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INTERACTIONS BETWEEN CACTUS-SPECIALIST SOLITARY BEES  
AND THEIR HOST CACTI

by

Margrit Elizabeth McIntosh

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A Dissertation Submitted to the Faculty of the  
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GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Margrit Elizabeth McIntosh entitled Interactions between cactus-specialist solitary bees and their host cacti

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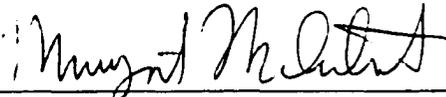
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SIGNED: 

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## **DEDICATION**

I dedicate this work to my mother, Marie-Louise Schuler McIntosh, who gave me a scientist's love for and fascination with the natural world, and to my father, Donald Spencer McIntosh, who gave me a scientist's unshakable faith in the truth. I also dedicate this work to all those beings throughout space and time who reverence infinite diversity in infinite combinations.

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## ABSTRACT

Although bees are herbivorous insects, they are seldom studied as such. My dissertation research characterizes the interactions between a guild of solitary cactus-specialist bees and the cacti they visit, examining these bees both as herbivores and as pollinators.

I first examined the reproductive biology of two species of *Ferocactus* as background for the pollination study. Both *F. cylindraceus* and *F. wislizeni* were obligate outcrossers, and neither species was pollen-limited. Fruit set was high in both species, and reproductive output was limited by architectural constraints.

Whereas most other herbivorous insects are antagonists of the plants they feed on, bees are usually regarded as mutualists (pollinators) of their host plants. However, not all floral visitors are pollinators, and pollen-foraging specialists have been postulated by some to be particularly antagonistic to the plants they visit. To test whether cactus-specialist bees are actually mutualists of the cacti they visit, I examined both the quantity and quality components of pollinator effectiveness of bees visiting *F. cylindraceus* and *F. wislizeni*. Despite the generalized morphology of the flowers of these plants, there were very few floral visitors other than three species of cactus-specialist bees. Flowers of both species were pollinated almost exclusively by cactus-specialist bees, primarily *Diadasia rinconis*.

I also tested the pollen preferences of four species of cactus bees. Bees were offered flowers from their normal host cactus in which the stamens had been removed and replaced with novel pollens. Novel cactus pollens were accepted to some degree by all four species, but *Diadasia rinconis* and *D. opuntiae* accepted more novel non-cactus pollens than did either *Lithurge apicalis* or *Idiomelissodes duplocincta*. Both species of

*Diadasia* showed significant acceptance of *Sphaeralcea* (Malvaceae) pollen, thought to be the ancestral host plant for the genus *Diadasia*. Caged, naive *D. rinconis* bees did not initiate nesting when presented with *Sphaeralcea* flowers alone: nesting began immediately when cactus flowers were offered. Once nesting was underway, most *D. rinconis* bees switched to *Sphaeralcea* if cactus flowers were removed. In both *D. rinconis* and *D. opuntiae*, there were striking differences in pollen preferences among individuals.

## CHAPTER 1

### INTRODUCTION

Herbivorous insects and the plants they feed on together comprise more than half of the described species of terrestrial eukaryotes (Farrell et al. 1992), and the interactions between them are thought to have contributed in large part to this remarkable diversity (Thompson 1998). Studies of diet breadth and processes of host selection in herbivorous insects are providing biologists with insights into the evolution and maintenance of these crucial interactions.

Determining the host range of insect herbivores is the first step in documenting current patterns of host use, and can suggest future trajectories of host use, such as host shifts. A population with measurable variation in host preference, for example, might be able to shift to a new host if a preferred host becomes unavailable, whereas a population in which all individuals exhibit a genetically based, strict preference for the same host might not be able to survive the loss of the preferred host. Further, comparisons among herbivorous insects that have converged on using the same host plant can suggest the degree to which host shifts are constrained by factors intrinsic to the insect (e.g., neurological constraints in information processing, or the phylogenetic history of host use in the clade) or to the plant (e.g., plant secondary compounds, or ecological correlates such as geographic range). Finally, studying the effects of different herbivores on their host plants can indicate whether the negative impact of an herbivore is significant or negligible.

*Bees as pollinators versus bees as herbivores*

Bees are usually thought of as pollinators, enjoying a mutualistic relationship with the flowering plants they visit. However, bees are also herbivorous insects in that they feed on pollen (reproductive tissue) and nectar (secretion) as both larvae and adults. Hence they are potentially also antagonists of the plants they visit. For example, there is an inherent conflict of interest between the needs of pollen-foraging bees – i.e., to remove pollen and feed it to the offspring – and the needs of the plant, whose priority is to ensure that the pollen arrives at the stigma of a conspecific plant, not in a bee nest (Westerkamp 1996).

The observation that the majority of herbivorous insects are relatively specialized to their host plants has long intrigued biologists, because the disadvantages of resource limitation via specialization seem obvious (Jaenike 1990, Bernays and Weislo 1994). Generalists should perform better than specialists when plant availability is unpredictable in space and time, for example, as generalists can switch to a different plant if the preferred host is unavailable, whereas specialists may not be able to switch. As is the case with most other herbivorous insects, bees are relatively specialized, often collecting pollen from only a few closely related species or genera of plants (Weislo and Cane 1996, Michener 2000). Although host-plant selection and host range has been comparatively well-studied in many herbivorous insects, we know virtually nothing about pollen-host selection in bees. The little we know comes from intensive studies of social, generalist bees such as *Apis mellifera* (the domesticated honeybee) and species of *Bombus* (bumblebees), whose foraging behavior is determined in large part by their sociality. The observation that most solitary bees are pollen-specialists, however, remains just that: an observation. The actual host range of pollen-specialist bees has

almost never been tested in an experimental setting, e.g., by offering foraging bees a choice of pollens (but see Guirguis & Brindley 1974; Cripps & Rust 1989).

Further, we have little information as to whether pollen-specialist bees are more likely than generalist bees to be antagonists of the plants they visit. One could argue that generalists should be better pollinators than specialists, because specialists persistently and efficiently remove and sequester pollen rather than dispersing it. The negative impact of pollen-foraging generalists might be lessened by the fact that they (presumably) visit fewer flowers of any one species than a specialist does. Conversely, one could argue that specialists should be better pollinators because of their constancy to the flowers of one plant species, a constancy that does not vary with the abundance of pollen available from other plants. Very few pollination studies have addressed the question of whether specialist bees are better or worse pollinators than generalists: however, Cane and Payne (1993) found that *Vaccinium* flowers were most effectively pollinated by a “buzz-pollinating” (pollen-foraging) *Vaccinium*-specialist bee.

