed self-pollination as the corolla abscises in early afternoon and drags the adnate anthers past the persistent stigma (Chap. 1). Flowers are visited by butterflies, skippers, bombyliid flies, wasps, honeybees, and primarily solitary bees. This last group accounts for most of the insect pollination in the populations used in this study. The capsular fruits, containing up to 13 seeds (Geiger, personal observation), are explosively dehiscent. The seeds may be dispersed several meters by the aid of retinaculae and perhaps even further by floating in seasonally flooded habitats.

METHODS

Stem tip cuttings of mature plants were collected on 15 July 1999 from three sites each separated by several kilometers in Long Pine Key, Everglades National Park. The sites will be referred to as EG (entrance gate), PT (pineland trail), and RF (Redd fingerglade). One stem tip cutting was removed from each of 25 individual plants at the three sites; cuttings were propagated in the greenhouse. The hand pollination treatments were performed in the insect free FIU greenhouse from 7 December 1999 through 29 February 2000. I used clean, wooden toothpicks to collect and deposit a large quantity of self and outcross pollen on the flower's stigma for the self and outcross treatments, respectively. Outcross pollen was obtained from flowers of plants from one of the other populations to avoid crossing possible siblings or even same genotypes within each population. To consider genotypic and site differences in inbreeding depression, an attempt was made to perform at least two replicates of each of the floral treatments on the maternal plants. Data on fruit set and seed set were recorded for all hand pollinations and pooled per maternal plant for the self- and outcross-treatments.

Inbreeding Depression

To quantify inbreeding depression at different life-history stages, I performed controlled hand pollinations on plants derived from the field and grew the resulting offspring in the greenhouse. Inbreeding depression values were computed using the formula:

$$\delta = 1 - w_s / w_x ,$$

where w_s and w_x denote the two separate mean fitnesses of self- and outcross-progeny, respectively (Charlesworth and Charlesworth 1987). The maternal-family plant values are pooled in this calculation, i.e. the values are not segregated by maternal family (Johnston and Schoen 1994). Using this formula, positive inbreeding depression values show outcrossed individuals outperform selfed individuals, while negative values show selfed individuals outperform outcrossed individuals.

Seed Quality

Studies of F1 seed viability, offspring growth and offspring survival, including F1 reproduction, were performed on the progeny resulting from the hand pollinations. Seeds were removed from the mature fruits and stored in individually labeled paper coin envelopes. A random sample of 25 seeds from each of the two hand pollination treatments (self- and outcross-) from each of the three sites (EG, PT, and RF) was chosen for the progeny fitness tests. This resulted in a total of 150 seeds representing, on average, 22 maternal plants from each site out of a possible 25 maternal plants. Each seed was weighed on a Mettler balance in the laboratory to the nearest 0.1 mg. The seeds were planted in individually labeled 6-celled plastic pots with soil-less potting mix in the greenhouse under ambient light on 31 May 2000. Data on whether seeds germinated, and, if yes, days to germination were recorded for each individual seed. Emergence date was also recorded for each seedling. As seeds germinated, the seedlings were transferred into individually labeled 13 cm pots with the same soil-less potting mix.

Seedling Fitness

For 80 days post-germination the number of flowers produced, fruit set and the number of seeds per fruit were recorded for each seedling. A sub sample of 10 randomly chosen plants per each pollination treatment per site (for a total of 60 plants) was sequentially harvested after 80 days to investigate total plant biomass (both roots and shoots). Any remaining fruit were removed and the soil was gently washed from the roots. Plants were placed in individually labeled aluminum foil bags and dried in a drying oven at 35 C for one month. The dried plants were then weighed on a Mettler balance to the nearest 0.001 g.

Data Analysis

Statistical analyses were conducted using SPSS version 10.0 (SPSS Inc. 1999). Data for percent fruit set from hand pollinations, percent germination of seeds, and percent fruit set of F1 plants were arcsine square-root transformed (Sokal and Rohlf 1981). For all of the variables, two-way ANOVAs were performed to test the main effects of pollination treatment and site as well as the interaction between these two main effects. As pollination treatment only had two groups (self and outcross), t-tests were also performed but the results were qualitatively no different from the ANOVA tests. Multiple comparisons tests using the Bonferroni procedure were necessary to investigate site differences for the variables of seed set of the hand pollinations and seed weight.

RESULTS

Hand Pollinations

Percent fruit set did not differ significantly for the two cross types, self and outcross (Table 1, Table 5). Fruit set following hand pollinations was high for both treatment groups (Figure 1). There was no significant difference for seed set per fruit for the two cross types (Table 5), but there was a significant site difference for this variable (Table 1). Mean seed set per fruit was significantly different between the sites EG and RF (Figure 3). The mean seed set was marginally higher for the outcross treatment when the data were pooled by pollination treatment (Figure 2).

Seed Quality

There were significant differences in the seed weight of the two pollination treatments (Table 2, Table 5) with the outcross seeds weighing more than the self seeds (Figure 4). There was also a significant site difference among the seed weights (Figure 5). However, the interaction between pollination treatment and site was not significant (Table 2). Seed germination exceeded 95% for both pollination treatments (Figure 6).

Percent germination and days to germination did not vary significantly among the treatments (Table 2; Figures 6 and 7).

Seedling Fitness

The number of flowers produced and percent fruit set were not significantly different by pollination treatment, site or their interaction (Table 3) for the F1 plants. Self progeny on average flowered more than outcross progeny (Figure 8), although the differences were not significant (Table 5). The outcross offspring had marginally higher percent fruit set than the self-seedlings (Figure 9), and more seeds per fruit than the self-seedlings (Figure 10), but these differences were not significant (Table 5). There were no significant differences of seed set per fruit by pollination treatment or site. Significant differences were found in the interaction of pollination treatment by site for this variable (Table 3). For sites PT and RF, the outcross offspring had greater numbers of seeds per fruit than the self-seedlings, but for site EG, the opposite was true. There was no mortality of any of the seedlings. The final variable of total seedling biomass showed no significant differences by pollination treatment, site, and their interaction (Table 4). The outcross offspring weighed more, on average, than the self offspring, but the difference was not significant (Figure 11).

Inbreeding Depression

Pollination treatment did not significantly influence either fruit set or seed number per fruit for the hand pollinations, and the inbreeding depression measures were low for both variables (Table 5). The nonsignificant results for these two variables always favored outbreeding reproduction. Seed weight was the only variable that showed a significant effect of cross type, still, the inbreeding depression measure was a low 6% (Table 5). There were no significant differences of cross type for both percent germination and days to germination of seeds (Table 5). The inbreeding depression measure of -0.036 for days to germination and –0.251 for number of flowers per offspring shows selfed progeny outperformed outbred progeny. For the final variables taken on the 80-day-old offspring, there were no significant differences between the two pollination treatments (Table 5). The inbreeding depression measures for fruit set and number of seeds per fruit for the offspring were the highest of all the variables, but were still relatively low. The cumulative inbreeding depression over three life stages (seed production on maternal plants + germination + growth and reproduction of offspring) was 0.171. Including the fourth life stage of seedling survival would not change the value of 0.171, as inbreeding depression at this stage was zero.

DISCUSSION

In this study, self- and cross-pollinated flowers had near identical fruit set from controlled hand pollinations, both near 100%. Selfed and outcrossed flowers produced statistically indistinguishable numbers of seeds. Several other self-compatible species show no effect of inbreeding on number of seeds per fruit: *Costus guanaiensis* (Schemske 1983), *Gilia achilleifolia* (Schoen 1983), *Astragalus linifolius, A. lonchocarpus* (Karron 1989), and *Lobelia cardinalis* and *L. siphilitica* (Johnston 1992). On the other hand, an inbreeding effect at this early stage was detected in *Delphinium nelsonii* (Price and Waser

1979), *Costus allenii* and *C. laevis* (Schemske 1983), *Sabatia angularis* (Dudash 1990), and *Amsinckia grandiflora* (Weller and Ornduff 1991). There were significant site differences for mean number of seeds per fruit when the pollination treatments were pooled by site (Fig. 3). This result may point to genetic differences in reproduction between plants from these three populations as care was taken to maintain equivalent environmental conditions in the greenhouse. For fully self-compatible *Ruellia succulenta,* inbreeding depression appears to be lacking at these two earliest stages of the life cycle.

The only life history parameters that showed statistically significant inbreeding depression was seed weight. Pooled by pollination treatment, the outcrossed seeds were 6 % heavier than the selfed seeds. Significant differences in mean seed weight were also found when the data were pooled by site (Figure 5). In contrast, Karron (1989) and Dudash found no significant differences in mean seed mass, while Kalisz (1989) did. It seems that these significant seed-mass differences did not cause detectable fitness differences following germination, as none of the subsequent stages revealed statistically significant inbreeding depression. This is a surprising result, as seed weight has been shown to be an important determinate of seedling performance for other species (Winn 1988; Kalisz 1989).

For the seed germination, there were no differences in mean percent germination or days to germination. Although the selfed seeds weighed less, on average, than the outcrossed seeds, they tended to germinate more quickly. Similarly, no inbreeding effect on germination was found in *Gilia achilleifolia* (Schoen 1983), *Costus guanaiensis* and *C. laevis* (Schemske 1983), and *Sabatia angularis* (Dudash 1990). In contrast, a significant effect was shown for *Costus allenii* (Schemske 1983), and *Collinsia verna* (Kalisz 1989).

The final measurements taken on the 80-day-old F1 plants revealed no further evidence of significant inbreeding depression at these later stages of growth and reproduction. Survival of the offspring was 100 % in the benign conditions in the greenhouse. Johnston (1992) reported very different results from his study carried out in the field, rather than the greenhouse. Both *Lobelia cardinalis* and *L. siphilitica*, had significant inbreeding depression in survival. Perhaps if the *Ruellia succulenta* seeds had been sown in the field, there may have been a greater chance for inbreeding depression to be expressed at this stage. The rest of the stages measured, while not significant, did reveal the outbred progeny to outperform the inbred progeny, on average.

My results seem to support those obtained by Husband and Schemske (1996) in their review article. The value of cumulative inbreeding depression for *Ruellia succulenta* is 0.21, which includes the stages of maternal plant seed set, percent germination of seeds, and fruit set and biomass of F1 plants. Husband and Schemske (1996) offered a range of cumulative inbreeding depression values for predominantly selfing species (N = 54 species) from – 0.15 to 0.51. While evidence suggests *R. succulenta* has a mixed-mating breeding system (Chap. 3), its cumulative measure of inbreeding depression is in the range of the predominantly selfing species considered (species with intermediate mating systems were excluded from the analysis). This result agrees with the theoretical prediction that selfing reduces the magnitude of inbreeding depression. Again, as far as the timing of inbreeding depression, my results support those of Husband and Schemske (1996). Most self-fertilizing species expressed the majority of their inbreeding depression late in their life cycle, at the stage of growth and reproduction; this was the case for my study species.

Both genetic and ecological factors may have been important for the evolution of selfing in *Ruellia succulenta*. This species is associated with frequently disturbed (i.e. pyric subclimax) habitats and occurs in a wide range of population densities. It is likely that high levels of inbreeding in local populations and population bottlenecks may have purged deleterious recessive alleles and hence, favored the evolution of selfing (e.g., Lande and Schemske 1985; Agren and Schemske 1993). Finally, "reproductive assurance" through delayed self-pollination may have contributed to the success of this colonizing species in newly disturbed areas. Evidence shows that a large minority of stigmas have low pollen loads by insect vectors alone (Chap. 3). This indicates that pollinators can limit female reproductive success. Self-pollination is expected to evolve if pollinator limitation is sufficiently high (Lloyd 1979).

