

BREEDING SYSTEM AND POLLINATION OF A NARROWLY ENDEMIC HERB OF THE LOWER FLORIDA KEYS: IMPACTS OF THE URBAN-WILDLAND INTERFACE¹

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We examined the breeding system and pollination of *Chamaecrista keyensis* Pennell (Fabaceae: Caesalpinioideae) and the effects of urban edge and mosquito control on reproduction of this rare endemic herb of the Lower Florida Keys. Controlled hand-pollination treatments were applied to plants in the field. Although *C. keyensis* flowers are self-compatible, they are not capable of automatic selfing. Inbreeding depression was observed in both seed set and percentage seed germination. Bees of seven genera were observed visiting *C. keyensis* flowers during the peak flowering season (June to July). Only *Xylocopa micans* and *Melissodes* spp. may be effective pollinators for *C. keyensis*, as they were the only bees that “buzz pollinate” this species, which has poricidal anther dehiscence. *Chamaecrista keyensis* received substantially more visits by *X. micans*, but fewer visits from *Melissodes* spp. in urban-edge vs. forest sites. Aerial mosquito spraying may exacerbate the existing pollinator limitation suffered by *C. keyensis* by reducing the number of visits by the buzz-pollinating bees. Individuals of *C. keyensis* at urban edges produced fewer seeds per fruit than did individuals in a pristine forest mainly because of greater insect seed predation.

Key words: breeding system; buzz pollination; *Chamaecrista keyensis*; Fabaceae; Florida Keys; mosquito control; pesticide spray; pine rocklands; pollination; urban-wildland interface.

The continued existence of plants in changing habitats depends a great deal on their reproductive biology. Knowledge of both pollination biology and breeding systems of rare and endangered species is essential for successful management and recovery programs (Hamrick et al., 1991; DeMauro, 1993; Weller, 1994; Spira, 2001). Such information may also provide insights into the vulnerability of a species (e.g., Bowlin et al., 1993; Sipes and Tepedino, 1995; Carlsen et al., 2002).

Rarity and endangerment of a plant species may be due to intrinsic (related to the biology of the species) or extrinsic (related to environment) factors (Rabinowitz, 1981; Fiedler and Ahouse, 1992). While some extrinsic factors are natural, many others are caused by humans. Anthropogenic habitat fragmentation has been widely cited as a major threat to biodiversity (Simberloff, 1988). As a result, many studies have evaluated the direct and indirect biotic effects of fragmentation (e.g., Shreeve and Mason, 1980; Jennersten, 1988; Aizen and Feinsinger, 1994a). One of the many changes brought about by habitat fragmentation is increased edge habitat (Murcia, 1995). Most edge-effect studies have dealt with edge habitats that were created by agricultural fields (Sork, 1983; Murcia, 1995; Kapos et al., 1997; Fortin and Mauffette, 2001; Tscharrntke et al., 2002). Yet, urban edges are increasingly

common as a result of urban sprawl into natural areas. Effects of the urban matrix on natural populations and processes are likely different from those of an agricultural matrix, as effects of edge habitat vary depending on the degree of contrast between the forest and its surrounding matrix (Kapos et al., 1997). An urban matrix dominated by roads, houses, and artificial gardens is different from agricultural fields in both abiotic and biotic components. Few studies, however, have compared the biology of plants in an urban-edge habitat with those in a pristine habitat.

Another threat to natural populations and processes that comes from agricultural and urban development is the use of pesticides. Aerial pesticides on crop and forestry fields have been shown to negatively affect pollinator populations (Johansen, 1977; Johansen et al., 1983; Kearns and Inouye, 1997; Spira, 2001). Aerial insecticide spraying that coincides with the flowering of endangered entomophilous species threatens the continued existence of those species (Bowlin et al., 1993; Sipes and Tepedino, 1995). Pesticide spray is also used in urban areas to control mosquitos. Despite concerns about the use of mosquito spraying on natural insect populations, these effects on plant species are seldom examined.

Chamaecrista keyensis Pennell (Fabaceae), big pine partridge pea, is a narrowly endemic understory herb of pine rocklands, a fire-dependent ecosystem of the Lower Florida Keys. This species was formerly found on several of the Lower Keys (No Name, Big Pine, and Ramrod Keys [Irwin and Barneby, 1982]). However, a more recent survey by Ross and Ruiz (1996) found it only on Big Pine Key, indicating the extirpation of this species from parts of its former range. The most prominent threats to this species include habitat loss and degradation, especially long-term fire exclusion (Snyder et al., 1990). Although not yet recognized by the U.S. Fish and Wildlife Service (USFWS) as an endangered species, *C. keyensis* has been recommended for federal listing and is currently listed by the state of Florida (Florida Natural Areas Inventory, 2002).

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Big Pine Key has the largest pine rockland forest of the Lower Florida Keys (Ross and Ruiz, 1996) and many urban/wildland interface issues. Roads and residential and commercial buildings fragment the once continuous forest on the island. The many parcels of private property within the National Key Deer Refuge, a major site for *C. keyensis*, also provide many challenges for wildland managers. Peak mosquito season overlaps the flowering peak (June–July) of *C. keyensis*, and mosquito control includes aerial (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) and ground [Permethrin (3-phenoxyphenyl) methyl (\pm) *cis*, *trans*-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate and piperonyl butoxide)] sprayings throughout the island during the summer, even though most of the pine forest is federal property. Mosquito-control agents are sprayed in response to monitoring and resident complaints on no fixed schedule, but sometimes as often as daily.

Our goals in this research were twofold: to elucidate the breeding system and pollinators of *C. keyensis* and to assess the effects of urban wildland interface on the reproductive biology of *C. keyensis*. Specifically, we address the following questions: (1) Is *C. keyensis* self-compatible? (2) Is there inbreeding depression with self-pollination? (3) Does *C. keyensis* depend on insect pollinators for sexual reproduction? (4) Which insect species are effective pollinators? (5) Are there differences in pollinator visitation and fruit and seed set between forest and urban-edge populations? (6) What is the effect of aerial mosquito control on the pollination biology of *C. keyensis*?