Unlike honeybees and bumblebees, the majority (~87 to 90%; based on data in Michener 2000) of the ~20,000 species of bees are solitary. Each solitary female creates a nest with individual cells for her offspring, and does not interact with other nesting females. In most cases, the adult female creates a separate chamber or cell for each offspring. She stocks each cell with a provision mass, usually comprised of pollen and nectar, lays an egg on or near the mass, and seals the cell. After sealing the cell she has no further contact with her offspring. The egg hatches, the larva consumes the provision mass and then pupates in the cell, emerging as an adult. Nectar provides the larvae with carbohydrates for energy, and pollen provides protein for tissue growth. Even parasitic bees (~15% of all bees; Michener et al. 1994, Wcislo and Cane 1996) parasitize the

provisions of other bees. Consequently, all larval bees feed on pollen, and all non-parasitic female bees collect pollen.

Adult bees of both sexes ingest nectar (and some pollen) for their own nutritional requirements. Thus, foraging bees are comprised of males and females ingesting nectar and pollen for themselves, and nesting females collecting nectar and pollen for provision masses. I refer to "pollen-foraging" in bees as the behavior of adult females collecting pollen to provision a nest.

Because larval bees have no choice in what they eat, host use in bees is entirely under the control of the adult female as she provisions her nest. Hence, pollen-collecting in bees is the functional analogue of oviposition in many other herbivorous insects: it is via this behavior that the adult female selects the host plant for her offspring.

Pollen foraging in bees is not nearly as well studied as nectar foraging (but see Macior 1994, Rasheed and Harder 1997a, 1997b), and in particular there is little evidence as to whether nectar-collecting visits are more or less likely to effect pollination than pollen-collecting visits (but see Jacob-Remacle 1989, Wilson and Thomson 1991, Free 1993, Conner et al. 1995, Gómez and Zamora 1999). I did not have an a priori expectation as to which type of visit would be better for pollination, but I did hypothesize that there would be a significant difference.

### *Pollen-host specificity in bees*

Solitary bees are usually generalists on nectar hosts, but many specialize to some degree on their pollen hosts (Müller 1996). Pollen-specificity in bees, often referred to as "oligolecty" (in contrast to "polylecty," or generalized behavior in pollen

foraging) is a behavior that persists over many generations of a species. It is distinctly different from the short-term floral constancy exhibited by nearly all foraging bees in that preferences are believed to be genetically based, and are not affected by the presence or relative abundance of other available pollen hosts. Pollen specialization is widespread in bees: more than 50% of North American bees for which host records are known collect pollen from only one plant genus or species (Schemske 1983). Another 12% specialize on plants of one family.

As in other herbivorous insects, the distribution of specialists among bee clades is quite variable. Some lineages contain mostly (or only) oligoleges, some mostly (or only) polyleges, while others are not dominated by either oligoleges or polyleges. This diversity of habit is seen at all levels: family, subfamily, tribe, genus, subgenus, and species group. Oligolecty is widespread in the Andrenidae, Melittidae, and Colletidae (except Hylaeinae), and less prevalent in the Megachilidae and Apidae (including the paraphyletic "Anthophoridae"). Most bees of the family Halictidae are polylectic. Systematic studies of bees using modern phylogenetic methods have only just begun to appear (Alexander 1990, Roig-Alsina 1991, Roig-Alsina and Michener 1993), and we cannot yet draw any firm conclusions about how specialization and generalization have evolved in bees overall. It does seem clear, however, that there have been multiple independent origins of oligolecty.

Abundant plants that produce copious amounts of pollen may in general host more pollen-specialist bees than less abundant and less rewarding plants (Wcislo and Cane 1996). Of the 280 pollen-foraging bees that visit *Helianthus* in the U.S.A., 131 species (49%) are reportedly specialists on *Helianthus* (Hurd et al. 1980; Minckley et al. 1994). Guilds of pollen-collectors and other flower visitors have also been studied on *Larrea* (creosote bush; Hurd and Linsley 1975), *Prosopis* (mesquite; Simpson et al.

1977), *Vaccinium* (blueberries and allies; Cane et al. 1985), and the Onagraceae genera *Oenothera* (Linsley, et al. 1963, Linsley et al. 1964, Linsley et al. 1973), *Camissonia* (Linsley et al. 1973), and *Clarkia* (MacSwain et al. 1973). Plants of these genera have the attributes of “predictable plethora” plants (Weislo and Cane 1996): (a) large amounts of easily available pollen and nectar; (b) persistent local patches (either perennials, or reseeding annuals); (c) flowering phenology that is reliable relative to that of other co-occurring plants; (d) flowering coincides with bee phenology. These attributes also characterize many common species of Cactaceae.

#### *Cactus-specialist bees*

In the deserts of North America, flowers of many species of cacti are visited by a guild of pollen-specialist bees that have convergently evolved the habit of collecting pollen and nectar only from cactus flowers. My dissertation research investigates the interactions between these “cactus bees” and their host plants. In particular, I focus on five species that are common in Arizona: *Diadasia rinconis* and *D. opuntiae* (Apidae: Emphorini), *Lithurge apicalis* (Megachilidae: Lithurgini), *Idiomelissodes duplocincta* (Apidae: Eucerini), and *Ashmeadiella opuntiae* (Megachilidae: Osmiini). There are about forty-six species of *Diadasia* (a New World genus), all of which are apparently oligolectic: most specialize on plants in the Malvaceae, but four specialize on cacti (Sipes & Wolf 2001). *Lithurge* is a world-wide genus of about fifty species; all (about ten) North American species specialize on cacti. *Idiomelissodes* is monotypic, and its affinities within the Eucerini are uncertain. *Ashmeadiella* contains about fifty-five North American species. Other cactus specialist bees in North America include at least seven species of *Perdita* (Andrenidae: Panurginae), one species of *Dufourea* (Halictidae:

Rophitinae), and two species of *Melissodes* (Apidae: Eucerini) (Hurd 1979, Snelling and Danforth 1992, Michener et al. 1994). Because these genera are not closely related, the habit of specializing on cactus pollen has arisen independently at least six times in Apoidea (Roig-Alsina and Michener 1993, Alexander and Michener 1995).

### *Explanation of Dissertation Format*

My dissertation research investigates the biology of cactus bees from two perspectives: as pollinators of barrel cacti and as herbivorous pollen-foragers selecting pollen-host plants for their offspring. To provide the requisite background information for the pollination study, I examined the reproductive biology of two species of the barrel cactus genus *Ferocactus*. I then studied the pollination of these two cacti, using the technique of single-pollinator visits to individual flowers, to determine if cactus bees pollinate the cactus flowers they visit. Finally, I investigated pollen choice in female cactus bees foraging for larval provisions by placing different kinds of pollen into emasculated cactus flowers, and observing whether or not the bees collected the pollen.