Table 1. Two-way ANOVA testing main effects of pollination type, site, and their interaction on fecundity components (fruit set and seed set) from hand pollination treatments. Significant p-values are in bold.

Fecundity components	Source of variation	*df*	*F*	*P*
Fruit set	Pollination type	1	0.531	0.467
	Site	2	0.206	0.814
	Pollination x site	2	0.593	0.554
Seed set	Pollination type	1	0.148	0.701
	Site	2	3.605	**0.03**
	Pollination type x site	2	0.026	0.971

Table 2. Two-way ANOVA testing main effects of pollination type, site, and their interaction on seed quality (seed weight, percent germination, and days to germination). Significant p-values are in bold face.

Seed quality	Source of variation	*df*	*F*	*P*
Seed weight	Pollination type	1	4.856	**0.029**
	Site	2	4.339	**0.015**
	Pollination type x site	2	0.155	0.856
Percent germination	Pollination type	1	0	1
	Site	2	1.043	0.355
	Pollination type x site	2	0.379	0.971
Days to germination	Pollination type	1	0.769	0.382
	Site	2	0.393	0.676
	Pollination type x site	2	1.626	0.2

Table 3. Two-way ANOVA testing main effects of cross type, site, and their interaction on fecundity components (number of flowers, fruit set, and seed set) of 80-day-old F1 plants. Significant p-values are in bold face.

Fecundity components	Source of variation	*df*	*F*	*P*
Number of flowers	Pollination type	1	1.859	0.175
	Site	2	0.298	0.743
	Pollination type x site	2	1.574	0.211
Fruit set	Pollination type	1	2.055	0.155
	Site	2	1.738	0.181
	Pollination type x site	2	1.194	0.307
Seed set	Pollination type	1	3.393	0.068
	Site	2	1.018	0.365
	Pollination type x site	2	4.915	**0.009**

Table 4. Two-way ANOVA testing main effects of cross type, site, and their interaction on total plant biomass of 80-day-old F1 plants.

Source of variation	*df*	*F*	*P*
Cross type	1	0.424	0.518
Site	2	0.697	0.502
Cross type x site	2	0.579	0.564

Table 5. Inbreeding depression measures (δ) and results of t-tests (self vs outcross) for several life-history stages of maternal plants and their progeny. Significant p-values are in bold face, * p< .05.

Life-history stage	Generation	Inbreeding depression (δ)	Sig. of t-test (*p*-value)
Fruit set	Maternal plants	0.01	ns (0.463)
Seed set	Maternal plants	0.011	ns (0.690)
Seed weight	Progeny	0.061	*** (0.032)**
Percent germination	Progeny	0	ns (1.000)
Days to germination	Progeny	-0.036	ns (0.404)
Number of flowers	Progeny	-0.251	ns (0.175)
Fruit set	Progeny	0.162	ns (0.155)
Seed set	Progeny	0.211	ns (0.119)
Biomass	Progeny	0.038	ns (0.512)

Figure 1. Mean percentage of flowers producing fruits following two controlled pollination treatments. Percent treatment means shown above bars. N = number of plants and (flowers).

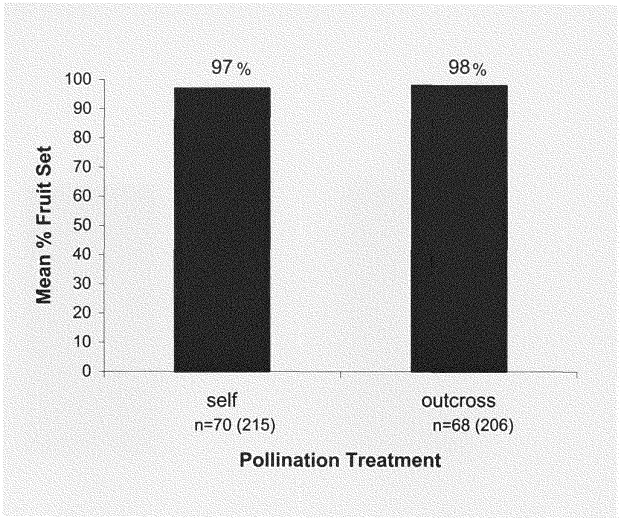


Figure 2. Mean number of seeds per fruit following two controlled pollination treatments. Treatment means shown above bars. N = number of plants and (fruits).

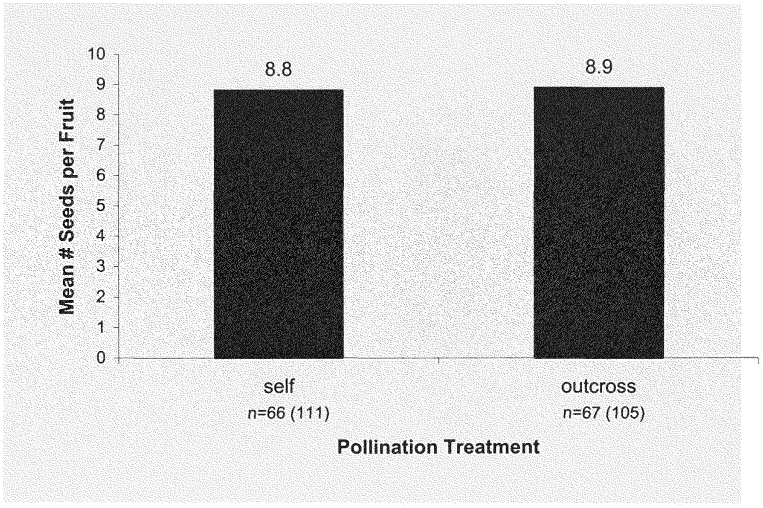


Figure 3. Mean number of seeds per fruit following two controlled pollination treatments pooled by site. Sites with different uppercase letters were shown to be significantly ($p < 0.05$) different. Site means shown below uppercase letters. N = number of plants and (fruits).

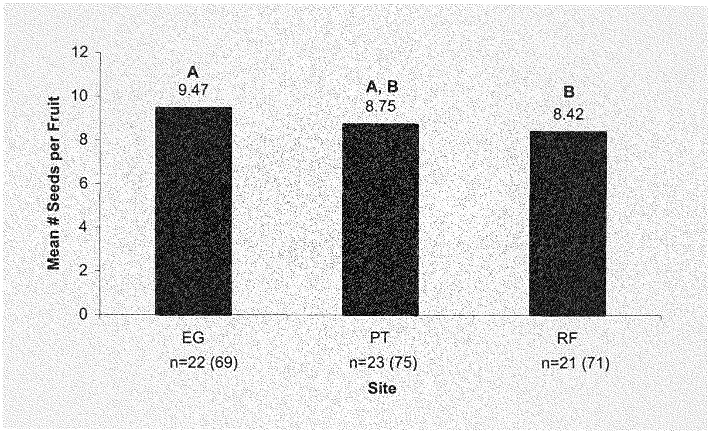


Figure 4. Mean seed weight (mg) following two controlled pollination treatments. Pollination treatments with different uppercase letters were shown to be significantly ($p < 0.05$) different. Pollination treatment means shown below uppercase letters. N = number of seeds.

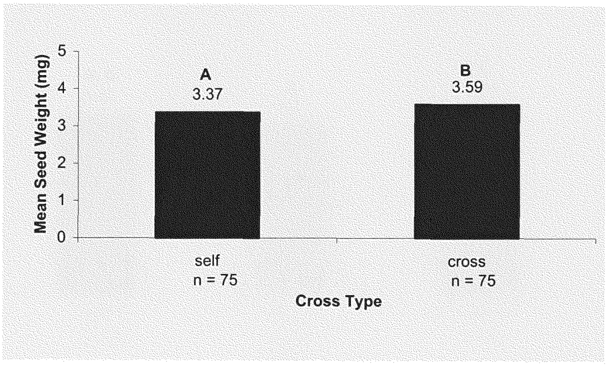


Figure 5. Mean seed weight (mg) following two controlled pollination treatments pooled by site. Sites with different uppercase letters were shown to be significantly ($p < 0.05$) different. Site means shown below uppercase letters. N = number of seeds.

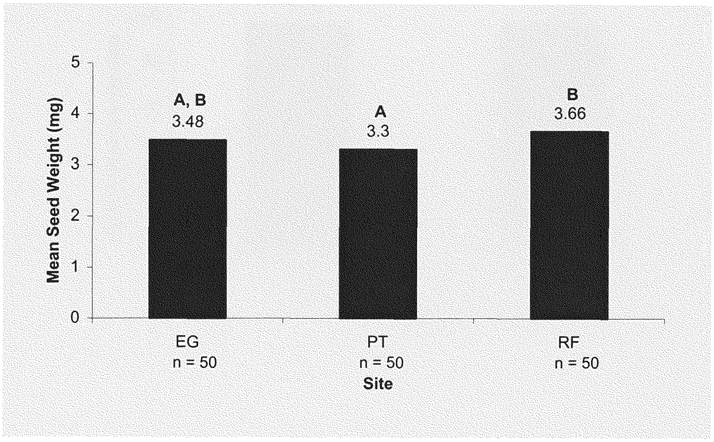


Figure 6. Mean percent seed germination of progeny from controlled pollination treatments. Crosstype means shown above bars. N = number of seeds.

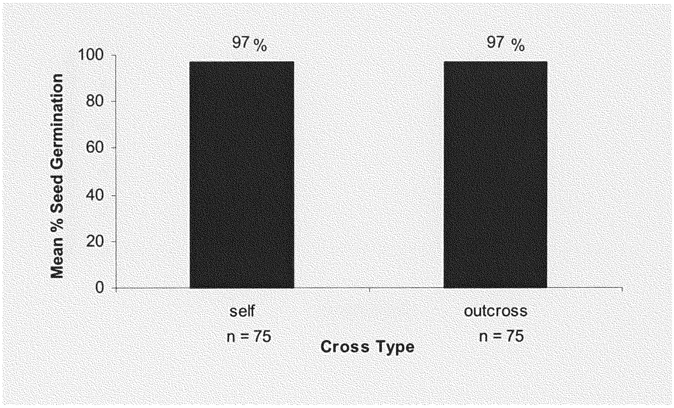


Figure 7. Mean number of days to germinate for progeny from controlled pollination treatments. Pollination treatment means shown above bars. N = number of seeds.

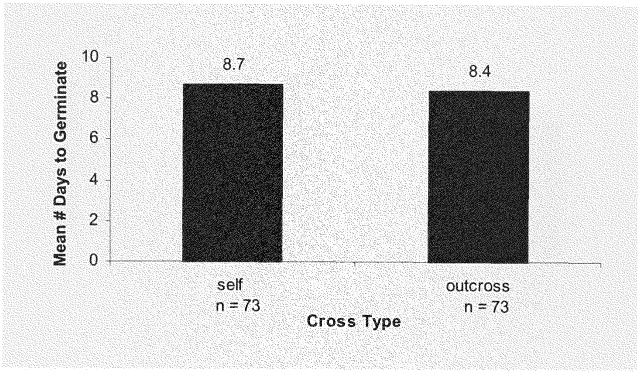


Figure 8. Mean number of flowers produced by 80-day-old F1 plants. Pollination treatment means shown above bars. N = number of plants.