MATERIALS AND METHODS

Species description—*Chamaecrista keyensis* produces large, showy, yellow, slightly asymmetrical flowers (2.3×2.7 cm, $N = 12$) from May to August, the wet season (Fig. 1). Individual plants produce one to many flowers per day. The 1-d flowers open right before dawn and wilt before dusk. Each flower has one pistil and nine stamens (Fig. 1). Anthers of *C. keyensis*, as do all other species in the genus, dehisce by apical pores, a condition termed poricidal dehiscence (Buchmann, 1983). Because access to the pollen is severely limited by the small terminal pores, pollination of poricidal flowers usually rely on bees that can carry out “buzz pollination” (Buchmann, 1978). Buzz pollination refers to a bee shivering its indirect flight muscles to generate a specific frequency of vibration that effectively releases the pollen for collection (Buchmann, 1978). Pollination of *C. keyensis* is therefore expected to be insect dependent.

Study area—Pine rocklands, a fire-dependent habitat, occur on outcroppings of limestone in extreme southern Florida (Snyder et al., 1990). Habitat destruction, fragmentation, and degradation (i.e., long-term fire exclusion) have made pine rocklands a globally endangered ecosystem (Snyder et al., 1990). Pine rocklands on Big Pine Key, the only home of *C. keyensis*, is also an important habitat for the federally endangered key deer. The canopy of pine rocklands is monotypic, composed of slash pine (*Pinus elliottii* var. *densa*). The relatively open canopy allows the growth of a diverse shrub and herb layer, with many rare and endemic species (Alexander and Dickson, 1972). The 665 ha of pine rocklands on Big Pine Key is fragmented into many tracts by roads, houses, and firelanes.

Our breeding system study was carried out at two sites, Orchid and Loma Lane. Orchid represents “pristine” pine rocklands located at the northeast end

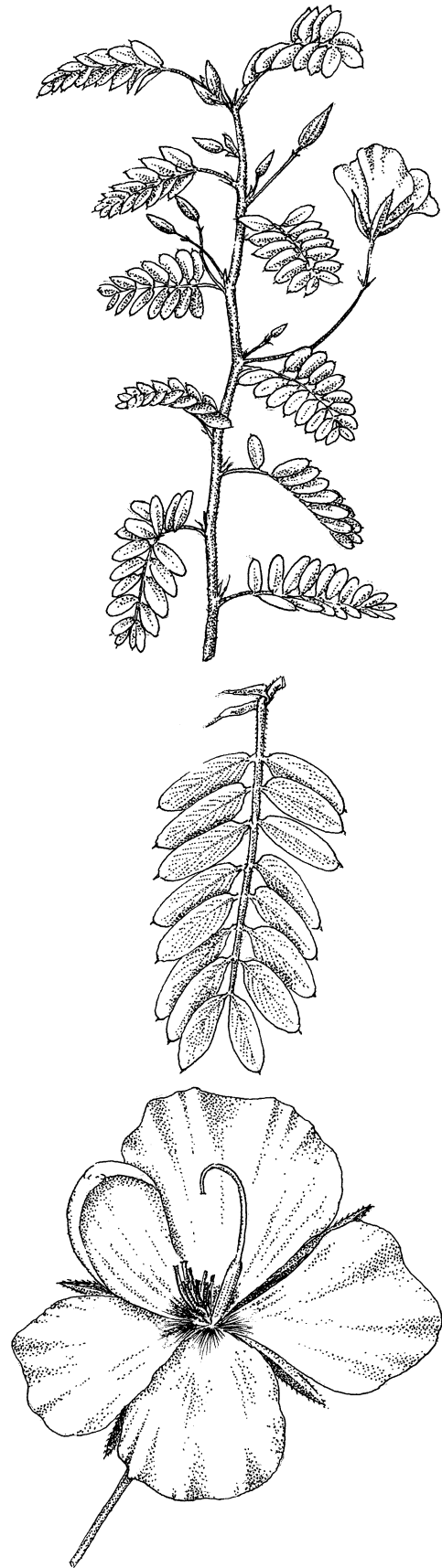


Fig. 1. Floral and plant morphology of *Chamaecrista keyensis*. Drawings by Wes Jurgens.

TABLE 1. Experimental protocol of hand pollination and the number of flowers and plants used in each treatment for breeding system study of *Chamaecrista keyensis* at Orchid (forest) and Loma Lane (urban edge) sites on Big Pine Key in the Lower Florida Keys. Pollen supplement, in conjunction with control treatment, is to determine the existence of pollen limitation.

	Autogamy	Self-pollination	Cross-pollination	Pollen supplement	Control
Bagging	Yes	Yes	Yes	No	No
Hand pollination	No	Yes	Yes	Yes	No
No. flower/no. plant at Orchid	64/31	72/23	91/34	40/21	102/35
No. flower/no. plant at Loma Lane	21/16	22/11	19/10	7/3	33/17

of Big Pine Key in a relatively large (20 ha) continuous piece of pineland (the forest population). This area has burned at various intervals (7–10 yr) during the last two decades; prior to that, its fire history is uncertain. Loma Lane represents degraded pine rocklands in the southwest part of Big Pine Key, adjacent to roads and homes, mowed occasionally and managed by homeowners (the urban-edge population). Pollinator watches were conducted throughout the island, including the Orchid and Loma Lane sites.

Breeding system—To determine the breeding system of *C. keyensis*, we performed controlled hand-pollination experiments (Kearns and Inouye, 1993) on plants in their natural environments using five treatments: autogamy (automatic self-pollination), self-pollination, cross-pollination, pollen supplement, and control (Table 1). We did not include emasculation (removal of anthers) to test apomixis because it is difficult to remove all the anthers without damaging the ovary of the flower. We bagged the flowers with fine mesh cloth bags in the early morning, prior to any insect visits and as flowers were just opening. In this way we prevented flower deformation and unwanted insect visits. Hand pollinations began after 0800, when anthers released pollen with tapping (see later). A flower was self-pollinated by applying pollen from another flower of the same plant and then bagging it until dusk to prevent additional pollen deposition by insects. Similarly, a flower was cross-pollinated by applying pollen collected that same morning from one or more plants at least 10 m away. The pollen-supplement (pollen +) treatment was performed by applying pollen from another plant but without bagging the treated flower afterwards (Table 1). This treatment was to test the existence of pollen limitation. For the control treatment, we did nothing to the flower to allow for natural pollination.