The chapters in this dissertation are included as four appendices. All four are single authored papers that have been or will be submitted for publication. A summary of the major findings of these four papers is presented in Chapter 2, Present Study. A general discussion of the strengths, weaknesses, and implications of the four papers is presented in Chapter 3, General Discussion.

Appendix A describes a multi-year study documenting breeding systems, reproductive output, and plant size and growth in two species of barrel cacti, *Ferocactus cylindraceus* and *F. wislizeni* (Cactaceae), at study sites in southeastern Arizona.

Appendix B is a continuation of the above study, investigating the flowering phenology of these two cacti. *F. cylindraceus* and *F. wislizeni* have been documented as sister species, which means that differences between them are the result of evolutionary processes other than shared phylogenetic history. By documenting self-compatibility, degree of pollen limitation, and patterns of fruit set and seeds per fruit of these cacti in natural, open-pollinated settings, the studies described in Appendices A and B provide the requisite background to a study of the pollination of these two cacti.

Appendix C describes a study of the pollination of *F. cylindraceus* and *F. wislizeni*, using the technique of single-pollinator visits. The goal of this study was to discover which floral visitors are pollinating these plants, and to determine whether pollen-foraging in specialist bees is good or bad for plant reproduction. I quantified the results of each visit in terms of probability of fruit set and number of seeds per fruit. Comparisons were drawn between visitors of different taxa, pollen-collecting visits versus nectar-collecting visits, and male versus female bees. I also described the relative importance of per-visit quality and pollinator quantity (relative abundance of visits) to the realized fecundity of the host plants.

Appendix D presents data from experiments in which I offered a choice of different pollens to four species of cactus bees foraging for larval provisions. The goals of this study were to determine how strictly specialized to cactus pollen these bees actually are, whether they have preferences among the pollens of different cacti, and whether there is variation in preference among individuals of each species. Such variation is the raw material for evolutionary processes, and may suggest whether these species have the potential for future host shifts. In other words, if there is no variation in (presumably genetically-based) preferences, then there is nothing for natural selection to act upon, and if the preferred host plant is unavailable, the bee will be unable to

reproduce. If variation in preference is present, however, changes in host availability could eventually lead to a host shift. To facilitate bee-flower contact and control for floral effects other than pollen, I placed novel pollens in cactus flowers whose stamens had been removed. I then recorded the numbers of pollen-collecting visits to control and manipulated flowers, and described the pollen preferences of the four bee species based on their foraging behavior.

## CHAPTER 2

### PRESENT STUDY

The methods, results, and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the major findings in these papers.

In Appendix A, I investigated the reproductive biologies of two species of unbranched short-columnar cacti, *Ferocactus cylindraceus* and *F. wislizeni*. I recorded female reproductive output (flowers produced, fruit set, seeds per fruit and seed mass), plant size and growth, and used hand-pollination experiments to determine breeding systems and pollen limitation. In both species, the ability to self varied among individuals, but self-pollination resulted in very few seeds, suggesting strong inbreeding depression. Neither species was pollen-limited. Numbers of flowers produced increased with plant size for both species, and seeds per fruit may also be related to plant size. Flower production was similar in both species, but *F. cylindraceus* produced fewer seeds per fruit than *F. wislizeni*, and its seeds weighed less. Fruit set by *F. cylindraceus* was heavily impacted by a florivorous lepidopteran. Fruit set was very high (94 to 96%) in *F. wislizeni*. Architectural constraints (e.g., meristem limitation) are probably more limiting to reproductive output for both species than resource levels or the level of pollinator services. In *F. cylindraceus*, numbers of seeds per fruit were positively correlated with mean seed mass per seed per fruit, whereas in *F. wislizeni*, the relationship was negative (the relationship indicated a possible tradeoff between seed size and seed number). The growth rates of *F. wislizeni* are affected by rainfall the previous season, and growth rates increase as the plant ages. *Ferocactus cylindraceus* and *F. wislizeni* are thought to be sister species, meaning that observed differences

between them are more likely to be the result of recent evolutionary processes in their lineages rather than differing phylogenetic histories.

In Appendix B, I investigated flowering phenology and reproductive output of *Ferocactus cylindraceus* and *F. wislizeni*. *Ferocactus cylindraceus* began blooming in May, and continued until early or mid-October, with a bimodal pattern of flowering amplitude. Individuals in the study population were moderately synchronized phenologically. *Ferocactus wislizeni* began blooming in July, and like *F. cylindraceus*, continued until early or mid-October, with a single peak of intensity: individuals in the study population were well synchronized phenologically. In both species, nearly all individuals of reproductive size bloomed every year. Plant size was positively correlated with flowering amplitude in both species, and with flowering onset in *F. wislizeni* (i.e., larger plants began blooming later than smaller plants). The study population of *F. cylindraceus* was strongly affected by a flower-eating caterpillar in all years, with the earliest flowers most likely to be destroyed. For *F. wislizeni*, seed number per fruit was highest for flowers open in the middle of the blooming season in 1998. Other components of individual plant phenology, including among-plant synchrony, had little influence on reproductive output.

In Appendix C, using the method of single pollinator visits, I examined both the quantity and quality components of pollinator effectiveness of bees visiting *F. cylindraceus* and *F. wislizeni*. The goals of this study were to determine which of their flower visitors actually pollinate these plants, and to detect whether nectar-collecting visits are better or worse than pollen-collecting visits in terms of pollination success. Pollen-collecting visits and nectar-collecting visits did not differ in their per-visit pollination effectiveness. However, nectar-collecting visits were more abundant than any other visit type and accounted for the majority of seeds produced for both species of

cacti. For *F. cylindraceus*, the cactus-specialist bee *Diadasia rinconis* was by far the most abundant and effective visitor. For *F. wislizeni*, the three cactus specialist bees (*D. rinconis*, *I. duplocincta*, *A. opuntiae*), and generalists in the family Halictidae each accounted for a quarter of all visits. However, all three cactus bees acted as pollinators of *F. wislizeni*, whereas the small halictids never pollinated the flowers. *D. rinconis* visits were more effective (on a per-visit basis) than visits by the other two cactus bees. Despite the fact that the large open flowers of barrel cacti do not exclude any type of visitor, flowers of both species were pollinated almost exclusively by cactus-specialist bees: 99% (*F. cylindraceus*) and 94% (*F. wislizeni*) of all seeds produced resulted from visits by cactus-specialist bees. Apart from the halictids, the flowers were not visited by generalist bees that are commonly found at the same locality and time on other flowers.