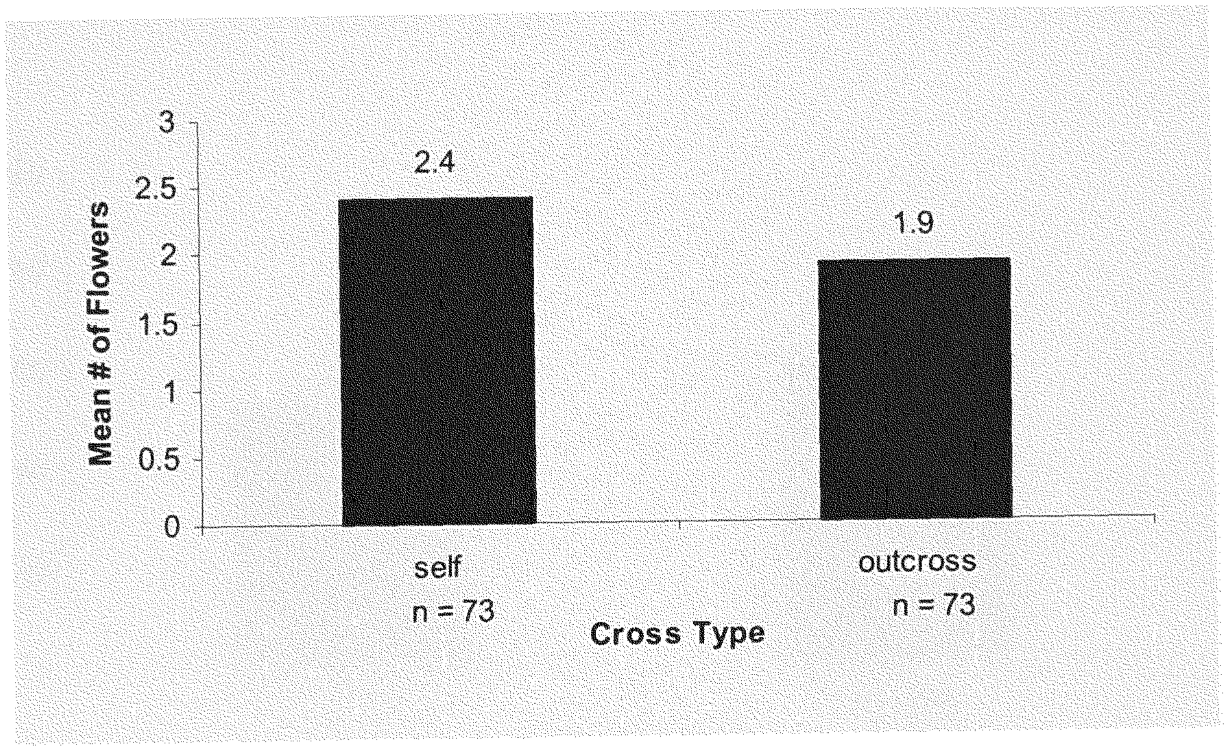


Figure 9. Mean percent fruit set for 80-day-old F1 plants. Pollination treatment means shown above bars. N = number of plants.

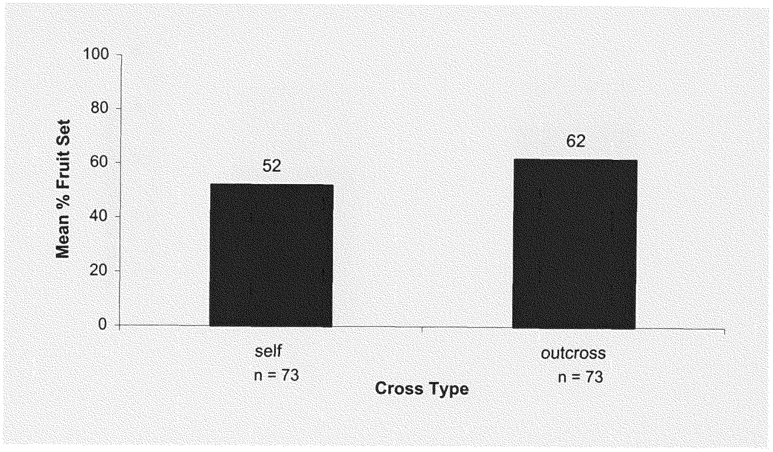


Figure 10. Mean number of seeds per fruit produced by 80-day-old F1 plants. Pollination treatment means shown above bars. N = number of plants.

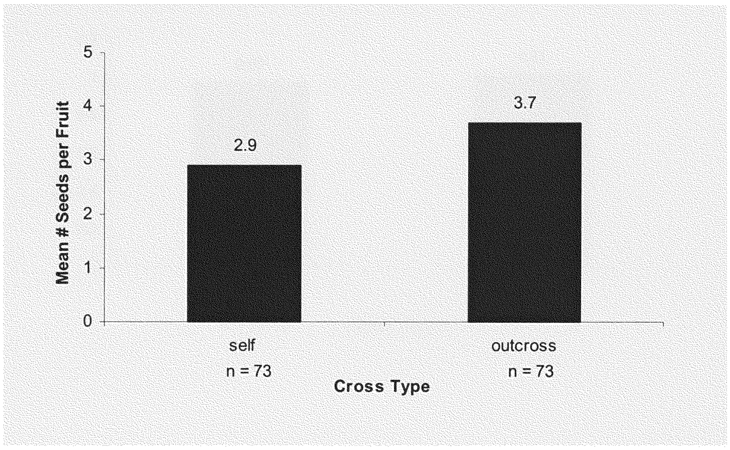
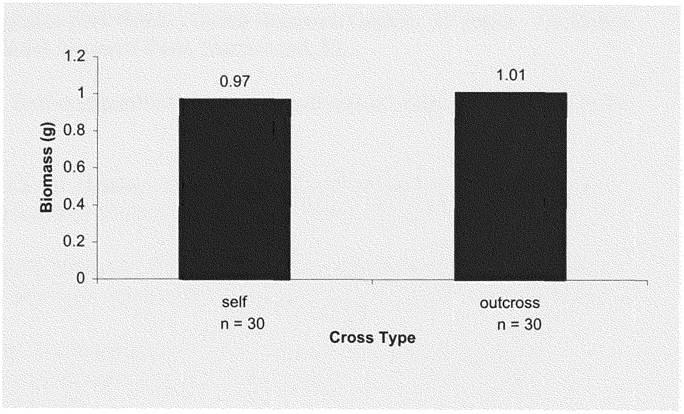


Figure 11. Mean biomass (g) of the 80-day-old F1 plants. Pollination treatment means are shown above the bars. N = number of plants measured.



LITERATURE CITED

Agren, J., and D. W. Schemske. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. Evolution 47: 125-135.

Avery, G. N., and L. L. Loope. 1980. Endemic taxa in the flora of South Florida. Report T-558, Everglades Park South Florida Research Center. 39 pages. Available from: Everglades National Park, Homestead, FL.

Baker, H. G. 1955. Self-compatibility after "long-distance" dispersal. Evolution 11: 449-460.

Barrett, S. C. H., and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Research in Ecology and Evolution 11: 73-79.

Barrow, D. A., and R. S. Pickard. 1985. Estimating corolla length in the study of bumble bees and their food plants. Journal of Apicultural Research 24: 3-6.

Charlesworth, B., and D. Charlesworth. 1979. The evolutionary genetics of sexual systems in flowering plants. Proceedings of the Royal Society of London 205: 513-530.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding Depression and its Evolutionary consequences. Annual Review of Ecology and Systematics 18: 237-268.

Darwin, C. 1876. The Effects of Cross and Self-Fertilization in the Vegetable Kingdom. John Murray, London.

Dole, J. A. 1990. Role of corolla abscission in delayed self-pollination of *Mimulus guttatus*. American Journal of Botany 77: 1505-1507.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments. Evolution 44: 1129-1139.

Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) II. Gene establishment. Evolution 45: 410-422.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. Annals of Eugenics 11: 53-63.

Holsinger, K. E. 1986. Dispersal and plant mating systems: The evolution of self-fertilization in subdivided populations. Evolution 40: 405-413.

Holsinger, K. E. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. The American Naturalist 138: 606-622.

Holsinger, K. E., M. W. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants: A population genetic model. The American Naturalist 124: 446-453.

Jain, S. K. 1976. The evolution of inbreeding in plants. Annual Review of Ecology and Systematics 7: 469-495.

Johnston, M. O. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. Evolution 46: 688-702.

Kalisz, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. Evolution 43: 1263-1272.

Karron, J. D. 1989. Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of *Astragalus* (Fabaceae). American Journal of Botany 76: 331-340.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39: 24-40.

Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. The American Naturalist 113: 67-79.

Lloyd, D. G. 1980. Demographic factors and mating patterns in angiosperms, pp. 67-88. in O. T. Solbrig, editor. Demography and Evolution in Plant Populations. Blackwells, Oxford.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press, London.

Price, M. V., and N. M. Waser. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsonii*. Nature 277: 294-296.

Schemske, D. W. 1983. Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). Evolution 37: 523-539.

Schoen, D. J. 1983. Relative fitnesses of selfed and outcrossed progeny in *Gilia achilleifolia (*Polemoniaceae). Evolution 37: 292-301.

Snyder, J. R., A. Herndon, and W. B. Robertson. 1990. South Florida rockland. Pages 230-277, in R. L. Myers, and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, University Presses of Florida, Orlando.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, San Francisco.

SPSS Inc. 1999. SPSS Base 10.0 User's Guide. SPSS Inc., Chicago.

Uyenoyama, M. K., and D. M. Waller. 1991. Coevolution of self-fertilization and inbreeding depression. II. Symmetric overdominance in viability. Theoretical Population Biology 40: 47-77.

Waller, D. M. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. Evolution 38: 427-440.

Waller, D. M. 1986. Is there disruptive selection for self-fertilization? The American Naturalist 128: 421-426.

Weller, S. G., and R. Ornduff. 1991. Pollen tube growth and inbreeding depression in *Amsinckia grandiflora* (Boraginaceae). American Journal of Botany 78: 801-804.

Winn, A. A. 1988. Ecological and evolutionary consequences of seed size in *Prunella vulgarus*. Ecology 69: 1537-1544.

EFFECTS OF HABITAT FRAGMENTATION ON THE POLLINATION ECOLOGY OF *RUELLIA SUCCULENTA* (ACANTHACEAE)

ABSTRACT

I investigated the effects of habitat fragmentation on the pollination ecology of *Ruellia succulenta* Small (Acanthaceae). This plant is a facultatively autogamous, herbaceous perennial commonly found in the pine rockland habitat of southern Florida. Outside of Everglades National Park, there is less than 2 % of the original habitat in Miami-Dade county. The once nearly continuous habitat now exists as small fragments in a matrix of urban, suburban, and agricultural areas. Twelve field sites were chosen, three in each of four size classes of habitat: small < 3 ha, medium 3-9 ha, large > 10 ha, and intact habitat in Everglades National Park. Timed pollinator watches were conducted over the course of a year at the field sites and both number and identification of floral visitors was recorded. A sample of stigmas from open flowers was collected at the same time to indirectly gauge insect visitation rate to flowers. There were no differences in visitation rates to flowers by size class of habitat from the pollinator watch data; floral visitation rates averaged *ca* 20 % for all size classes. There were highly significant differences in the proportion of Hymenoptera (bees) and Lepidoptera (butterflies and skippers) floral visitors by habitat size class. Hymenoptera made up an increasing proportion of the total floral visitors from the small to intact size classes. Lepidoptera represented an increasing proportion of the total floral visitors from the intact to the small size classes. There were no significant differences in the proportion of insect visited flowers by habitat size class as evidenced from the stigma data.