Pollen, collected by tapping the anthers gently into a petri dish, was applied to the target stigma with a cosmetic brush. The petri dish and brush were carefully cleaned with alcohol and dried between flowers to prevent contamination. All flowers were tagged with jewelry tags to record the date, plant number, flower number, and treatment. All five treatments were applied randomly on each individual plant on the same day or across several days if there were insufficient flowers to complete all treatments in one day. Fruiting information was collected for each tagged flower over several weeks. Fruit

abortion was obvious within several days. The number of seeds per fruit was counted for subsamples of the tagged fruits from each treatment. Presence or absence of insect seed predation was also noted. Mature fruits that had been attacked by predispersal insect seed predators appeared black and flat (either in part or wholly). Healthy fruit were brown and filled.

Seed germination—Replicates of 10 seeds from each different pollination treatment (with sites and plants pooled) were used to test seed germination. Seeds were nicked to break seed dormancy and then put in petri dishes with moist filter paper. Seeds with any radicle or cotyledon growth were considered germinated. Percentage germination was compared across treatments with all sites combined.

Floral visitors—We carried out 10-min floral visitor watches at arbitrarily selected patches of *C. keyensis* at four forest sites (Orchid, Dogwood, Locustberry, and Buttonwood) and two urban-edge sites (Loma Lane and Wilder Road). From preliminary observations, on sunny days, floral visits start around 0800 and peak between 0900 and 1000, then taper off after 1100. We carried out the watches from 0800 to 1200 on sunny days for two consecutive weeks in early July, when aerial mosquito spraying (adulticide) had just started for the season. The number of visits by different insects and the number of flowers of each watched patch were recorded during the watches. Insect behavior on a flower, such as buzz pollination, was noted. Samples of flower visitors were collected for determination. The captured insects were sampled for pollen types by touching fuchsin gel to the insect's body. The gel was then melted onto a slide for examination under a microscope. Dates of aerial mosquito spraying during the floral visitor watches were recorded and confirmed with the Lower Keys Mosquito Control Unit.

Statistical analysis—We used chi-square tests to assess the differences in fruit set (proportion of flowers that developed fruits) and percentage fruit with insect predation between sites and among treatments. Individual plants subjected to pollination treatments cannot be used as replicates for the described variables because of the small number of flowers available for each treatment per plant. Two-way ANOVA was used to test the effect of site, treatment, and their interaction on seed set (number of seeds per fruit). However, because the error variances were significantly different (Levene's test, $F_{7,195} = 3.933$, $P < 0.001$), we also used nonparametric tests (Mann-Whitney U and Kruskal-Wallis) to verify and report the differences between sites and among and between treatments in seed set (post-hoc tests). We used nonparametric tests to determine the differences in percentage seed germination among and between treatments because no transformation could improve the data for parametric assumptions. Mann-Whitney U tests were used to test the differences in the number of visitors per watch per flower between forest vs. urban-edge sites. We used SPSS 10.0 (SPSS, Chicago, Illinois, USA) for these tests.

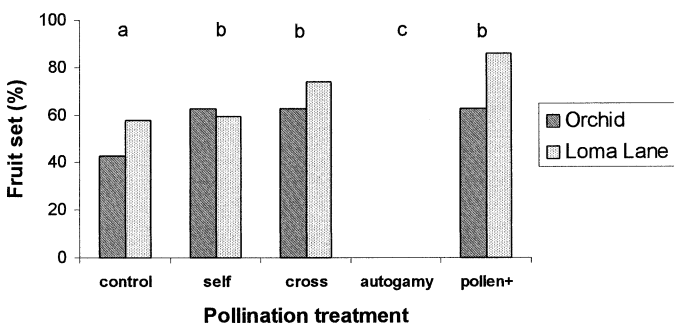


Fig. 2. Percentage fruit set by *Chamaecrista keyensis* from two sites on Big Pine Key after five pollination treatments. Pollen + = pollen-supplement treatment. Treatments with different letters indicate a significant difference at 0.05 level with chi-square tests. Between-site differences not statistically significant. Please note that the autogamy treatment had zero fruit set at both sites.

RESULTS

Fruit set—Fruit set pooled across treatments did not differ significantly between the two sites ($\chi^2 = 0.845$, $df = 1$, $P = 0.358$). Similar results were found between sites for all treatments ($\chi^2 = 2.088, 0.083, 0.838, 1.430$, $df = 1$; $P = 0.148, 0.773, 0.360, 0.232$, for control, selfed, crossed, and pollen-supplement treatments, respectively) (Fig. 2). All flowers in the

TABLE 2. Two-way ANOVA table for seed set of *Chamaecrista keyensis* under four pollination treatments at Orchid (forest) and Loma (urban edge) sites on Big Pine Key.

Source	Type III SS	df	Mean square	F	P
Corrected model	886.646	7	126.664	13.444	<0.001
Intercept	1710.220	1	1710.220	181.518	<0.001
Site	304.866	1	304.866	32.358	<0.001
Treatment	183.506	3	61.169	6.492	<0.001
Site × treatment	14.700	3	4.900	0.520	0.669
Error	1837.245	195	9.422		
Total	10213.000	203			
Corrected total	2723.892	202			

autogamy treatment aborted except for one, the bag of which was removed from the flower by an unknown agent (we suspect a key deer) and was excluded from the analysis. Because there was not a significant site difference, we analyzed the differences among and between treatments with sites pooled. There were significant differences among the treatments, with (Pearson $\chi^2 = 100.932$, $df = 4$, $P < 0.001$) or without the autogamy treatment (Pearson $\chi^2 = 10.779$, $df = 3$, $P = 0.013$) (Fig. 2). The control treatment yielded significantly fewer fruit than the selfed (Pearson $\chi^2 = 5.027$, $df = 1$, $P = 0.025$), crossed (Pearson $\chi^2 = 7.819$, $df = 1$, $P = 0.05$), and pollen-supplement treatments (Pearson $\chi^2 = 5.195$, $df = 1$, $P = 0.023$).