In Appendix D, I tested the pollen preferences of four species of solitary, cactus-specialist bees. The goal of the study was to determine how strictly specialized to cactus pollen these bees are, and to see if they prefer some cactus pollens to others. Bees were offered cactus flowers in which the stamens had been removed and replaced with novel pollens in a choice assay that always included unmanipulated cactus flowers. *Diadasia rinconis*, *Lithurge apicalis*, and *Idiomelissodes duplocincta* were tested in a free-flying setting, and marked individuals of *D. rinconis* and *D. opuntiae* were tested in a flight cage. A no-choice experiment was also performed on naive, newly-emerged *D. rinconis* females in a flight cage. Non-host cactus pollens were accepted to some degree by all four species, although the two species of *Diadasia* accepted more non-cactus pollens than did either *L. apicalis* or *I. duplocincta*. Both species of *Diadasia* showed significant acceptance of *Sphaeralcea* (Malvaceae) pollen, a host plant used by most other species of *Diadasia*. Caged, naive *D. rinconis* bees did not initiate nesting when presented with *Sphaeralcea* flowers alone; nesting began immediately when *Opuntia*

(Cactaceae) flowers were offered. However, once nesting was underway, six of seven bees switched readily to *Sphaeralcea* when cactus flowers were removed. In both *D. rinconis* and *D. opuntiae*, there were striking differences in degree of pollen specificity and in pollen preferences among the caged individual bees. These results highlight differences among bees that have converged on specializing on the same resource, and suggest that host shifts to non-cactus hosts might be especially likely in cactus-specialist *Diadusia*.

### CHAPTER 3

#### GENERAL DISCUSSION

In my dissertation research, I aimed to build up an ecological portrait of the interactions between cactus-specialist bees and the cacti they visit. The unifying theme of my work is to integrate the study of bees as potentially mutualistic (pollinating) flower visitors with the study of bees as potentially antagonistic herbivores. Despite predictions by some bee biologists that pollen-foraging bees should be antagonistic to the plants they visit (Westerkamp 1991, Harder and Barclay 1994, Westerkamp 1996), my results indicate that pollen-foraging visits by pollen-specialist bees to the flowers of two species of *Ferocactus* are equally effective at pollinating these flowers as other visit types (true for all three species of cactus bees). I found that all three cactus bees were good pollinators in terms of per-visit quality, and further, that they were virtually the only pollinators of both species of *Ferocactus*. Thus I found that at least for these three species of pollen-specialist bees, their interactions with their host plants are primarily mutualistic.

My study of two species of *Ferocactus* allowed me to place the importance of these bees as pollinators into the context of the overall reproductive biology of these plants. I also investigated pollen preferences of several cactus-specialist bees, to determine their degree of host specificity, and found that they are indeed strongly specialized to cacti. Now that a well-supported phylogeny of *Diadasia* has been published (Sipes and Wolf 2001), we can interpret the responses of the species of *Diadasia* I studied to non-cactus pollens used by their congeners in the light of the history of pollen host use in the lineage.

I found that *Ferocactus cylindraceus* and *F. wislizeni* are completely dependent on insect visitation to their flowers for reproduction (Appendix A). This characteristic made them appropriate subjects for a study of the interactions between cactus-specialist bees and their host plants, because it meant that the effects of flower visits by cactus bees, whether negative or positive, are likely to have significant consequences for the fitness of the plant. In other cacti such as prickly pears and chollas, which reproduce vegetatively via detached joints, it is unclear how important flower visitation is to the immediate reproductive success of the plant (Bowers 1996, Mandujano et al. 1996).

Both species had a very high rate of fruit set (~ 93% to 95%), and set fruit with as few as fifteen seeds (Appendix A). This meant that fruits that were aborted after a single pollinator visit (Appendix C) probably had not received enough pollen to set fruit. In other plants, flowers or fruits are aborted due to resource limitations or other factors not related to the pollinator effectiveness of the visitor. This high fruit set rate does not mean that these plants are free from resource limitation, but rather that flowering is likely to be limited by the production of new meristems since the last flowering episode ("meristem limitation"). In these plants, flowers are produced in a specialized short-shoot structure called an areole. Each areole produces only one flower in its lifetime, and hence production of flowers depends on the production of new areoles (meristems). Production of new meristems in turn may be limited by the availability of resources, so that even if flowering is not directly limited by resources, resource limitation may act indirectly on flower production via meristem production.

Both species routinely produce several hundred to several thousand seeds per fruit and, assuming that the typical plant produces a mean of thirty flowers a year (Appendix A), an individual plant may produce ~17,000 (*F. cylindraceus*) to ~22,000 (*F. wislizeni*) seeds a year. Summed over the reproductive lifespan of the plant (which is

unknown but estimated to be 50 to 100 years), this adds up to a large number of seeds. Such fecundities are not unusual in desert plants, because the chance of any one seed surviving to become a reproductive adult is negligible (Shreve 1917, Steenberg and Lowe 1977, Bowers et al. 1995).

Plants of both species will set a fruit with as few as fifteen seeds, and the variance in seeds per fruit is such that it proved difficult to detect factors that significantly affected the number of seeds per fruit. This was, however, another characteristic that made these plants good study subjects: in pollination studies of plants with few seeds per fruit, fruit set can be equivalent to seed set in terms of the outcome of a single visit. In these plants, however, the probability of fruit set does not predict the number of seeds per fruit. Thus, there are two separable components to the outcome of a visit, allowing for an additional dimension in quantifying the pollinator effectiveness of different visitors or visit types.

In both species plant size had a strong, positive effect on the number of flowers produced, as it does in many other plants (Appendix A). Flower production is especially crucial in these plants because of the extremely high fruit set rate: every flower counts (i.e., contributes a large proportion of seeds to yearly fecundity). My estimates of yearly growth rates for *F. wislizeni* seemed high in comparison to other estimates for species of *Ferocactus* (Appendix A); this could be due to higher rainfall than usual during the study years, or it could be because the location of the study population on average receives more rain than the sites of previous studies.

The compact, unbranched growth form of these plants made size relatively easy to measure. However, in retrospect it would have been preferable to additionally use a method suggested by Nobel (1986), which is to count the number of new areoles (specialized axillary buds that produce spines, ephemeral leaves, and flowers) produced

over time. This could easily be done by marking the central spine of each areole. This measure would have the advantage of being more directly tied to the production of flowers, because each areole produces only one flower in its lifetime. Hence, flower production is dependent on the production of new areoles. It would also enable one to answer the question of whether areoles always flower within a year of being produced, or whether areoles are held over for flowering in future years. This would make it possible to address the question of whether there is a tradeoff between growth and reproduction in these cacti, a question I did not address in my study. It would also be of value to continue to monitor the study populations of these two plant species I set up in the course of my research. Long-term studies of plant demography are few, and almost unknown for cacti.