INTRODUCTION

Concern is mounting that widespread anthropogenic modifications of the landscape will cause a cascade of disruptions to natural ecosystem function, particularly to the loose mutualistic relationships between plants and their pollinators. Such disruptions may lead to declines in plant reproduction (Rathcke and Jules 1993: Murcia 1996; Kearns and Inouye 1997; Kearns et al. 1998). A sense of urgency has been adopted by many researchers to focus attention on the potentially devastating effects of the "pollinator crisis" (Buchman and Nabhan 1996; Allen-Wardell et al. 1998). However, there have been very few published reports of unequivocal cases where pollination of wild plants declines, resulting in diminished fruit or seed production due to habitat fragmentation (but see Cunningham 2000).

Proponents of the "pollinator crisis" claim that reductions in the abundance of pollinators will cause reductions in fruit and seed production as plants are visited at declining rates in fragmented sites. This has been shown to be a likely outcome of the habitat fragmentation process (Steffan-Dewenter and Tscharntke 1999; Cunningham 2000). To verify the link between declines in pollination and declines in reproduction, evidence must prove that reproduction in habitat fragments is pollen limited. Zimmerman and Pyke (1988) have presented a rigorous test to conclusively determine the causes of limited seed set, but few studies are comprehensive enough to prove pollen limitation of reproduction per se, let alone in fragmented habitats. Even the landmark study by Aizen and Feinsinger (1994) that surveyed reproduction in 16 species of the fragmented chaco forest offered no conclusive evidence of pollen limitation, although their results suggested this possibility. Several other studies have documented reduced reproduction in low-density or small populations without the direct link to habitat fragmentation or without data on pollination (Beattie 1976; Sih and Baltus 1987; Spears 1987; Jennersten 1988; Sowig 1989; Husband and Barrett 1992; Agren 1996; Ghazoul et al. 1998; Kery et al. 2000). In many of these cases, changes to the resource environment of the fragments may equally be the cause of reproduction declines. While reproduction declines may lead to serious declines in plant populations, even possible collapses, they do not necessarily offer proof of pollination decline. Indeed, there have been several empirical investigations that demonstrate autogamous reproduction can partially or wholly compensate for a lack of pollinator visits in small or low-density populations (Sih and Baltus 1987; Jennersten 1988; Vaughton 1988; Karoly 1992; Bond 1994).

In this paper I report results of a study considering the effects of habitat fragmentation on pollination ecology of the facultatively autogamous plant, *Ruellia succulenta* (Acanthaceae). The hypotheses tested in this study are: 1) both diversity and abundance of flower visitors will decrease with decreasing size of habitat fragments, and 2) a greater proportion of flowers will show evidence of insect pollination in large than in small fragments.

METHODS

Study Species

Ruellia succulenta, the pineland petunia, is an herbaceous perennial endemic to southern Florida (Avery and Loope 1980) that most commonly occurs in the pine rockland habitat of Miami-Dade county (Snyder at al. 1990). Plants are trailing to ascending, usually less than 50 cm tall, with one (most often) to more than 30 (rare) stems (full details, Chap. 1). Parameters were recorded over a year during weekly surveys to 240 tagged plants, 20 per field site. The 12 field sites included 9 pine rockland fragments at Miami-Dade county parks, three sites in each of 3 fragment size classes: small < 3 ha, medium 3-9 ha, and large > 10 ha (Figure 1). The fourth size class, intact, consisted of three field sites in the intact pine rockland habitat in Long Pine Key, Everglades National Park.

Study Area

The pine rockland habitat occurs on elevated areas of limestone rock in southern Florida. The largest outcropping of limestone rock is the Miami Rock Ridge that runs northeast to southwest from downtown Miami into Everglades National Park. The habitat is characterized by a monospecific overstory of widely spaced Dade-county slash pine (*Pinus elliottii* var. *densa* Little and Dorman)) with an understory containing more than 300 taxa, nearly 15 % of the species endemic to pine rockland (Snyder et al. 1990). This sub-climax community is maintained by fire. In the absence of fire, pine rockland develops into tropical hardwood hammock, in as few as two decades (Robertson 1953).

The Miami Rock Ridge originally contained 52, 754 ha of pine rockland but today, less than *ca* 6,000 ha remain (O'Brien 1998). Excluding the large piece of intact pine rockland preserved in Everglades National Park, less than 2 % of the pre-settlement habitat exists on the Miami Rock Ridge. The once continuous forest now exists as *ca.* 400 isolated fragments (most less than 10 ha) in a matrix of agricultural, urban, and suburban areas with only three remnants greater than 50 ha left (O' Brien 1998).

Pollinator Watches

During weekly surveys of the tagged plants at the 12 field sites, timed pollinator watches were performed if open flowers were present. Three 10 minute long watches were conducted and the following variables were recorded: number of open flowers monitored, and number, identification, and behavior of floral visitors. Samples of floral visitors were collected for identification over the course of the study.

Stigma Collection

Besides the data resulting from the pollinator watches, additional data indirectly measuring pollinator activity was gained by collecting a sample of stigmas from flowers of untagged plants at the sites. If available, stigmas were plucked from flowers that retained their corollas. Back in the laboratory, the stigmas were mounted in Fuchsin gel (Kearns and Inouye 1993) on microscope slides and individually labeled. The slides were viewed under a light microscope and the number of *Ruellia succulenta* pollen grains and other species of pollen was recorded. For *R. succulenta* pollen grain numbers of equal to or greater than 50, a count of 50 pollen grains was scored.

Statistical Analysis

Several Chi-Square tests were run on the count data by both sites and size classes. A second set of tests was performed on the variable order (all five orders) of flower visiting insect by sites and size classes. The results of this second set of tests were invalid because the assumptions of the Chi-Square test were not met. However, when the orders Orthoptera, Diptera, and Coleoptera were excluded from the analysis, the assumptions were met and the tests were performed. A Chi-Square test was performed on the number of *Ruellia succulenta* pollen grains on stigmas by size class of habitat fragment. All statistical analyses were conducted using procedures of SPSS© version 10.0 (SPSS inc. 1999).

RESULTS

Flower and Plant Features

Plants in the greenhouse flowered continuously as did plants in the field, except for a marked decrease in flowering of the field plants during the dry season months from December-April. Stems on plants in the field lasted a full year. Senescing stems were replaced by new stems that began to sprout in early December at all field sites. The new stems elongated over the course of a year, attaining maximum height at the end of the wet season in October/November. The number of stems on each individual tagged plant was consistent from one season to the next. Only 5 plants out of the 240 added one stem, while no plants produced fewer stems the following season. Greenhouse plant stems elongated more quickly and new stems were produced in early December, as the field plants did.

Pollinator Watches

The list of floral visitors to *Ruellia succulenta* blossoms included 24 different species from five orders of insects (Table 1). Insects from the order Hymenoptera (bees, wasps, and ants) had the dominant proportion of floral visitors with Lepidopteran (butterflies, and skippers) visitors, a distant second (Table 1). Together these groups accounted for nearly 90 % of floral visitors. Coloeptera (beetles) and Diptera (flies) were infrequently seen on flowers, while Orthopteran (grasshoppers) visitors were rarely observed. The behaviors of insects from the five orders were, in general, distinct. Most Hymenoptera collected pollen from the anthers; their body width and short tongues restricted their access to nectar at the base of the corolla tube. There was no evidence of nectar robbing via holes chewed through the base of the corolla tubes. In addition, many species of Hymenoptera carried large amounts of *R. succulenta* pollen on their bodies, not only in their corbiculae. Lepidopteran visitors probed for nectar with their long probosces. Often, by plunging their heads into the throat of the corolla tube, they would leave the flower with ample pollen deposited on their heads. The Coleopteran visitors comprised two species, a blister beetle and a weevil. The former was most often observed eating the entire flower, both corolla and ovary. The latter was seen in groups inside the flared throat of the corolla tube. The final order, Orthoptera, consisted of grasshoppers that, like the blister beetles, were seen eating the entire flower.

Sites differed significantly in the number of visits ($X^2 = 23.3$, $df = 11$, $p = 0.016$), but size classes did not ($X^2 = 0.717$, $df = 3$, $p = 0.869$). There were minor differences among size classes in the proportion of watches with a floral visitor (Table 2). Differences among orders in visits (Hymenoptera or Lepidoptera) by sites were highly significant ($X^2 = 34.8$, $df = 11$, $p < 0.001$). Size classes also differed significantly in the order (Hymenoptera or Lepidoptera) of insect visitors ($X^2 = 21.4$, $df = 3$, $p < 0.001$). The data show interesting patterns in proportions of both Hymenopteran and Lepidopteran floral visitors by size class (Table 3). The proportion of Hymenopteran visitors steadily increases from small to intact habitat size classes, while the proportion of Lepidopteran visitors increases from intact to small habitat size classes.

Stigma Collection

The stigma data were collected as an adjunct to the pollinator watches to help determine the frequency of insect visits to open flowers. The results offer stronger support of frequent insect visitation than those obtained from the pollinator watches. The majority of flowers examined had pollen on their stigmas. It seems likely that, before abscission of the corolla, an insect vector is needed to deposit pollen on the stigma (Chap. 1). In other words, a stigma with zero pollen grains is assumed to have gone unvisited by an insect. A stigma that has any pollen on the stigma was most likely visited by an insect. From the total sample of 2,207 stigmas, 23 % of stigmas had zero pollen grains, while *ca.* 60 % had five or more pollen grains (29 % had 50 pollen grains). There were no significant differences in number of pollen grains on stigmas by size class ($X^2 =$

1.057, *df* = 3, *p* = 0.714). The average number of *Ruellia succulenta* pollen grains on the 2,207 stigmas was 21.8.

DISCUSSION

The results from the pollinator watches do not show significant differences in the abundance of floral visitors at different sized sites, contrary to theoretical expectations. Overall, about 20 % of the pollinator watches resulted in the observation of a floral visitor; this proportion was nearly equivalent regardless of the size class of habitat. While the diversity of flower-visiting insects was greater in the large and intact size classes than in the medium and small size classes, the differences also were not statistically significant. However, there were marked differences in the composition of the pollinator guild from the small fragments to the intact habitat. There were highly significant differences in the proportion of Hymenopteran *vs* Lepidopteran visitors in the size classes of habitat. Hymenopteran visitors steadily increased in proportion from the small to the intact habitat, coming to represent 88 % of all floral visitors in the intact habitat in Everglades National Park. On the other hand, Lepidoteran visitors steadily increased in proportion from the intact to the small habitat (opposite to the trend seen in Hymenoptera), coming to represent 71 % of all floral visitors in the small size class of fragments. This striking trend echoes changes to the pollinator guild from intact to fragmented habitat seen in other plant species (Sih and Baltus 1987; Spears 1987; Jennersten 1988; Lamont and Klinkhamer 1993; Aizen and Feinsinger 1994; Steffan-Dewenter and Tscharntke 1999). There are potentially many reasons for these observed patterns. It may be that below some threshold area, populations of solitary bees become absent. There was some evidence that several species of solitary bees were absent from some of the small sites. This absence may be due to a lack of resources to support these bees as the small sites tended to have a depauperate sub-set of native plant species. All species of solitary bees were observed in Everglades National Park, which contains the largest area of intact pine rockland. The pine rockland in Everglades National Park is also well managed by prescribed burns and contains the greatest diversity of plant species. It seems likely that Lepidoterans, especially the skippers, were more frequent at small sites because they were less affected by resource limitation in the small sites, being more mobile than bees. Skippers are known to be strong fliers and it is possible that they are able to more easily travel from small fragment to small fragment searching for resources because of their superior energetics.