Seed set—The autogamy treatment was excluded from this analysis because it did not produce any fruit. Because results from nonparametric tests agreed with ANOVA, we report the two-way ANOVA table on seed set (Table 2). Seed set of the Loma Lane (urban edge) population was significantly lower than that of the Orchid (forest) population. Seed set was also significantly different among the four pollination treatments (Fig. 3). Post-hoc Mann-Whitney tests indicated that seed set of selfed flowers was significantly lower than control ($Z = -3.928$, $P < 0.001$), crossed ($Z = -5.096$, $P < 0.001$), and pollen-supplement treatment flowers ($Z = -4.111$, $P < 0.001$). Seed set of the control treatment was significantly lower than crossed flowers ($Z = -2.772$, $P = 0.006$) and pollen-supplemented flowers ($Z = -2.356$, $P = 0.018$).

Seed predation—The percentage of fruit suffering predation was significantly higher in Loma Lane than in Orchid (67.6%

vs. 4.3%, Pearson $\chi^2 = 89.825$, $df = 1$, $P < 0.001$). However, there was no significant difference in seed predation among the four pollination treatments that produced fruits (66.7%, 62.5%, 77.8%, and 50% for control, selfed, crossed, and pollen-supplement treatments, respectively, Pearson $\chi^2 = 0.810$, $df = 3$, $P = 0.847$).

Seed germination—Percentage germination differed significantly among seeds produced by different pollination treatments (Kruskal-Wallis $\chi^2 = 19.044$, $df = 3$, $P < 0.001$). Significantly fewer selfed seeds germinated than in the three other treatments (Fig. 4).

Floral visitors and urban-edge habitat—We observed 153 bees of seven genera visiting *C. keyensis* flowers during a total of 185 watches (104 and 81 for forest and urban sites, respectively) (Table 3), among which only *Xylocopa micans* and *Melissodes* spp. bees carried out buzz pollination when visiting *C. keyensis*. Bees of these two genera constitute 50% of the total observed visitors (Fig. 5). However, *Megachile* bees were the most frequent visitors (Fig. 5). Although all examined visitors carried pollen of *C. keyensis*, probably not all are effective pollinators. Only the buzz-pollinators landed on and grabbed the anthers while vibrating, with their bodies touching the stigma (Fig. 6). The nonbuzz bees, most of which were less than 9 mm tall (Table 3), usually landed on the flower petals near the base of the flower without touching the stigma, which is 1.0 cm ($N = 12$) above the flower base (Figs. 1, 6) to gather pollen that was accidentally scattered by the buzz bees. Therefore, the buzz bees are the only likely pollinators for *C. keyensis*.

Because the number of visitors was influenced by the number of flowers in each observed patch, analysis of the number of flower visitors was adjusted on a per-flower basis. The num-

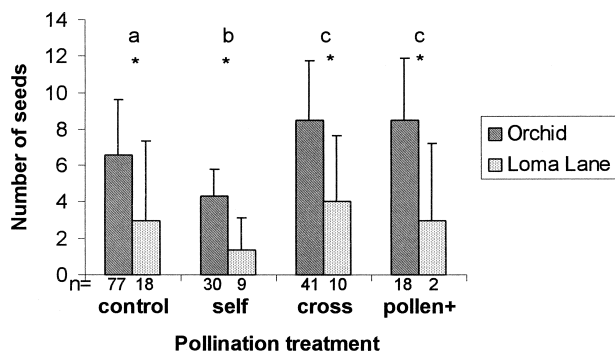


Fig. 3. Mean and standard deviation of seed set of *Chamaecrista keyensis* after four pollination treatments at two sites on Big Pine Key. Pollen + = pollen-supplement treatment. Treatments with different letters indicate significant difference at 0.05 level (Mann-Whitney *U* tests). An asterisk indicates significant difference between sites at 0.05 level within treatments.

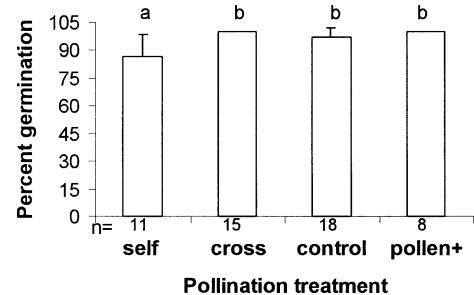


Fig. 4. Percentage germination of *Chamaecrista keyensis* seeds after four pollination treatments at two sites on Big Pine Key. Pollen + = pollen supplement, n = the number of germination trial replicates. Note that the standard deviations of crossed and pollen-supplement treatments are zeros.

TABLE 3. Floral visitors to *Chamaecrista keyensis* and number of pollen types carried by each on Big Pine Key. All examined visitors carried pollen from *C. keyensis* along with pollen from one or more other plant species. Flower visitors are listed in order of their body size, largest bee first.

Flower visitor ^a	Mean body size \pm SE (cm)			N	Buzz pollination	Range of no. of pollen types found (N bees examined)
	Length	Width	Height ^b			
<i>Xylocopa micans</i>	1.68 \pm 0.037	0.8 \pm 0	1.22 \pm 0.102	5	Yes	2–4 (5)
<i>Melissodes</i> spp. ^c	1.26 \pm 0.04	0.5 \pm 0.045	0.92 \pm 0.02	5	Yes	3–4 (3)
<i>Centris errans</i>	1.43 \pm 0.033	0.6 \pm 0	1.0 \pm 0.058	3	No	2 (1) ^d
<i>Apis mellifera</i>	1.3 \pm 0.041	0.43 \pm 0.025	0.83 \pm 0.025	4	No	2–3 (3)
<i>Megachile</i> spp. ^e	1.16 \pm 0.031	0.36 \pm 0.016	0.59 \pm 0.023	11	No	3–7 (8)
<i>Augochloropsis anonyma</i>	0.775 \pm 0.048	0.263 \pm 0.013	0.4 \pm 0.041	4	No	3–5 (2)

^a *Dianthidium curvatum floridiense* was observed but not listed here because of lack of specimens.

^b Standing height includes leg length.

^c Includes *M. communis* and *M. comptaoides*.

^d Only one individual caught on *C. keyensis* flowers.