My study of the flowering phenology of these two species of *Ferocactus* revealed that although they begin flowering at different times (April to May for *F. cylindraceus* and July to August for *F. wislizeni*, in the two populations I studied), there is considerable overlap in their flowering periods: both continue flowering until early October (Appendix B). The population of *F. cylindraceus* I studied had a bimodal pattern of flowering, with the first peak in late May and the second in August (coinciding with the peak flowering of *F. wislizeni*). Because I only studied one population of *F. cylindraceus*, I cannot say whether this pattern is characteristic of the species as a whole, or determine what mechanisms underlie it. Because flowering onset is thought to be triggered by a rainy period (winter rains for *F. cylindraceus*, summer rains for *F. wislizeni*), this population of *F. cylindraceus* may be responding to summer rains, which are not common in most other parts of its range. A study of the impact of climate on flowering onset in these plants would be an important advance in our knowledge of their biology.

The results of the pollination study (Appendix C) were striking in that cactus-specialist bees dominated the visitor fauna to both species of *Ferocactus*, and were virtually the only pollinators observed. This was unexpected because the flowers are large, bowl- or cup-shaped, and freely offer abundant pollen and nectar to any visitor, suggesting that the visitor fauna (if not the pollinator fauna) would be very diverse. The cactus bees were not only abundant and effective pollinators, they were reliably present in all three years of the study.

The absence of visits by generalist bees that commonly visit other flowers that co-occur in space and time with *Ferocactus* flowers was especially mystifying. Of 624 floral visitors observed, for example, only five were *Apis mellifera*, the introduced honey bee. It is tempting to speculate that these flowers possess visual or chemical cues not obvious to humans that somehow screen out visitors other than cactus bees (and non-pollinating small bees in the family Halictidae, which were the only other visitors significant in numbers: Appendix C). My methods did not directly measure visitation rates, and so my observations are confined to relative abundances of different taxa of visitors. Explicit study of visitation rates to flowers of these two plant species in other localities would be necessary to determine if the dominance of the cactus bees that I observed is a peculiarity of the populations I studied, or the years I studied them in, or whether cactus bees are as important in other parts of their ranges.

I predicted that the per-visit pollination effectiveness of nectar-collecting visits would be different than that of pollen-collecting visits. I reasoned that, while pollen-collecting, female bees are removing pollen from the pool available for deposition on stigmas, by sequestering it into their pollen brushes, and therefore may not be passively transferring as much pollen as nectar-foragers. In addition, pollen-collecting bees remain on the tops of the stamens, whereas nectar-collectors must burrow down

between the stamens to access the nectar: I expected that these two very different behaviors in the flower would result in differential pollination success. I measured two components of pollination effectiveness: quantity (number of visits), and per-visit quality (probability of fruit set, and mean seeds per fruit, resulting from a single visit).

In fact, there were no per-visit differences in the pollination quality of the two kinds of visits. In light of the fact that I recorded data from over four hundred visits, I do not believe that sampling error masks differences in nectar- versus pollen-collecting visits. However, nectar-collecting visits were more abundant than any other visit type for all three species of cactus bee and, in general, I found that this type of “quantity” effect was more important to the overall realized fecundity of the plants (total number of seeds produced) than the per-visit “quality” effects.

In the pollen-transfer experiments (Appendix D), I shifted my focus from bees as pollinators to bees as herbivores. I found that tested individuals of *Lithurge apicalis* and *Idiomelissodes duplocincta* showed a strong or invariant preference for cactus pollen, whereas tested individuals of the two species of *Diadasia* were more likely to collect non-cactus pollens. Because these experiments involved experienced bees, it could be that the strong specificity to cactus pollen displayed by *L. apicalis* and *I. duplocincta* is the result of adult conditioning, not innate preference. Based on studies of patterns of host use in other herbivorous insects (Thompson 1994) I would also expect to find geographical variation in the host preferences of all these bees.

Of the six non-cactus pollens offered, the one preferred most often by both species of *Diadasia* was the pollen of *Sphaeralcea* (globe-mallows). This is particularly interesting because *Sphaeralcea* and other plants in the family Malvaceae are thought to be the ancestral host plants for the genus *Diadasia* (Sipes and Wolf 2001). Futuyma et al. (1995: p. 798) predicted that if host shifts are constrained by lack of genetic

variability in preference. "...species with derived host associations might retain more variation for response to a recently ancestral host plant than species with ancestral host associations display in response to the hosts of their 'apomorphic' relatives." In the case of *Diadasia*, this principle would translate into a prediction that the cactus-using species, which form a monophyletic clade nested within the other species of the genus (most of which are mallow specialists) would likely respond to mallow pollen, whereas the mallow-using species of *Diadasia* would not respond to cactus pollen, unless they were very close relatives of the cactus clade. This could be tested by offering Malvaceae-using species of *Diadasia* cactus pollen: a lack of response would support the prediction. On the other hand, if host shifts are not constrained by genetic variation in preference behavior, but are solely due to chemical or other convergent similarities between cactus pollen and mallow pollen, one would expect mallow-using bees to respond to cactus, and vice versa.

Apart from *Diadasia*, host shifts (of bees) between Malvaceae, Cactaceae and Convolvulaceae are found in other lineages of bees as well. Species of *Perdita* use Cactaceae and Malvaceae, species of *Ancylloscelis* use Cactaceae and Convolvulaceae, and species of both *Diadasia* and *Lithurge* use Cactaceae, Malvaceae, and Convolvulaceae. This strongly suggests that there are "hidden similarities" (sensu Futuyma and Moreno 1988) shared by the pollens of these three plant families. Therefore I was surprised when both *D. rinconis* and *I. duplocincta* definitively rejected pollen from *Ipomoea* (Convolvulaceae) flowers in my choice tests. I have limited anecdotal data to suggest that *Ipomoea* pollen may be acceptable to species of *Lithurge*, and hope to test this in future experiments.

It has been suggested that the large size of pollen grains produced by plants in these families is a cue used by foraging females (Houston 1971, O'Toole and Raw

1991, Schlindwein 1998). However, the pollen grains of *Sphaeralcea* used in my experiments were not especially large (mean of 41  $\mu\text{m}$ , as opposed to 111  $\mu\text{m}$  for *Opuntia* and 94  $\mu\text{m}$  for *Ipomoea*; Appendix D). Other large-grained pollens were rejected (e.g., *Zea mays*, 82  $\mu\text{m}$ , and *Cucurbita pepo*, 135  $\mu\text{m}$ ), whereas a cactus pollen with medium-sized grains (*Ferocactus wislizeni*, 50  $\mu\text{m}$ ) was acceptable to all four cactus bees. This does not support the idea that pollen grain size is an overriding factor in host choice in these bees. Rather, pollen chemistry, particularly as it may be expressed in pollen odor, seems the most likely “hidden similarity” that facilitates host shifts among these three plant families. The next step in this study would be to describe the chemical profiles of these pollens, to determine if there is a common element linking them. Of course, it is plausible that there is more than one chemical or group of chemicals involved – perhaps *Diadasia* cactus bees recognize a compound shared by cacti and mallows, one that does not occur in Convolvulaceae, whereas *Lithurge* bees recognize a compound shared by cacti and Convolvulaceae that does not occur in mallows.