The stigma data results are not consistent with the proposed hypothesis of greater proportions of insect visitation to flowers in larger size classes of habitat. There were no differences in the number of pollen grains deposited by insect visitors by size class of habitat. The stigmas collected in the intact habitat of Everglades National Park contained, on average, an equivalent number of pollen grains as the stigmas collected in the small fragments. In light of the different pollinator guilds of these size classes, there may be important ramifications of the type (self- vs. outcross-pollen) of pollen on these stigmas. It seems likely that Lepidoptera carry pollen from widely scattered individuals while Hymenoptera carry pollen from spatially close conspecifics. The resulting genetic diversity of the offspring produced via these distinct groups of floral visitors may be quite different. Potential population genetic dynamics may show marked variability through the compounding factors produced by these different mating events.

Table 1. Summary list of flower visiting insects to *Ruellia succulenta* recorded during 608 (10 minute long) pollinator watches.

Order	% of floral visitors	# of species	Species identification
Orthoptera	0.7	1	unknown grasshopper
Diptera	2.8	2	*Ornidia obesa*
			Systoechus spp., bombyliid fly
Coleoptera	7	2	*Lytta aenea* , blister beetle
			unknown Curculionid beetle
Lepidoptera	26.6	8	*Agraulis vanillae* , Gulf fritillary
			Hylephila phyleus , skipper
			Lerema accius , skipper
			Phoebis sennae , cloudless sulphur
			Urbanus proteus proteus , skipper
			unknown brown skipper
			unknown orange skipper
			unknown yellow skipper
Hymenoptera	62.9	14	*Agapostemon splendens* , bee
			Augochlorids, 4 different bee spp.
			Apis mellifera , honeybee
			Dialictus nymphalis , sweat bee
			Dialictus tegularis , sweat bee
			Dianthidium curvatum floridiense , bee
			Halictus ligatus , bee
			Megachile xylocopoides , bee
			unknown Megachilid bee
			unnknown yellow/black wasp
			unknown quick-flying bee

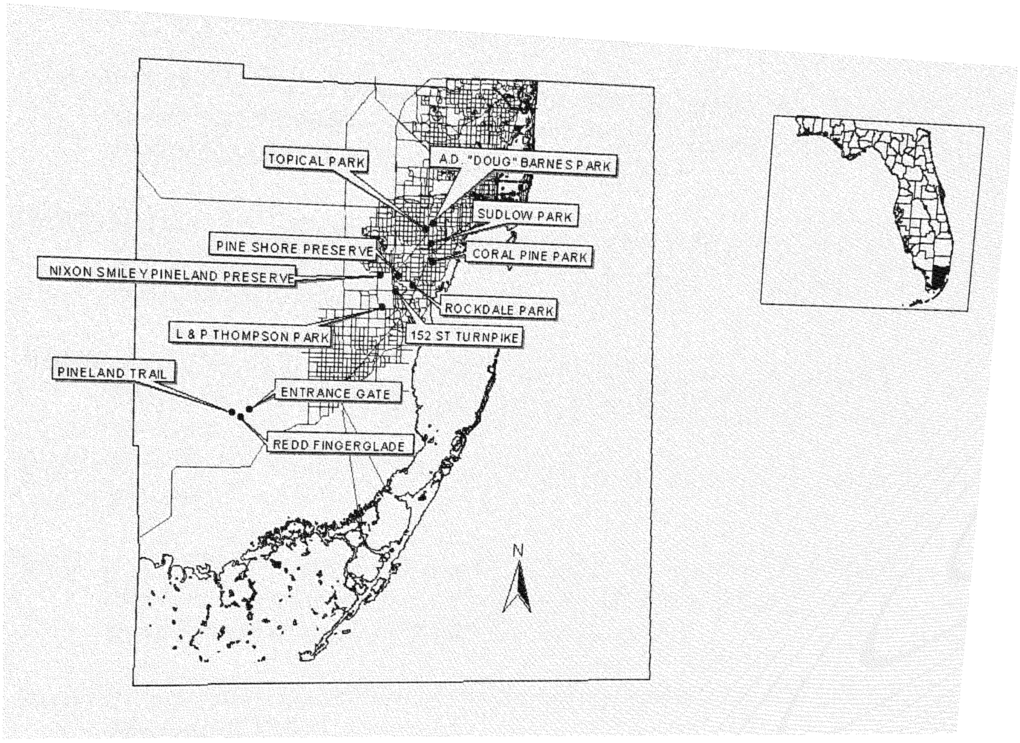
Table 2. Pollinator watch components by size class of habitat.

Size class	# of poll. watches	# of spp. flr.visitors	% poll. watches w/ visitor
Small	76	7	19.7
Medium	136	11	23.5
Large	192	15	22.9
Intact	204	12	23.5

Table 3. Comparison of % of Hymenopteran visitors and % of Lepidopteran visitors by size class of habitat.

Size class	% of Hymenoptera	% of Lepidoptera
Small	28	71
Medium	58	42
Large	77	23
Intact	88	12

Figure 1. Locations of 12 field sites in Miami-Dade county, Florida.



LITERATURE CITED

Agren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77: 1779-1790.

Aizen, M. A., and P. Feinsinger. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75: 330-351.

Allen-Wardell, G., (and 17 others). 1998. The potential consequences of pollinator declines on the conservation on the conservation of biodiversity and stability of food crop yields. Conservation Biology 12: 8-17.

Avery, G. N., and L. L. Loope. 1980. Endemic taxa in the flora of South Florida. Report T-558, Everglades Park South Florida Research Center. 39 pp. Available from: Everglades National Park, Homestead, FL.

Barrow, D. A., and R. S. Pickard. 1985. Estimating corolla length in the study of bumble bees and their food plants. Journal of Apicultural Research 24: 3-6.

Beattie, A. J. 1976. Plant dispersion, pollination and gene flow in *Viola*. Oecologia 25: 291-300.

Bond, W. J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London Series B 344: 83-90.

Buchman, S. L., and G. P. Nabhan. 1996. The forgotten pollinators. Island Press, Washington, D. C.

Cunningham, S. A. 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in Mallee woodland. Conservation Biology 14: 758-768.

Ghazoul, J. A., K. A. Liston, and T. J. B. Boyle. 1998. Disturbance induced density dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest Tree. Journal of Ecology 86: 462-473.

Husband, B. C., and S. C. H. Barrett. 1992. Pollinator visitation in population of tristylous *Eichornia paniculata* in northeastern Brazil. Oecologia 89: 365-371.

Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2: 359-366.

Karoly, K. 1992. Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). American Journal of Botany 79: 49-56.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado. Colorado.

Kearns, C. A., and D. W. Inouye. 1997. Pollinators, flowering plants, and conservation biology. BioScience 47: 297-307.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29: 83-112.

Kery, M., D. Matthies, and H. Spillman. 2000. Reduced fecundity and offspring performance of the declining grassland plants *Primula veris* and *Gentiana lutea.* Journal of Ecology 88: 17-30.

Lamont, B. B., P. G. L. Klinkhamer, and E. T. F. Witkowski. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* – a demonstration of the Allee effect. Oecologia 94: 446-450.

Murcia, C. 1996. Forest fragmentation and the pollination of neotropical plants. *in* Forest patches in tropical landscapes. J. Schelhas, and R. Greenberg, editors, pp. 19-36. Island Press, Washington, D. C.

O'Brien, J. J. 1998. The distribution and habitat preferences of rare *Galactia* species (Fabaceae) and *Chamaesyce deltoidea* subspecies (Euphorbiaceae) native to southern Florida pine rockland. Natural Areas Journal 18: 208-222.

Rathcke, B., and E. S. Jules. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65: 273-277.

Robertson, W. B., Jr. 1953. A survey of the effects of fire in Everglades National Park. Everglades National Park Service, Homestead, FL.

Sih, A., and M. S. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68: 1679-1690.

Snyder, J. R., A. Herndon, and W. B. Robertson. 1990. South Florida rockland. pp. 230-277, in R. L. Myers, and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, University Presses of Florida, Orlando, FL.

Sowig, P. 1989. Effects of flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera: Apidae). Oecologia 78: 550-558.

Spears, E. E. 1987. Island and mainland pollination ecology of *Centrosema virginianum* and *Opuntia stricta*. Journal of Ecology 75: 351-362.

Steffan-Dewenter, I., and T. Tscharntke. 1999. Effects of habitat isolation on pollinator communities and seed set. Oecologia 121: 432-440.

Vaughton, G. 1988. Pollination and seed set of *Banksia spinulosa*: evidence for autogamy. Australian Journal of Botany 36: 633-642.

Zimmerman, M., and G. H. Pyke. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. The American Naturalist 131: 723-738.

HABITAT FRAGMENTATION EFFECTS ON REPRODUCTION OF *RUELLIA SUCCULENTA* SMALL (ACANTHACEAE)

ABSTRACT

This paper reports the results of a study to gauge the effects of habitat fragmentation on *Ruellia succulenta* (Acanthaceae), an herbaceous perennial commonly found in the fire-dependent pine rockland habitat of southern Florida. Reproduction of this plant at 12 field sites was surveyed for one year. The 12 field sites consisted of nine habitat fragments (in three size classes) and three sites in the intact habitat of Long Pine Key, Everglades National Park. Several site parameters that might influence reproduction were recorded for each site. These included: area of habitat, time since last fire, and the density of conspecifics. Hypotheses were formulated using the assumption that plant reproduction decreases with concomitant reductions in habitat area. The results of this study were contrary to these *a priori* expectations. All the reproductive variables measured showed no pattern of monotonic decline with reductions in habitat area, rather some of the lowest reproduction was reported from the intact habitat in Everglades National Park. Density of conspecifics did not affect reproduction, contrary to expectation. Time since last fire appeared to be the most important factor positively affecting all measures of reproduction. In general, the most recently burned sites had the highest measures of reproduction.

INTRODUCTION

Anthropogenic changes to the landscape have significantly affected the distribution and functioning of plant populations. Fragmentation has resulted in once widely distributed species being confined to smaller, isolated "islands" of suitable habitat in a sea of incompatible environments (Jennersten et al. 1992; Vitousek 1994). Plants in these newly created habitat patches often are subject to different biotic and abiotic conditions (Harris 1988; Saunders et al. 1991; Young & Mitchell 1994; Murcia 1995).

The novel abiotic conditions in the fragments are termed edge effects. Deterioration of environmental quality in small fragments, such as environmental changes in resource availability for flowering or fruiting, has been linked to decreased reproduction in some plant species (Widen 1993; Oostermeijer et al 1994). Several studies have suggested plant resource limitation as a possible factor resulting in reduced reproduction after pollinator limitation and genetic erosion had been ruled out (Bierzychudek 1981; Horvitz & Schemske 1988; and Burd 1994).