^e Includes *M. georgica*, *M. brevis pseudobrevis*, *M. mendica*, and *M. pruina*.

ber of *Xylocopa micans* visits per watch per flower was substantially higher in the urban-edge habitat than in the forest (Mann-Whitney *U*, $Z = -2.215$, $P = 0.027$) (Figs. 5, 7). Opposite but nonsignificant trends were observed for *Melissodes* spp., other bees, and all bees pooled (Fig. 7).

Patterns of floral visits and aerial mosquito spray—Visits by *Xylocopa* and *Melissodes* peaked between 0900 and 1100, while *Megachile* bee activity remained high all morning (Fig. 8). The other bees' activities declined through the morning hours (Fig. 8). The number of visits per watch per flower by *Melissodes* bees declined to zero following the first of three every-other-day aerial sprayings and only partially recovered after three consecutive spray-free days (Fig. 8). Flower visits by *X. micans* bees also declined, although to a lesser degree than *Melissodes* spp., after each aerial spray (Fig. 9). Flower visits by *Megachile* spp. and other bees were apparently little affected (Fig. 9).

DISCUSSION

Breeding system and urban-edge effects—*Chamaecrista keyensis* is clearly self-compatible: 61% of the self-pollinated flowers set fruit, no different from cross-pollinated flowers. Because none of the flowers in the autogamy treatment set fruit, we conclude that *C. keyensis* relies on the services of insect pollinators for sexual reproduction. Although not tested, the same evidence also suggested that *C. keyensis* is not capable of apomixis (seed set without pollination). Seed set of the control flowers was between that of selfed and crossed (higher than the former and lower than the latter), suggesting that flowers were pollinated by a mixture of self and outcross pollens under natural conditions. *Chamaecrista keyensis* thus has a mixed mating system.

Although capable of selfing and probably doing so under natural conditions as suggested by the seed set data, *C. keyensis* suffers a certain degree of inbreeding depression, as self-pollination resulted in the lowest seed set and percentage germination of all treatments. Inbreeding depression may negatively affect plant demography and thus pose a threat to the conservation of endangered species (Menges, 1991; Burgman and Lamont, 1992; Oostermeijer, 2001). Only inbreeding depression of the very earliest parts of the life cycle was measured in this study. Because the expression of inbreeding depression may vary across a plant's life cycle (Schemske, 1983; Husband and Schemske, 1996), more research is needed to

quantify the extent of inbreeding depression of *C. keyensis* throughout its life cycle.

Although there was no difference in fruit set between Loma Lane (urban edge) and Orchid (forest) populations, Loma Lane had significantly lower seed set across all treatments (autogamy treatment excluded). This lower seed set was due to higher insect predation of seeds. We were unable to rear or determine the identity of two species whose larvae and pupae were found in many pods. We cannot make the generalized conclusion that urban-edge populations have lower seed set and higher insect seed predation than forest populations because of our limited quantitative data (the lack of replicate sites for each type of habitat). However, our results from two sites verified field observations that *C. keyensis* individuals at the urban edge suffer higher seed predation by insects than those inside the forest.

Reduced seed production has been documented in many fragmented plant populations from reduced pollinator services (Jennersten, 1988; Rathcke and Jules, 1993; Aizen and Feinsinger, 1994a; Kearns and Inouye, 1997; Kearns et al., 1998; Steffan-Dewenter and Tschamtkke, 1999; Spira, 2001). In this study, however, the effects of the urban edge on pollinator services were mixed. While the urban-edge habitat had a significantly higher frequency of *X. micans* (buzz-pollinating bees), it had fewer (but not statistically significant) visits by *Melissodes* spp. (also buzz-pollinating bees) and by all bees pooled. Thus, the overall effect of urban edge on pollination and seed production of *C. keyensis* is unclear. Increased visitation frequency by certain bees such as honey bees associated with fragmented habitats has been documented in other studies (Aizen and Feinsinger, 1994b).

Increased herbivory associated with habitat fragmentation has also been shown in many studies and is often attributed to small-area or isolation effects (Kruess and Tschamtkke, 1994, 2000; Lienert et al., 2002). However, results of edge effects on herbivory are mixed (Sork, 1983; Fortin and Mauffette, 2001; Tschamtkke et al., 2002). The increased herbivory has been attributed to reduced parasitism in fragments (Kruess and Tschamtkke, 1994), increased nutrient influx in small sites (Lienert et al., 2002), and enhanced nutritional quality of edge foliage (Fortin and Mauffette, 2001). For *C. keyensis*, increased seed predation may be due to lack of fire, as habitat near the urban edge is rarely burned. More study is needed to understand the mechanism of increased insect seed predation of *C. keyensis* associated with the urban edge.