A surprising result was the level of variation in pollen preferences among individuals in the experiments on caged, marked bees. This was true for both *D. rinconis* (seven bees) and *D. opuntiae* (five bees). In both cases there was at least one individual that took pollen only from control (i.e., normal host cactus) flowers, several individuals that accepted some non-cactus or novel cactus pollens, and at least one individual that actually preferred novel pollens over the control flowers (Appendix D). If these differences are genetically based, it could indicate the presence in these populations of sufficient variation to allow future host shifts.

On the other hand, in the test on naive newly-emerged adult female *D. rinconis* bees, all seven bees refused to initiate nesting when presented with *Sphaeralcea* flowers

only: nesting began when cactus flowers were made available. When cactus flowers were introduced, all seven began nesting within a two-day period, although they individually had emerged at different times and had been caged for different periods. Nevertheless, once nesting was underway, six of the seven switched to collecting pollen from *Sphaeralcea* when cactus flowers were withdrawn. This suggests the possibility that contact with cactus flowers may be required for oogenesis or egg maturation in these bees, as shown in a number of other herbivorous insects (Papaj 2000), or may act as a trigger for nesting behavior. A literature review of host-shifts in specialist bees suggests that switching hosts may be more common during or at the end of the nesting period than at the beginning (Appendix D). An extension of the present study would be to cage naive cactus bees with either *Sphaeralcea* or cactus flowers, and to dissect their ovaries after elapsed intervals to determine the relationship between pollen resources and oogenesis.

Modern phylogenetic studies of bee lineages are just beginning to proliferate, but the utility of such studies for understanding host use in bees is already clear. A 1996 paper by Müller was the first to present a hypothesis of the phylogenetic relationships in a clade of bees with pollen host plants mapped onto the phylogeny; he found that specialization is ancestral and the derived strategy of generalization had repeatedly evolved in the clade. Sipes and Wolf (2001) inferred the phylogeny of the genus *Diadasia*, showing that the use of cactus pollen has evolved only once in the clade. In an unpublished dissertation on the phylogeny of a clade of *Perdita* bees, Danforth (1991) postulates that the use of cactus pollen is ancestral in this clade, and the use of *Sphaeralcea* evolved once, with one subsequent reversion to cacti.

R. L. Minckley, J. H. Cane and others have researched the use of *Larrea tridentata* flowers by pollen-foraging bees; their work has begun to unravel possible

ecological correlates of specialization in bees (Minckley et al. 1999, Minckley et al. 2000). As yet unpublished research by N. M. Williams (pers. comm) into pollen choice in both specialized and generalized bees – a much more rigorous study than the one described here – should be a significant advance in our understanding of host use in bees. Ground-breaking work on the nutritional components of pollen by T. Roulston and others is setting the stage for incorporating pollen nutrition into studies of pollen host choice in bees (Roulston and Cane 2000, Roulston and Buchmann 2000, Roulston et al. 2000). The work of H. E. M. Dobson and others into the role of pollen odor in host recognition in specialist bees has laid the foundation for future studies of pollen odor and chemistry (references summarized in Dobson and Bergström 2000). Finally, an exciting new study by B. N. Danforth (1999) demonstrates conclusively what many bee biologists have long suspected, namely that desert bees are capable of multi-year diapause. That is, these organisms can form “bee banks” (analogous to “seed banks”), thus employing the same bet-hedging strategies that plants do. This finding has important implications for host specialization and phenological matching in desert bees. In sum, bees have much to teach students of other herbivorous insects.

My contributions to the field of plant-insect interactions are, first, demonstrating that pollen-specialist bees can be important mutualists of the plants they visit, even as they are depleting them of pollen. Second, I have shown that the identification of *D. rinconis*, *D. opuntiae*, *L. apicalis*, and *I. duplocincta* as “cactus bees” is well-founded: in the populations tested, bees of all four species showed a strong preference for cactus pollen and, in many cases, cactus pollens were the only ones accepted. Finally, the results of the caged bee experiments suggest that some specialist bees may require contact with their host plant in order to mature eggs and initiate nesting. Although these

results are suggestive rather than conclusive, this phenomenon has not previously been shown in bees, and clearly warrants further investigation.

My research shows that cactus-specialist bees are mutualists of the cacti they visit. If most pollen-specialist bees are mutualists of their hosts, then bees may have an especially important role to play in the study of plant-insect interactions. By comparing mutualistic and antagonistic herbivores, we can learn which aspects of plant-insect interactions are specific to each type of interaction, and which are general for herbivores, whether mutualistic or antagonistic. For example, it has long been thought that the prevalence of specialization in herbivorous insects is driven by an "escape and radiate" arms race between insects and their hosts. This scenario would not be relevant to specialization in bees, however, because if bees are mutualists, plants should not need to defend themselves against them. Other hypotheses for the prevalence of specialization by herbivores, such as genetic constraints in host adaptation (Futuyma et al. 1995), or neural constraints in information processing (Bernays and Wcislo 1994), would apply equally to mutualistic and antagonistic herbivores, however. In sum, the study of host use in pollen-foraging bees has the potential to bring new insights into the field of plant-insect interactions. It is my hope that my work and the work of others will help to bring bees into the mainstream of plant-insect studies.

**APPENDIX A**

**PLANT SIZE, BREEDING SYSTEM, AND LIMITS TO REPRODUCTIVE  
SUCCESS IN TWO SISTER SPECIES OF *FEROCACTUS* (CACTACEAE)**

Plant size, breeding system, and limits to reproductive success  
in two sister species of *Ferocactus* (Cactaceae)

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## Abstract

Plant reproductive output can be limited by a variety of factors, both intrinsic and extrinsic. I investigated the reproductive biologies of two species of unbranched short-columnar cacti, *Ferocactus cylindraceus* and *F. wislizeni*. I recorded female reproductive output (flowers produced, fruit set, seeds per fruit and seed mass), plant size and growth, and used hand-pollination experiments to determine breeding systems and pollen limitation. In both species, the ability to self varied among individuals, but self-pollination resulted in very few seeds, suggesting strong inbreeding depression. Neither species was pollen-limited. Numbers of flowers produced increases with plant size for both species, and seeds per fruit may also be related to plant size, although the relationship is unclear. Seed mass is not correlated with plant size. Flower production was similar in both species, but *F. cylindraceus* produced fewer seeds per fruit than *F. wislizeni*, and its seeds weighed less. Fruit set by *F. cylindraceus* was heavily impacted by a florivorous lepidopteran. Fruit set was very high (94 to 96%) in *F. wislizeni*, suggesting that architectural constraints (e.g., meristem limitation) are more limiting than resource levels or the level of pollinator services. In *F. cylindraceus*, numbers of seeds per fruit was positively correlated with seed mass, whereas in *F. wislizeni*, the relationship was negative (tradeoff). The growth rates of *F. wislizeni* are affected by rainfall the previous season, and growth rates increase as the plant ages. *Ferocactus cylindraceus* and *F. wislizeni* are thought to be sister species, meaning that observed differences between them are more likely to be the result of recent evolutionary processes in their lineages rather than differing phylogenetic histories.