Both the biotic and abiotic environments are altered by fragmentation, but the biotic environment is affected as well. Any disruptions of the plant-animal interactions as a consequence of fragmentation can have serious affects on plant populations (Olesen & Jain 1994; Bond 1995). One interaction that may be affected is the mutualism between plants and their animal pollinators (see reviews, Kearns et al 1998; Spira 2001). Several studies have shown changes to the pollinator guild, in terms of both diversity and abundance, comparing intact *vs.* fragmented habitat (Jennersten 1988; Jennersten et al. 1992; Aizen and Feinsinger 1994b). Both reduced quality of pollen and decreased quantity of pollen has been linked to reduced reproduction in many plant species (Rathcke 1983; Sowig 1989; Klinkhamer and de Jong 1990; Lamont et al. 1993; Rathcke & Jules 1993; Matthies 1995; Murcia 1995; Didham 1996; Dewenter & Tscharntke 1999; Kery et al 2000; Cunningham 2000). The decreased reproduction seen in the fragmented areas was attributed to 'pollinator limitation'. It has been hypothesized that pollinator limitation of reproduction will be more severe for self-incompatible rather than self-compatible species (Byers 1995). Fragmented plant populations also may show increased reproduction due to shifts in the pollinator guild by newly invaded pollinators (Aizen & Feinsinger 1994a, 1994b). Pollination is not the only component of reproduction affected by fragmentation. Predation rates of reproductive structures (flowers, fruits, and seeds) have been shown to vary with fragmentation also (Pudlo et al. 1980; Santos & Telleria 1994; Zabel & Tscharntke 1998).

Another factor that affects the reproduction is plant density. The density of reproductive conspecifics is very important for pollinator attraction (Sih & Baltus 1987) as well as mate sources for outcrossing and autogamous species (Karoly 1992). Studies of decreasing reproduction with decreasing density have focused on either pollinator limitation (Silander 1978; vanTreuren et al. 1994; Agren 1996; Roll et al. 1997; Morgan 1999) and/or inbreeding depression (Fischer & Matthies 1998; Kery et al. 2000) as the causative factors.

For this project, I examined the effect of habitat fragmentation on the self-compatible plant *Ruellia succulenta* (Acanthaceae) common to the pine rockland habitat of southern Florida. I related several reproductive variables (flowers, fruits, and seeds) to site parameters (area of habitat, time since last fire, and density of conspecifics). I expected the following results: 1) reproduction should increase monotonically with increasing area of fragment, 2) recently burned sites should have higher reproduction (via increased resources of light and nutrients), and 3) reproduction should increase as the density of conspecifics increases (via facilitation for this facultatively autogamous species).

METHODS

Study Species

Ruellia succulenta Small (Acanthaceae) is a self-compatible (Chap. 1), long-lived perennial endemic to southern Florida (Avery and Loope 1980). The plant most commonly occurs in pine rockland (Snyder et al 1990) but also is found in ecotones into short hydroperiod sawgrass/muhley grass marshes. Stems originate from adventive buds located on the top portion of the relatively large rootstock. The flowering period is all year, with a marked decrease during the cool dry months from December to April. Flowers occur in clusters in the leaf axils (Wunderlin, 1998). The five petaled lavender (rarely white or pink) flowers have a funnelform corolla from 2-5 cm long, the tube about 2 cm long with a flower face diameter from 3-5 cm (Chap. 1). In general, flowers open at sunrise and abscisse in the early afternoon. Floral rewards are pollen, and nectar that is secreted at the base of the corolla tube. The insect pollinators of *R. succulenta* include: butterflies, bombyliid flies, wasps, honeybees, and primarily, solitary bees (Chap. 3). Carpels mature into glabrous capsules within one to three weeks containing up to 13 seeds. The fruit is explosively dehiscent, and the seeds are dispersed several meters by the aid of retinaculae (Geiger, personal observation).

Study Area

Pine rockland occurs on elevated areas of limestone rock in southern Florida. The largest outcrop is the Miami Rock Ridge that runs northeast to southwest from downtown Miami into Everglades National Park. The habitat is characterized by a monospecific overstory of widely spaced Dade county slash pine (*Pinus elliottii* var. *densa* Little and Dorman) with an understory shrub/herb layer containing over 300 taxa, nearly 12% of them endemic to pine rockland (Snyder et al. 1990).

Fire is essential for the maintenance of this sub-climax community. In the absence of fire, pine rockland develops into the climax community, hardwood hammock, in as few as two decades (Robertson 1953). Natural, lightning induced fires most often occur during the wet season months of May-September and human ignited fires tend to occur during the dry season months of November to April (Taylor 1981). Estimates of fire return intervals range from 2-10 years. The pines are fire resistant as adults, but the juveniles and seedlings suffer high rates of mortality. The perennial (with very few exceptions) shrubs and herbs lose above ground biomass during fires but are capable of resprouting from below ground soon afterwards, experiencing very low rates of mortality.

The Miami Rock Ridge originally contained 52, 754 ha of pine rockland but today, only ≈6,000 ha remain (O'Brien 1998). Excluding the intact pine rockland preserved in Everglades National Park, less than 2 % of the pre-settlement habitat exists on the Miami Rock Ridge. Because of relatively higher elevation, these areas were the first to be built on during development. The once continuous forest now exists as ≈400 isolated fragments (most less than 10 ha) in a matrix of agricultural and suburban areas with only 3 remnants greater than 50 ha left (O'Brien 1998).

Study Sites

The 12 study sites are located on the Miami Rock Ridge (Table 1; Chap. 3, Figure 1). The 3 Everglades National Park sites are located in the intact pine rockland of Long Pine Key, separated by several kilometers. Three sites in each of 3 fragment size classes were chosen outside of Everglades National Park. The small, medium, and large size classes are: < 3 ha, 3-9 ha, and > 10 ha, respectively.

Floristically the sites are fairly similar. Most sites have an overstory of widely spaced Dade county slash pines with an understory dominated by saw palmetto (*Serenoa repens*) and, to a lesser extent, cabbage palm (*Sabal palmetto*). Newly burned sites and those with frequent fires tend to have many bare patches of ground along with a species rich herbaceous/grass layer. Long unburned sites have a thick understory of saw palmetto, few bare patches of ground, and a shrub/small tree layer of hardwood hammock species. The herbaceous/grass layer is much reduced or absent. In addition, long unburned pine rockland often becomes dominated by several invasive exotic plant species, including, *Schinus terebinthifolius, Neyraudia reynaudiana, Pennisetum purpurea, Albizia lebbeck*, *Waltheria indica*, and *Lantana camara.*

Data on the time since last fire (Table 1) was available for most of the fragments (Hazelton, Department of Environmental Resource Management, personal communication) and for all 3 sites in Everglades National Park (Ainslie, ENP Fire Cache, personal communication). Fire data for Coral Pine, A.D. Barnes, and Pineshore don't exist. These sites showed evidence of being long unburned and were given the conservative value of 60 months since last fire, but the actual time may be longer.

Measurements of Reproduction

At each of the 12 sites, 20 haphazardly chosen plants were tagged. I recorded the following parameters weekly for a year for each plant: number of open flowers, number of flower buds, number of immature fruit, number of mature fruit, and number of seeds. Weekly surveys were necessary as mature fruit can be produced from flowers in one week (most fruits took 2 weeks to mature but others stayed on the plant for up to a month). Mature fruit were opened and the number of plump, healthy-appearing seeds was recorded. The variable of interest was net reproduction, the total number of viable seeds produced by each plant over the course of a year.

Measurements of Population Characteristics

I recorded density of conspecifics, both adults and seedlings, at each of the 12 sites. Because *Ruellia succulenta* plants grew in clumps, I made density measures using the 20 tagged plants at each site as focal plants. All adult (reproductive) plants were counted within the circular area created using a 2 m radius sweep centered on each tagged plant, and seedlings (usually < 10 cm with no evidence of reproduction) were counted using a 1 m radius sweep.

The number of stems on each tagged plant was recorded every month. Stems last a full season (unless damaged). They began to senesce at the beginning of the dry season in December and new basal resprouts appeared within ~2 weeks at all sites. Stems expanded during the next several months. The number of stems remained constant from season to season for most plants with additional stems being added in late April/early May. Data for number of stems used in statistical analyses were taken from May 2000 survey.

Statistical Analyses

Statistical analyses of the data were performed using the software SPSS for Windows version 10.0 (SPSS 1999). To show an effect of fragment size, reproductive variables would have to differ in their means across the sites. These differences may be manifested by a monotonic increase or decrease of the means as the area of the fragment progressively increases. There may also be a critical size threshold, above which the site means are significantly different. ANOVAs were used to test for differences between two or more sites for the following reproductive variables: sum of open flowers, sum of mature fruits, and sum of seeds. These variables were square root transformed to approach normality. The ANOVA tests were robust, even with heteroscedasticity, because of equal sample sizes (no mortality of the 240 tagged plants during the study). Dunnett's C post-hoc was used to test for significant pairwise comparisons due to heteroscedasticity. Plots of the site means for the reproductive variables were examined to determine the effect of increasing fragment size and to search for a critical size threshold.

An ANOVA test was run to look for differences in plant size by site using the variable number of stems per plant. This variable did not need transformation. To account for differences in plant size that might have translated into differences in reproduction, an ANCOVA test was performed using stems as the covariate on square root sum of seeds. Linear regressions were performed for each site with number of stems as the predictor of square root sum of seeds.

To investigate relationships between the reproductive variables and several other site parameters (area, time since last fire, adult plant density, seedling density), Spearman's rank correlations were performed. Nonparametric tests were used because violations of the Pearson's test assumptions could not be overcome. Hypothesized effects of habitat fragmentation on reproduction would be shown by positive correlations between the area of habitat and the reproductive variables. Time since last fire may have had a significant effect on reproduction, shown by negative correlations with the reproductive variables. The correlations of the density of both adults and seedlings with the other variables were performed to infer historical effects of fragmentation and fire regime.

RESULTS

Effects of Fragment Size

The ANOVA's of the three reproductive variables tested (sums of open flowers, mature fruit, and seeds) by site were all highly significant. The plots of the site means did not show the hypothesized trend of either a monotonic increase with increasing area or a critical size threshold (Figure 1). The means plots were similar for all three reproductive variables. Surprisingly, means of sum of seeds for the intact habitat in Everglades National Park had some of the lowest values (Figure 1).

The ANOVA test on number of stems per plant by site was highly significant. The results of the ANCOVA test using stems as the covariate on the reproductive variable square root sum of seeds were inconclusive. The test was invalid because the site by stems interaction was highly significant. The necessary prerequisite for the ANCOVA test, homogeneity of the slopes of the grouping factor (site), was not met. To determine the relationships of the variables, linear regressions were then performed for each site on the square root sum of seeds with number of stems as the predictor variable. Of the 12 sites, five had significant (at the 0.05 level) positive relationships. A second ANCOVA was run just using the 5 sites with significant linear relationships. Again, the test was invalid because the homogeneity of slopes assumption could not be met.