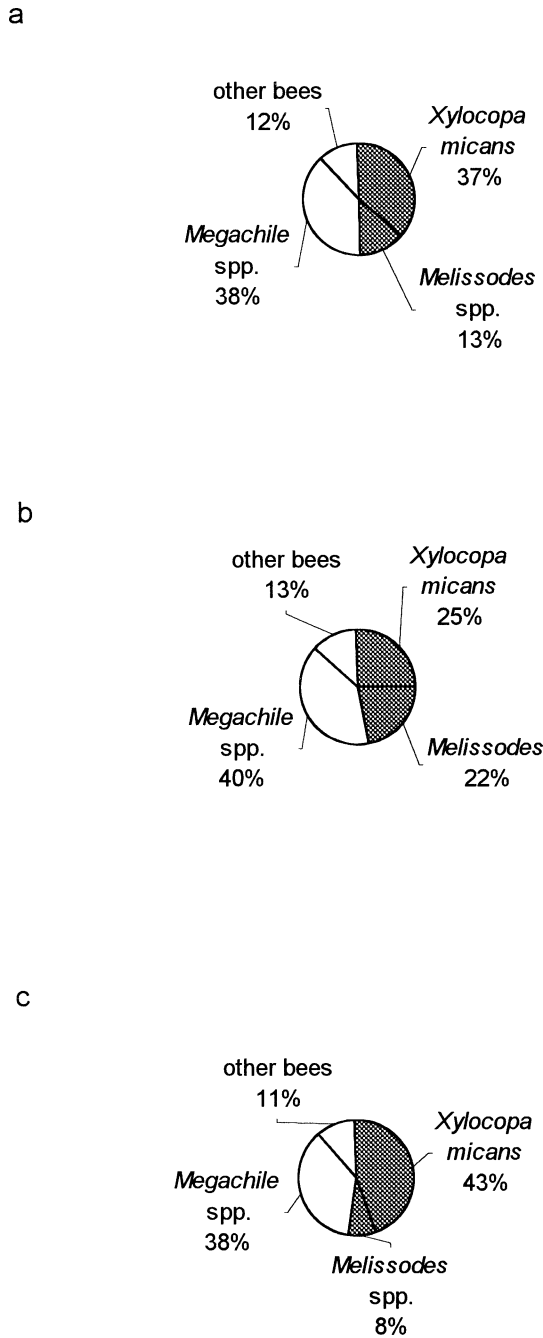


Fig. 5. (a) Observed proportions of *Chamaecrista keyensis* floral visitors at all sites pooled, $N = 153$ bees; (b) four forest sites pooled, $N = 60$ bees; (c) two urban edges pooled, $N = 93$ bees. Shaded areas represent proportions of buzz-pollinating bees.

Pollinators and the effects of aerial mosquito spraying—Percentage fruit set of the open-pollinated treatment was lower than the three hand-pollinated treatments (selfing, crossing, and pollen supplement) at both sites, suggesting that fruit production in natural populations of *C. keyensis* was pollen/pollinator limited. Pollinator limitation is widespread in natural plant populations (Bierzychudek, 1981) and may be due to natural factors such as stochasticity in flower visits by insects, low insect numbers, or low floral rewards (Burd, 1994; Johnson and Bond, 1997).

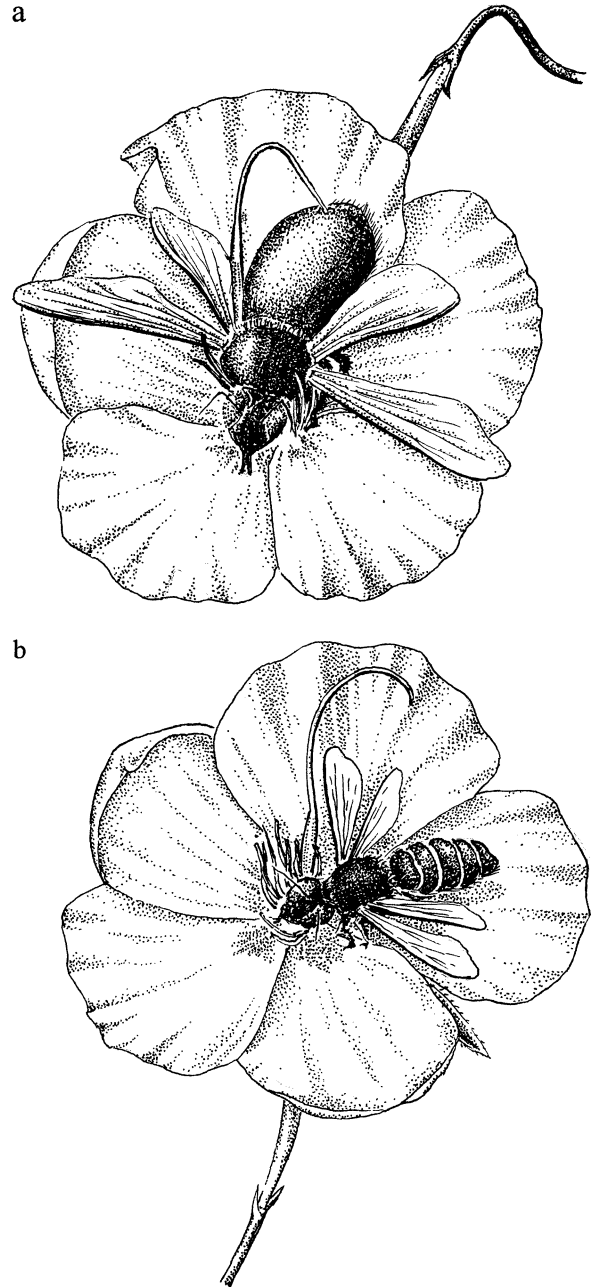


Fig. 6. *Xylocopa micans* (a) and *Megachile georgica* (b) visiting *Chamaecrista keyensis* flowers. Note that *M. georgica* is not in contact with stigma because of its small body size and its nonbuzzing foraging behavior (see detailed description in text). Drawings by Wes Jurgens.

Pollinator limitation in *C. keyensis* may also be due to natural causes. While many species of bees visit *C. keyensis* flowers, only three (*Xylocopa micans* and two *Melissodes* spp.) are capable of buzz pollination. Visits from the buzz pollinators constituted only 50% of the total flower visits. The floral morphology of *C. keyensis* (poricidal anthers and stigma held well above the anthers; Fig. 6) calls for buzz pollination behavior of the bees for effective pollination. The relatively large size of the flowers limits the range of bee sizes that can transfer pollen to the stigmas. The relatively low number of available bee species that are capable of buzzing in *C. keyensis* habitat

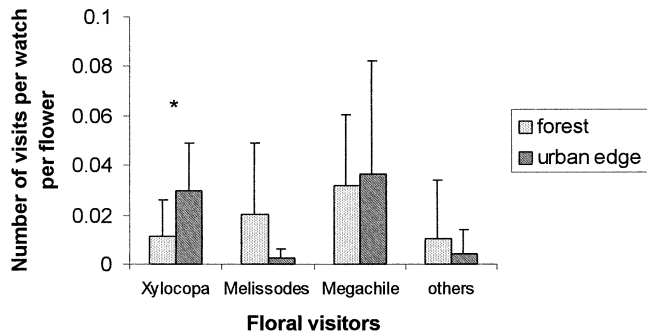


Fig. 7. Mean number (+1 SD) of floral visitors per watch per flower of *Chamaecrista keyensis* at forest vs. urban edge on Big Pine Key. An asterisk indicates significant difference at the 0.05 level between sites. The number of replicates (days of observation) are 11 and 7 for forest and urban-edge habitat, respectively.

may contribute to pollinator limitation. In addition, although the most abundant type of pollen carried by *C. keyensis* visitors was that of *C. keyensis*, all *C. keyensis* visitors, especially *Megachile* spp. (the most frequent, but nonbuzzing, bees) carried pollen of several other plant species. The lack of fidelity may also reduce the pollination efficiency of the pollinators and may even lead to stigma clogging by foreign pollen (Waser, 1978a, b), but this possibility has not yet been examined.