## Introduction

In plants, the flexibility inherent in the modular nature of their growth and reproduction has allowed them to evolve a rich diversity of reproductive strategies, making them of particular interest to biologists investigating the evolution of life histories. Many plants reproduce simultaneously from multiple meristems, and new meristems may be produced throughout the life of the plant, leading to complex relationships between plant size, resource allocation, and overall reproductive effort.

Meristem limitation (in which the number of reproductive structures is limited by the production of new meristems since the last reproductive episode: Watson 1984, Geber 1990) and other size-dependent constraints are only one category of intrinsic limits to reproductive output and success. Others include genotype, levels of stored resources, developmental constraints (e.g., number of ovules per ovary, determinate flowering), and architectural factors (Diggle 1995). Extrinsic limits may include climatic factors, pollen limitation, competition, anthropogenic effects, and flower and seed predation. Investigating the relative importance of these different factors in plants under natural conditions can help elucidate their impact on the overall reproductive success of these plants.

Plants in the Cactaceae possess some unusual modifications in growth and architecture. One such structure is the areole, a specialized axillary bud that gives rise to a highly condensed short shoot (Gibson and Nobel 1986). The areolar meristem produces primordia that result in spines and tiny, ephemeral leaves, and sometimes branches (leading to the production of new areoles). The areolar meristem can also give rise to a single flower, but this usually ends its reproductive lifespan -- no new flower primordia arise. In a few cacti, additional meristems are formed within the areole, such

that an areole can flower more than once (Gibson and Nobel 1986). An areole can branch (creating new meristems) or flower, but not both.

In unbranched cacti (including most species of *Ferocactus*) all vegetative growth takes place in the solitary stem: there is no clonal reproduction. These plants consist of a single stem studded with areoles (modules) which contribute little to growth in size. Flowering in *Ferocactus* is determinate (one flower per areole), and constrained by meristem limitation (production of new areoles). Flowering is also apical: areoles flower when they are newly formed and still near the stem tip (Gibson and Nobel 1986). Unlike in saguaros, *Ferocactus* areoles are not held in reserve for later flowering or branching. In addition, the vast majority of biomass exists above ground (Gibson and Nobel 1986), where it is easy to measure.

The simplified architecture of unbranched cacti make them excellent subjects for exploring the relationships between plant size, growth, and reproductive output. In these plants, resource limitation may affect reproduction in at least two ways: directly, by limiting the ability to produce and mature flowers and fruits (resulting in bud, flower or fruit abortion), and indirectly, by limiting production of new areoles via vegetative growth at the apex.

The physiology and environmental biology of *Ferocactus cylindraceus* (Engelm.) Orcutt has been extensively investigated (Lewis and Nobel 1977, Ehleringer and House 1984, Nobel 1986, Geller and Nobel 1987, Nobel 1989). Growth, size structure and seedling establishment of *F. cylindraceus* have also been studied (Jordan and Nobel 1981, Jordan and Nobel 1982, Nobel 1986). Work has also been done on the demography of this cactus, primarily at the Deep Canyon site in California (Nobel 1977, Jordan and Nobel 1981), and at the Grand Canyon in Arizona (Bowers et al. 1995, Bowers 1997, Bowers et al. 1997). The reproductive biology of this plant has not yet

been documented, however. For *Ferocactus wislizeni* (Engelm.) Britt. and Rose, previous studies are limited to documentation of size structure of populations. (Reid et al. 1983, Helbsing and Fischer 1992), some aspects of demography (Goldberg and Turner 1986), and reproductive output and minimum reproductive size (Bowers 1998).

A recent study of phylogenetic relationships within *Ferocactus* indicates that *F. cylindraceus* and *F. wislizeni* are sister species (Cota and Wallace 1997). This means that any differences observed are not due to different phylogenetic histories, but are solely the result of recent evolutionary processes.

I used hand-pollinations to determine the breeding systems of these two species, and I recorded several components of female reproductive success, including flower production, fruit set, seeds produced per fruit, and seed mass. I also determined plant size and growth rates to investigate the relationship between size, growth, and reproduction. Specifically, the aims of this study were to determine if these species are self-compatible and if they are they pollen-limited, to elucidate what factors limit their reproductive output, and to determine how plant size influences female reproductive output.

## **Methods**

### *Study organisms*

The genus *Ferocactus* (Cactaceae) contains 25 to 30 species (Cota and Wallace 1997), and is one of several genera of short-columnar, unbranched (usually) cacti called "barrel cacti." *Ferocactus* occur only in North America, with most species in México.

Four species occur in the U.S., and 3 of these are in Arizona. Flowering takes place in the spring or in the summer, depending on the species.

*F. cylindraceus* ranges from Sonora and Baja California, México, into California, far southern Nevada and Utah, and western Arizona (lat 28-37°N, long 110-117°W, elevation 0 to 1750 m; Turner et al. 1995). Within this range, it may be found in the Upper Division of the Sonoran Desert region, and in parts of the Mojave desert. Flowering onset varies among locations, but usually occurs in March-June, and may continue sporadically throughout July-October. The species has been divided into several varieties by various authors (see Turner et al. 1995 for summary), but the population I studied did not fit any of the described varieties. Maximum life span may be around 55 years (Bowers et al. 1995).

*F. wislizeni* ranges from northern México into Arizona, New Mexico and Texas, in both the Sonoran and Chihuahuan deserts. It does not range as far north as *F. cylindraceus* (lat 25 to 34°N), and its elevational range is slightly more restricted (0 to 1500 m; Turner et al. 1995). It has been described as occurring where there are both summer and winter rains, specifically where summer rainfall is greater (Turner et al. 1995). Flowering begins in July or August. Maximum life span observed at the Desert Laboratory in Tucson was 46 years (Goldberg and Turner 1986).