None of the reproductive variables responded to fragment size as hypothesized. The results of the correlations between area and square root sums of open flowers, mature fruits, or seeds were not significant (Spearman's rank correlation = -0.009, $n = 240$, $p = 0.885$; S.r.c. = 0.007, $n = 237$, $p = .920$; S.r.c. = -0.084, $n = 240$, $p = 0.197$, respectively). There were positive, highly significant correlations between the size of the fragment and both adult plant and seedling density (Spearman's rank correlation = 0.429, $n = 240$, $p < 0.001$; S.r.c. = 0.455, $n = 240$, $p < 0.001$, respectively).

Effects of Time Since Last Fire

Time since last fire appeared to be an important factor positively affecting reproduction. For two of the reproductive variables tested with ANOVAs (square root sums of open flowers, and mature fruits), five of the six most recently burned sites (< 25 months ago) had the highest means. For the third reproductive variable tested with an ANOVA (square root sum of seeds), four of the six most recently burned sites had the highest means. There were highly significant negative correlations between time since last fire and square root sums of open flowers, mature fruits, and seeds (Spearman's rank correlation $= -0.429$, $n = 240$, $p < 0.001$; S.r.c. $= -0.461$, $n = 237$, $p < 0.001$; S.r.c. $= -0.397$, $n = 240$, $p < 0.001$, respectively). Plants at recently burned sites had higher levels of reproduction, while those at sites whose time since last fire exceeded two years seemed to return to a baseline level of reproduction. There was a highly significant negative correlation between time since last fire and adult plant density (Spearman's rank correlation $= -0.264$, $n = 240$, $p < 0.001$). There was also a highly significant negative correlation between time since last fire and seedling density (Spearman's rank correlation $= -0.501$, $n = 240$, $p < 0.001$).

Effects of Plant Density

Correlations were performed considering the effect of both adult and seedling density on the reproductive variable square root sum of seeds. The correlation of adult density and this reproductive variable was not significant. There was a significant positive correlation between the density of seedlings and the square root sum of seeds (Spearman's rank correlation $= 0.194$, $n = 240$, $p = 0.003$). The final correlation was between the density of adult plants and seedlings. This correlation was positive and highly significant (Spearman's rank correlation $= 0.611$, $n = 240$, $p < 0.001$).

DISCUSSION

In general, most fragmentation studies report a decrease in reproductive output with decreases in habitat area (Spears 1987; Jennersten 1988; Lamont et al. 1993; Aizen and Feinsinger 1994a; Oostermeijer et al. 1998; Morgan 1999; Cunningham 2000) or in population size (Silander 1978; Sih & Baltus 1987; Agren 1996; Kunin 1997; Roll et al. 1997; Fischer & Matthies 1998; Kery et al. 2000). The three main reasons to explain decreases in reproduction are pollinator limitation (~ pollen limitation, either quantity or quality), resource limitation, and genetic erosion/inbreeding depression.

Effects of fragment size on net reproduction would have been shown by the site means for the reproductive variables either showing a monotonic increase/decrease with increasing fragment size or a critical size threshold. There was little evidence in my data to support either scenario. The site means for the reproductive variables exhibited a sawtooth pattern. There was no critical size threshold above which the site means increased significantly. The correlations of the three reproductive variables with area also were not significant. Other authors have published similar results. Aizen and Feinsinger (1994a) reported median decreases of 20% in pollination levels and seed set from continuous forest to forest fragments for 16 species. In their study, the species most comparable to my study species (a self-compatible herbaceous perennial, *Justicia squarrosa*, Acanthaceae) had significantly fewer pollen grains on the stigmas of plants from forest fragments versus continuous forest. This indication of pollen/pollinator limitation in fragments did not translate into significant differences in seed set; there was no negative effect of fragment size on net reproduction for this species, similar to my findings. The reproductive variable of pollen deposition on stigmas did not appear to be affected by area of habitat for *Ruellia succulenta* as those sites with active flowering showed adequate pollen deposition for full seed set, regardless of size (Chap. 3). This suggests that pollen or pollinator limitation was not a factor explaining the low seed set at some of the sites. These results demonstrate that net reproduction is made up of several separate reproductive components, each component potentially susceptible to the effects of fragmentation. Each element may have positive, neutral, or negative scaling effects on final seed set (Cunningham, 2000). Cunningham's (2000) study found evidence for a fragment size effect in only one reproductive variable of the many considered for four common species in the Mallee woodland. Similarly, my study showed little effect of fragment size on net reproduction.

Net reproduction appeared to be most affected by time since last fire at the sites studied. All of the reproductive variables considered showed highly significant negative correlations with time since last fire. Flowering, fruiting, and seed set were all highest at the most recently burned sites. This is in line with other studies that documented an increase in flowering post-fire (Gill 1981; Glenn-Lewin et al. 1990; Robbins & Myers 1992; Whelan 1995), several of which were conducted in the pine rockland habitat (Robertson 1953; Wade et al. 1980; Snyder & Ward 1987; Snyder et al. 1990; Spier and Snyder 1998). It seems likely that the increased reproduction seen in recently burned sites is the result of release from resource limitation, both light and nutrients. Following fire, plants of the herbaceous layer are exposed to full sun and much of the above ground plant biomass is remineralized into a nutrient rich ash layer (Knapp & Seastedt 1986; Kutiel & Naveh 1987; Dudley & Lajtha 1993; Brewer 1995). The response of *Ruellia* *succulenta* to fire seems to fit the "Early Peak" model of allocation to reproduction proposed by Ostertag & Menges (1994); increased resources lead to increased flowering in early post-fire years. Indeed, plants are capable of resprouting and flowering within 2 weeks of fire and the increase in flowering is dramatic. The return to "baseline" levels of reproduction seems to occur within 2-3 years post-fire, which coincidentally is how long it takes the shrub layer to return to its pre-burn coverage. This strategy of increased flowering in the early post-fire years followed by a quick return to pre-burn output has been noted for other fire adapted herbaceous perennials (Menges & Dolan 1998; Lesica 1999). *R. succulenta* appears to fit this pattern. The long (>2-3 years) unburned sites exhibited lower levels of net reproduction because those plants did not produce as many flowers most likely as a result of resource limitation, both light and nutrients. Other studies have shown the opposite result, that is, decreased seed set in recently burned sites (Ne'eman & Dafni 1999, Ne'eman et al 2000). The mechanism for the reduction in seed set was pollinator limitation for the self-incompatible species studied. *R. succulenta* is self-compatible and facultatively autogamous, so one would expect it to be relatively less vulnerable to pollinator limited seed set. In my study system, recently burned sites had the highest pollinator activity. I believe the increased flowering of most perennial species, in addition to increased nesting availability for both cavity and ground nesting solitary bees, helped facilitate pollination in the post-fire environment of this habitat.

Another important demographic factor affected by fire was seedling recruitment. For *Ruellia succulenta*, there was a highly significant negative correlation between the number of seedlings and the time since last fire. Long unburned sites had almost no seedlings while most recently (<2 years) burned sites had large crops of seedlings.

Several studies have documented increases in seedling recruitment in the post-fire environment for resprouting perennials (Keeler 1991, Menges & Dolan 1998, Lesica 1999). Seedling recruitment was variable and fairly infrequent for the plants investigated in these studies but there were positive relationships between recruitment and burn-managed sites. It appears that for *R. succulenta*, the greater seed production seen in recently burned sites is coupled with increased seedling recruitment. The process may be the creation of open, bare patches of ground ("safe sites" for seedling recruitment) caused by the fire. Seedlings were noticed predominantly in these locations.

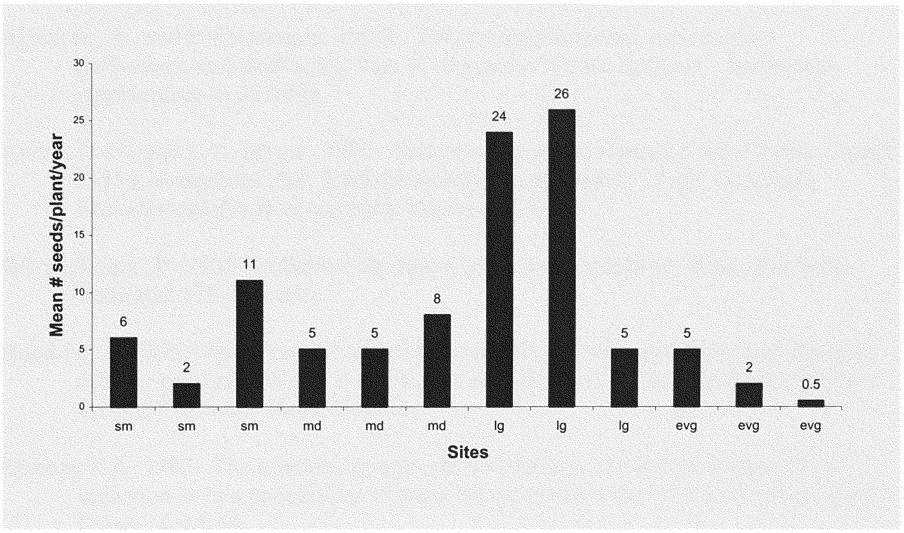
The final factor potentially affecting net reproduction that I examined was plant density. The strict definition of density is the number of individuals per unit area. Most studies have measured density in either one of two ways: the number of individuals in the population or the density of individuals in the population (distance to nearest conspecific or "local density", i.e. number of conspecifics surrounding chosen focal plants). The two methods of measuring density are often positively correlated (van Treuren et al. 1994) and yet, population size and population density can show different effects on species interactions (Kunin 1997). Because use of the first method, population size, is almost always confounded with population density, several authors have argued for using population density as a better parameter of density (Kunin 1997; Roll et al. 1997). I chose to use population density as the density measure in my study. Published studies of population size effects on pollination have shown both decreases (Sih & Baltus 1987; Lamont et al. 1993; Aizen & Feinsinger 1994a; Agren 1996) and increases (Sowig 1989; Aizen & Feinsinger 1994a) for small populations. These observed changes in pollination levels for small populations did not translate into any differences in reproductive output in some of these studies (Sowig 1989; Aizen & Feinsinger 1994a). On the other hand, most studies using population density measures have documented decreased pollination for low-density plant populations (Silander 1978; Klinkhamer & de Jong 1990; Kunin 1997; Roll et al. 1997). Many further show reduced reproductive output as a result of pollinator limitation. Self-compatible species were the anomalies for this general pattern. Self-compatible *Ruellia succulenta* also follows the exceptional pattern. There was no significant correlation between adult plant density and total seed production. This result is perhaps predictable; again because one would not expect a self-compatible facultatively autogamous plant to suffer from pollinator limited seed set due to population density differences. The correlation of seedling density and total seed production was positive and highly significant. This may point to the significant role of fire in driving the reproductive output of *R. succulenta*. It seems that plants maintain elevated levels of reproduction for several years post-fire, so the presence of seedlings suggests that the plants are still reproducing at higher, post-fire levels. The highly significant positive correlation between adult plant density and seedling density was expected; a higher density of adults should result in more seeds being produced, which translates into more seedlings.

My study is the first to investigate the effects of fragmentation on the reproduction of a perennial herb in the highly fragmented pine rockland ecosystem of southern Florida. It appears that area of the fragment does not have a negative effect on the reproductive output of self-compatible, facultatively autogamous *Ruellia succulenta* for the sites studied. Rather, fire management (prescribed burns) seems to be a very important factor that positively affects reproduction for this species of the herbaceous layer. It is likely that other plants of the herbaceous layer respond in a similar manner. Most of the plant diversity and endemicity of the pine rocklands occur in this vegetation layer. This should be an encouraging signal to the land managers of this globally imperiled ecosystem; indeed, proper fire management can lead to the proliferation of the plants in the herbaceous layer, regardless of the size of the fragment. The largest fragments should still be targeted for conservation as they generally contain the highest plant diversity.