On the other hand, the pollinator limitation experienced by *C. keyensis* may, in part, be due to aerial mosquito control. Mosquito spray seems to have suppressed the number of visits by insects, especially *Melissodes* spp. and *X. micans*, the only buzz-pollinating bees. All these species are solitary bees. Solitary bees are more susceptible to insecticides than social bees because of the lower fecundity of the former (Tepedino, 1979; Spira, 2001). Even if aerial mosquito spray is not the primary cause, it may exacerbate existing pollinator limitation.

Less frequent aerial mosquito spraying (no more than once a week) may be more pollinator-friendly than the spray frequency (every second day) during this study. Nevertheless, caution should be taken in interpreting short-term observational data, such as these, as factors other than mosquito spray (e.g., weather and the intrinsic biology of the bees) could have influenced the activities and abundance of insects (Frankie et al., 1998). Clearly, more research is needed to determine the effects of mosquito spray on *C. keyensis* pollinators and population dynamics.

We have provided evidence here that the endemic Big Pine

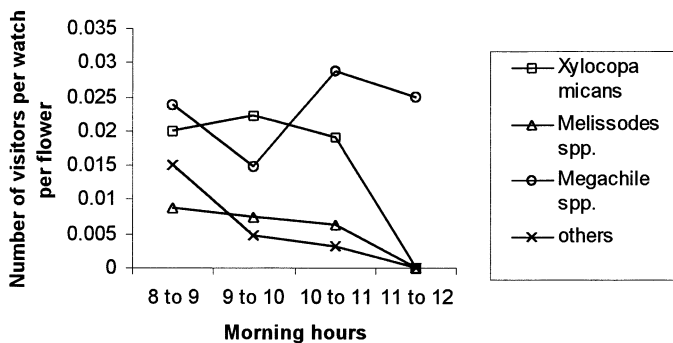


Fig. 8. Average number of visitors per watch per flower of *Chamaecrista keyensis* during the morning hours over 12 d on Big Pine Key. All sites pooled.

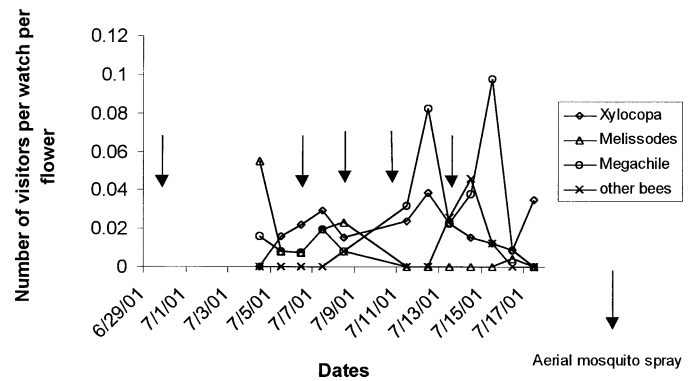


Fig. 9. Aerial mosquito adulticide spray dates and the average number of visitors per watch per flower of *Chamaecrista keyensis* over a 2-wk period in 2001. The first aerial mosquito adulticide spray of that year was on 30 June 2001.

partridge pea is self-compatible but requires insect visitation for pollination. Effective pollination can be performed by buzz-pollinating bees, though many kinds of bees collect pollen from their flowers. Plants may be pollinator limited, and urban-edge habitats have fewer bees visiting flowers (though *X. micans*, one of the buzz pollinators, is more common there) and lose more seed to insect predators. Bee numbers are depressed with aerial mosquito spraying (affecting both urban-edge and "pristine" woods habitats). Prevention of further fragmentation by limiting urban sprawl and more careful use of aerial insecticides are essential to maintain reproductive populations of this plant species.

LITERATURE CITED

- 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: 378–392.
- ALEXANDER, T. R., AND J. D. DICKSON. 1972. Vegetational changes in the National Key Deer Refuge. II. *Quarterly Journal of the Florida Academy of Sciences* 35: 85–96.
- BIERZYCHUDEK, P. 1981. Pollination limitation of plant reproductive effort. *American Naturalist* 117: 838–840.
- BOWLIN, W. R., V. J. TEPEDINO, AND T. L. GRISWOLD. 1993. The reproductive biology of *Eriogonum pelinophilum* (Polygonaceae). In R. Sivinski and K. Lightfoot [eds.], *Southwestern rare and endangered plants*, 296–302. Miscellaneous publication No. 2. New Mexico Forestry and Resources Conservation Division, Santa Fe, New Mexico, USA.
- BUCHMANN, S. L. 1978. Vibratile "buzz" pollination in angiosperms with poricidally dehiscent anthers. Ph.D. dissertation, Department of Entomology, University of California, Davis, California, USA.
- BUCHMANN, S. L. 1983. Buzz pollination in angiosperms. In C. E. Jones and R. J. Little [eds.], *Handbook of experimental pollination biology*, 73–113. Scientific and Academic Editions. Van Nostrand Reinhold, New York, New York, USA.
- BURD, M. 1994. Bateman's principal and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* 60: 83–139.
- BURGMAN, M. A., AND B. B. LAMONT. 1992. A stochastic model for the viability of *Banksia cuneata* populations: environmental, demographic, and genetic effects. *Journal of Applied Ecology* 29: 719–727.
- CARLSEN, T. M., E. K. ESPELAND, AND B. M. PAVLIK. 2002. Reproductive ecology and the persistence of an endangered plant. *Biodiversity and Conservation* 11: 1247–1268.
- DEMAURO, M. M. 1993. Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology* 7: 542–550.

- FIEDLER, P. L., AND J. J. AHOUSE. 1992. Hierarchies and cause: toward an understanding of rarity in vascular plant species. In P. L. Fiedler and S. K. Jain [eds.], *Conservation biology: the theory and practice of nature conservation, preservation and management*, 23–47. Chapman & Hall, New York, New York, USA.
- FLORIDA NATURAL AREAS INVENTORY. 2002. [Http://www.fnai.org](http://www.fnai.org).
- FORTIN, M., AND Y. MAUFFETTE. 2001. Forest edge effects on the biological performance of the forest tent caterpillar (Lepidoptera: Lasiocampidae) in sugar maple stands. *Ecoscience* 8: 164–172.
- FRANKIE, G. W., R. W. THORP, L. E. NEWSTROM-LLOYD, M. A. RIZZARDI, J. F. BARTHELL, T. L. GRISWOLD, J. Y. KIM, AND S. KAPPAGODA. 1998. Monitoring solitary bees in modified wildland habitats: implications for bee ecology and conservation. *Environmental Entomology* 27: 1137–1148.
- HAMRICK, J. L., M. J. W. GODT, D. A. MURAWSKI, AND M. D. LOVELESS. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In D. A. Falk and K. E. Holsinger [eds.], *Genetics and conservation of rare plants*, 75–86. Oxford University Press, New York, New York, USA.
- HUSBAND, B. C., AND D. W. SCHEMSKE. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.
- IRWIN, H. S., AND R. C. BARNEBY. 1982. The American Cassiinae, a synoptical revision of Leguminosae, tribe Cassieae, subtribe Cassiinae in the New World. *Memoirs of the New York Botanical Garden* 35: 1–918.
- JENNERSTEN, O. 1988. Pollination in *Dianthus deltooides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology* 2: 359–366.
- JOHANSEN, C. A. 1977. Pesticides and pollinators. *Annual Review of Entomology* 22: 177–192.
- JOHANSEN, C. A., D. F. MAYER, J. D. EVES, AND C. W. ISIOUS. 1983. Pesticides and bees. *Environmental Entomology* 12: 1513–1518.
- JOHNSON, S. D., AND E. J. BOND. 1997. Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. *Oecologia* 109: 530–534.
- KAPOS, V., E. WANDELLI, J. L. CAMARGO, AND G. GANADE. 1997. Edge-related changes in environment and plant responses due to forest fragmentation in central Amazonia. In W. F. Laurance and R. O. Bierregaard, Jr. [eds.], *Tropical forest remnants*, 33–44. University of Chicago Press, Chicago, Illinois, USA.
- KEARNS, C. A., AND D. W. INOUE. 1993. *Techniques for pollination biologists*. University of Colorado Press, Niwot, Colorado, USA.
- KEARNS, C. A., AND D. W. INOUE. 1997. Pollinators, flowering plants, and conservation biology—much remains to be learned about pollinators and plants. *Bioscience* 47: 297–307.
- KEARNS, C. A., D. W. INOUE, AND N. M. WASER. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics* 29: 83–112.
- KRUESS, A., AND T. TSCHARNTKE. 1994. Habitat fragmentation, species loss, and biological control. *Science* 264: 1581–1584.
- KRUESS, A., AND T. TSCHARNTKE. 2000. Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. *Oecologia* 122: 129–137.
- LIENERT, J., M. DIEMER, AND B. SCHMID. 2002. Effects of habitat fragmentation on population structure and fitness components of the wetland specialist *Swertia perennis* L. (Gentianaceae). *Basic and Applied Ecology* 3: 101–114.
- MENGES, E. S. 1991. Seed germination percentage increases with population size in a fragmented prairie species. *Conservation Biology* 5: 158–164.
- MURCIA, C. 1995. Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution* 10: 58–62.
- OOSTERMEIJER, J. G. B. 2001. Population viability analysis of the rare *Gentiana pneumonanthe*: importance of demography, genetics, and reproductive biology. In A. Young and G. Clark [eds.], *Genetics, demography, and viability of fragmented population*, 313–334. Cambridge University Press, Cambridge, UK.
- RABINOWITZ, D. 1981. Seven forms of rarity. In H. Synge [ed.], *The biological aspects of rare plant conservation*, 205–217. Wiley, New York, New York, USA.
- RATHCKE, B., AND E. S. JULES. 1993. Habitat fragmentation and plant–pollinator interactions. *Current Science* 65: 273–277.
- ROSS, M. S., AND P. RUIZ. 1996. A study of the distribution of several South Florida endemic plants in the Florida Keys. Report to the U.S. Fish and Wildlife Service. Florida International University, Southeast Environmental Research Program, Miami, Florida, USA.
- SCHEMSKE, D. W. 1983. Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). *Evolution* 37: 523–539.
- SHREEVE, T. G., AND C. MASON. 1980. The number of butterfly species in woodlands. *Oecologia* 45: 414–418.
- SIMBERLOFF, D. 1988. The contribution of population and community biology to conservation science. *Annual Review of Ecology and Systematics* 19: 473–511.
- SIPES, S. D., AND V. J. TEPEDINO. 1995. Reproductive biology of the rare orchid, *Spiranthes diluvialis*: breeding system, pollination, and implications for conservation. *Conservation Biology* 9: 929–938.
- SNYDER, J. R., A. HERNDON, AND W. B. ROBERTSON. 1990. South Florida rockland. In R. L. Myers and J. J. Ewel [eds.], *Ecosystems of Florida*, 230–277. University of Central Florida Press, University Presses of Florida, Orlando, Florida, USA.
- SORK, V. L. 1983. Distribution of pignut hickory (*Carya glabra*) along a forest to edge transect, and factors affecting seeding recruitment. *Bulletin of the Torrey Botanical Club* 110: 495–506.
- SPIRA, T. P. 2001. Plant–pollinator interactions: a threatened mutualism with implications for the ecology and management of rare plants. *Natural Areas Journal* 21: 78–88.
- STEFFAN-DEWENTER, I., AND T. TSCHARNTKE. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121: 432–440.
- TEPEDINO, V. J. 1979. The importance of bees and other insect pollinators in maintaining floral species composition. *Great Basin Naturalist Memoirs* 3: 139–150.
- TSCHARNTKE, T., I. STEFFAN-DEWENTER, A. KRUESS, AND C. THIES. 2002. Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. *Ecological Applications* 12: 354–363.
- WASER, N. M. 1978a. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* 59: 934–944.
- WASER, N. M. 1978b. Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia* 36: 223–236.
- WELLER, S. G. 1994. The relationship of rarity to plant reproductive biology. In M. L. Bowles and C. J. Whelan [eds.], *Restoration of endangered species*, 90–117. Cambridge University Press, Cambridge, UK.