Table 1. List of field sites and summary variables.

Site	Size Class	Area (ha)	Time since last fire (mo.)
152 Turnpike	Small	0.4	41
Coral Pine	Small	0.6	60
Tropical	Small	2	25
A. D. Barnes	Medium	4.9	60
Pineshore	Medium	3	60
Trinity	Medium	6.1	7
Rockdale	Large	12	3
Tamiami	Large	24.3	0.5
L. and P. Thompson	Large	58.7	1.5
ENP, Pine Island	Intact	15	24
ENP, Redd	Intact	367	47
ENP, Pineland	Intact	394	60

Figure 1. Mean number of seeds per plant by site.



LITERATURE CITED

Agren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77: 1779-1790.

Aizen, M. A., and P. Feinsinger. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75: 330-351.

Aizen, M. A., and P. Feinsinger. 1994b. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine "Chaco Serrano." Ecological Applications 4: 387-392.

Avery, G. N., and L. L. Loope. 1980. Endemic taxa in the flora of South Florida. Report T-558, Everglades Park South Florida Research Center. 39 pp. Available from: Everglades National Park, Homestead, FL.

Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. American Naturalist 117: 838-840.

Bond, W. J. 1995. Assessing the risk of local extinction due to pollinator and disperser failure. pp. 131-146 *in* J. H. Lawton and R. M. May, editors. Extinction Rates. Oxford University Press, Oxford.

Brewer, J. S. 1995. The relationship between soil fertility and fire stimulated floral induction in two populations of grass-leaved golden aster, *Pityopsis graminifolia.* Oikos 74: 45-54.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60: 83-139.

Byers, D. L. 1995. Pollen quantity and quality as explanations for low seed set in small populations as exemplified by *Eupatorium* (Asteraceae). American Journal of Botany 82: 1000-1006.

Cunningham, S. A. 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in Mallee woodland. Conservation Biology 14: 758-768.

Dewenter, I. S., and T. Tscharntke. 1999. Effects of habitat isolation on pollinator communities and seed set. Oecologia 121: 432-440.

Didham, R. K., J. Ghazoul, N. E. Stork, and A. J. Davis. 1996. Insects in fragmented forests: a functional approach. Trends in Ecology and Evolution 11: 255-260.

Dudley, J. L., and K. Lajtha. 1993. The effects of prescribed burning on nutrient availability and primary production in sandplain grasslands. American Midland Naturalist 130: 286-298.

Fischer, M., and D. Matthies. 1998. Effects of population size on performance in the rare plant *Gentianella germanica.* Journal of Ecology 86: 195-204.

Gill, A. M. 1981. Adaptive responses of Australian vascular plant species to fires. *in* A. M. Gill, R. H. Groves, and I. R. Noble, editors. Fire and the Australian Biota. Australian Academy of Sciences, Canberra.

Glenn-Lewin, D. C., L. A. Johnson, T. W. Jurik, A. Kosek, M. Leoscheke, and T. Rosburg. 1990. Fire in central North American grasslands: vegetative reproduction, seed germination, and seedling establishment. pp. 28-45 *in* S. L. Collins, and L. L. Wallace, editors. Fire in central North American Grasslands. University of Oklahoma Press, Norman, OK.

Harris, L. D. 1988. Edge effects and conservation of biotic diversity. Conservation Biology 2: 330-332.

Horvitz, C. C., and D. W. Schemske. 1988. A test of the pollinator limitation hypothesis for a neotropical herb. Ecology 69: 200-206.

Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2: 359-366.

Jennersten, O., J. Loman, A. P. Moller, J. Robertson, and B. Widen. 1992. pp. 396-425 *in* L. Hansson, editor. Conservation biology in agricultural habitat islands. Elsevier, London.

Karoly, K. 1992. Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). American Journal of Botany 79: 49-56.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29: 83-112.

Keeler, K. 1991. Survivorship and recruitment in a long-lived prairie perennial, *Ipomea leptophylla* (Convolvulaceae). American Midland Naturalist 126: 44-60.

Kery, M., D. Matthies, and H. Spillman. 2000. Reduced fecundity and offspring performance of the declining grassland plants *Primula veris* and *Gentiana lutea.* Journal of Ecology 88: 17-30.

Klinkhamer, P. G. L., and T. J. de Jong. 1990. Effects of plant size, plant density, and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57: 399-405.

Knapp, A. K., and T. R. Seastedt. 1986. Detritus accumulation limits productivity of tallgrass prairie. Bioscience 36: 662-667.

Kunin, W. E. 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica Kaber*. Journal of Ecology 85: 225-234.

Kutiel, P., and Z. Naveh. 1987. Soil properties beneath *P. halepensis* and *Q. calliprinos* trees on burned and unburned mixed forests on Mt. Carmel, Israel. Forest Ecological Management 20: 11-24.

Lamont, B. B., P. G. L. Klinkhamer, and E. T. F. Witkowski. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* – a demonstration of the Allee effect. Oecologia 94: 446-450.

Lesica, P. 1999. Effects of fire on the demography of the endangered, geophytic herb *Silene spaldingii* (Caryophyllaceae). American Journal of Botany 86: 996-1002.

Matthies, D., B. Schmid, and P. Schmid-Hempel. 1995. The importance of population processes for the maintenance of biological diversity. Gaia 4: 105-116.

Menges, E. S., and R. W. Dolan. 1998. Demographic viability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. Journal of Ecology 86: 63-78.

Morgan, J. W. 1999. Effects of population size on seed production in an endangered, fragmented grassland plant. Conservation Biology 13: 266-273.

Murcia, C. 1995. Edge effects in fragmented forests: implications for conservation. Trends in Ecology and Evolution 10: 58-62.

Ne'eman, G., and A. Dafni. 1999. Fire, Bees, and seed production in a Mediterranean key species *Salvia fruticosa* Miller (Lamiaceae). Israel Journal of Plant Sciences 47: 157-163.

Ne'eman, G., A. Dafni, and S. G. Potts. 2000. The effect of fire on flower visitation rate and fruit set in four core-species in east Mediterranean scrubland. Plant Ecology: 97-104.

O'Brien, J. J. 1998. The distribution and habitat preferences of rare *Galactia* species (Fabaceae) and *Chamaesyce deltoidea* subspecies (Euphorbiaceae) native to southern Florida pine rockland. Natural Areas Journal 18: 208-222.

Olesen, J. M., and S. K. Jain. 1994. Fragmented plant populations and their lost interactions. pp. 417-426 *in* V. Loeschcke, J. Tomuk, and S. K. Jain, editors. Conservation Genetics. Birkhauser, Basel.

Oostermeijer, J. G. B., M. W. van Eijck, J. C. M. den Nijs. 1994. Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). Oecologia 97: 289-296.

Oostermeijer, J. G. B., S. H. Luijten, Z. V. Krenova, and H. C. M. Nijs. 1998. Relationships between population and habitat characteristics of the rare *Gentiana pneumonanthe* L. Conservation Biology 12: 1042-1053.

Ostertag, R., and E. S. Menges. 1994. Patterns of reproductive effort with time since last fire in Florida scrub plants. Journal of Vegetation Science 5: 303-310.

Pudlo, R. J., A. J. Beattie, and D. C. Culver. 1980. Population consequences of changes in an ant-seed mutualism in *Sanguinaria canadensis*. Oecologia 46: 32-37.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. pp. 305-329 *in* L. Real, editor. Pollination Biology. Academic Press, Orlando, FL.

Rathcke, B., and E. S. Jules. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65: 273-277.

Robbins, L. E., and R. L. Myers. 1992. Seasonal effects of prescribed burning in Florida: a review. Tall Timbers Research Station Miscellaneous Publication No. 8, pp. 96, Tallahassee, FL.

Robertson, W. B., Jr. 1953. A survey of the effects of fire in Everglades National Park. Everglades National Park Service, Homestead, FL.

Roll, J., R. J. Mitchell, R. J. Cabin, and D. L. Marshall. 1997. Reproductive success increases with local density of conspecifics in a desert mustard *(Lesquerella fendleri*). Conservation Biology 11: 738-746.

Santos, T., and J. L. Telleria. 1994. Influence of forest fragmentation on seed consumption and dispersal of Spanish juniper, *Juniperus thunifera.* Biological Conservation 70: 129-134.

Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5: 18-32.

Sih, A., and M. S. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68: 1679-1690.

Silander, J. A. 1978. Density-dependent control of reproductive success in *Cassia biflora*. Biotropica 10: 292-296.

Snyder, J. R., and G. Ward. 1987. Effect of season of burning on the flowering response of subtropical prairie plants. Bulletin of the Ecological Society of America 68: 419.

Snyder, J. R., A. Herndon, and W. B. Robertson. 1990. South Florida rockland. pp. 230-277, in R. L. Myers, and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, University Presses of Florida, Orlando, FL.

Sowig, P. 1989. Effects of flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera: Apidae). Oecologia 78: 550-558.

Spears, E. E. 1987. Island and mainland pollination ecology of *Centrosema virginianum* and *Opuntia stricta*. Journal of Ecology 75: 351-362.

Spier, L. P., and J. R. Snyder. 1998. Effects of wet- and dry-season fires on *Jacquemontia curtisii*, a south Florida pine forest endemic. Natural Areas Journal 18: 350-357.

Spira, T. P. 2001. Plant-pollinator interactions: a threatened mutualism with the implications for the ecology and management of rare plants. Natural Areas Journal 21: 78-88.

SPSS Inc. 1999. SPSS Base 10.0 User's Guide. SPSS Inc., Chicago, IL.

Taylor, D. L. 1981. Fire history and fie records for Everglades National Park, 1948-1979. Report T-619, Everglades National Park South Florida Research Center. Available from: Everglades National Park, Homestead, FL.

van Treuren, R., R. Bijlsma, N. J. Ouborg, and M. M. Kwak. 1994. Relationships between plant density, outcrossing rates and seed set in natural and experimental populations of *Scabiosa columbaria*. Journal of Evolutionary Biology 7: 287-302.

Vitousek, P. M. 1994. Beyond global warming: ecology and global change. Ecology 75: 1861-1902.

Wade, D., J. Ewel, and R. Hofstetter. 1980. Fire in south Florida Ecosystems. Report SE-17, U.S. Forestry Service General Technical Report. Southeast Forest Experimental Station, Asheville, NC.

Whelan, R. J. 1995. The ecology of fire. Cambridge University Press, Cambridge.

Widen, B. 1993. Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio intergrifolius* (Asteraceae) Biological Journal of the Linnean Society 50: 179-195.

Wunderlin, R. P. 1998. Guide to the vascular plants of Florida. University Press of Florida, Gainesville, FL.

Young, A., and N. Mitchell. 1994. Microclimate and vegetation edge effects in a fragmented podocarp-broadleaf forest in New Zealand. Biological Conservation: 67: 63-72.

Zabel, J., and T. Tscharntke. 1998. Does habitat fragmentation of *Urtica* habitats affect phytophagous and predatory insects differentially? Oecologia 116: 419-425.