### *Study sites*

I studied a population of *F. cylindraceus* 45 km NW of Tucson in the summers of 1995 to 1998. The plot comprised ~1.5 hectares on an east-facing slope of "Desert Peak" (lat 32°36'N, long 111°14'W) a small ridge that rises 125 m above the 640 m elevation desert floor. The vegetation is typical Sonoran Desert -- Upper Subdivision

plant community, dominated by *Carnegiea gigantea* and *Cercidium microphyllum*. Mean annual rainfall (1893 to 1973) at nearby Red Rock (8 km WSW of study site) is 248 mm, about 47% of which falls in the summer (June through September; National Climatic Data Center: Western Regional Climate Center WWW Server: <http://www.wrcc.dri.edu/summary/climsmaz.html>). In this location, *F. cylindraceus* is restricted to the slopes of Desert Peak, whereas *F. wislizeni* occurs on the flats surrounding the ridge. I marked ca. 25 reproductively mature plants in 1995, an additional 25 in 1996, and an additional 95 in 1997. This 1997 survey aimed to include all reproducing plants within the plot ( $n = 128$ ); these plants were further studied in 1998 and 1999. I hand-pollinated plants in 1995 to 1996, and additional reproductive biology data were taken from a randomly selected subset of the population in 1997 to 1998. For hand-pollinations, I selected large healthy-looking plants (e.g., those with intact apices), because large plants produce many flowers, allowing replicates of the treatments.

I studied a small population of *F. wislizeni* located just west of the Tucson city limits in the summers of 1994 and 1995. The site, on the north side of Anklam Road (lat 32°14'N, long 111°02'W, 700 m elev., ~1 hectare), consisted of typical Sonoran Desert - Lower Subdivision plant community, dominated by *Carnegiea gigantea* and *Cercidium microphyllum*. Mean annual rainfall (1948 to 1998) in Tucson is 297 mm, about 53% of which falls in the summer (June through September; National Climatic Data Center – see above). I labeled approximately 45 mature (reproducing) plants in September 1994. The plants were located on a south-facing slope. In 1995 I hand-pollinated plants that I selected for their large size (more flowers).

The Anklam Road site was eradicated by urban sprawl, and I began studying another population of *F. wislizeni* located on the Santa Rita Experimental Range

(hereafter SRER), 40 km south of Tucson (lat 31°54'N, long 110°53'W, 914 m elev.) in the summers of 1996 to 1998. The study plot comprised 3 hectares along the north side of Santa Rita Road. The plant community is semi-desert grassland, dominated by *Prosopis velutina* and various cacti (especially prickly-pears and chollas). Mean annual rainfall (1948 to 1998) in nearby Sahuarita (9 km NW of study site) is 270 mm, about 60% of which falls in the summer (June through September; National Climatic Data Center – see above). I tagged approximately 55 mature plants in 1996, and an additional 50 plants in 1997. This 1997 tagging included all plants of reproductive age within the plot. At this site, and at the *F. cylindraceus* population, when a previously non-reproductive individual began flowering, I added it to the study. At SRER in 1996 I hand-pollinated plants selected for their large size, and other reproductive biology data were taken from a randomly selected subgroup in 1996 to 1998.

### *Breeding systems*

To determine the breeding systems of these plants, I covered the flowering crowns of selected plants with wire baskets covered with mesh cloth to exclude pollinators and then hand-pollinated flowers. The wire baskets were made secure by seating them down over a ring of cheesecloth placed below the crown: this prevented pollinators from gaining access to the flowers by walking up the grooves between the ribs. If the fit still appeared insecure, I additionally wrapped the body of the plant with cheesecloth. During hand-pollinations, I removed the cover, applied treatments, marked treated flowers with nail polish or paint pens, and replaced the cover. Later, I collected and dried the fruits, and counted the seeds. For *F. cylindraceus* in 1996, in order to reduce destruction of flowers and fruits by a lepidopteran florivore, covered flowers

were treated with insecticide following hand-pollinations. Open-pollinated flowers were not treated with insecticide.

To test the hypotheses that plants were self-incompatible, and that reproductive output is pollen-limited, I used four hand-pollination treatments: open-pollinated (no cover), covered but no manipulation (to test for spontaneous self-pollination), self-pollinated, and outcrossed. For the self-pollinations, I transferred pollen with a toothpick from the same flower or from a different flower on the same plant to the stigma lobes. For outcrossing, I removed a first-day flower (i.e., with freshly dehisced anthers) from a plant outside of the study plot, and transferred pollen with a toothpick from this flower to the stigma lobes of the treated flower. Donor plants were at least 15m distant from plot edge. Flowers were not emasculated (the hundreds of anthers dehisce before the flower opens). I did not apply mixed pollen loads. In 1995, to test for the timing of stigma receptivity, I hand-pollinated both first-day and second-day flowers.

To test the hypothesis that the two species are capable of hybridizing, I cross-pollinated plants at Desert Peak by applying pollen from *F. wislizeni* plants at that site to *F. cylindraceus* plants in the study plot. Because *F. cylindraceus* plants did not occur at Anklam Road or at the SRER, *F. cylindraceus* flowers were transported to these sites from Desert Peak in an ice chest, and then used for hand-pollinations of *F. wislizeni* plants.

#### *Female reproductive output*

For *F. cylindraceus* in 1997, I randomly selected 23 plants for study. In 1998, I added 5 plants to achieve a more even size distribution of selected plants (stratified

random design). For *F. wislizeni* in 1997, I selected 24 plants in a stratified random design that ensured that all size classes were sampled.

To quantify and determine the relative importance of different components of pre-dispersal (Wiens et al. 1987) female reproductive success, I used four measures: total number of flowers produced, fruit set, mean seeds per fruit, and mean seed mass. I surveyed plants at regular intervals throughout the blooming period, usually once a week. At each survey, the outer corolla of new flowers was marked with paint pens. These markings persisted into the fruiting stage. Fruits were collected when ripe and the seeds were counted and weighed in 1998. Seed counts were accurate to within  $\pm 1$  to 2 seeds for every 100 seeds counted (based on repeated counts: unpub. data). I measured seed mass, which is often important to female reproductive success (Rees 1997), for both species in 1998. After counting the seeds from a fruit, all the seeds were weighed together on a pan balance. The total weight was then divided by the number of seeds to obtain a mean mass per seed in that fruit. Unfilled seeds were not counted and were removed before weighing.

#### *Plant size*

To test the hypothesis that plant size affects reproductive output, I measured all *F. cylindraceus* plants within the plot at Desert Peak in April 1998, and all *F. wislizeni* plants within the plot at the SRER in July of 1997 to 1999. In both cases this meant plant size was measured just before flowering onset. When a previously non-reproductive individual began flowering, it was tagged and added to the study. Plant size was measured as height and width. Height was measured from base to crown by a folding rule or a metal tape measure. Width was measured with large aluminum

calipers. I measured the widest part of the plant, and some part of the width usually included some spines (spines unavoidably forced the calipers away from the body of the plant). When the cross-section was asymmetrical, I averaged the width measured along two axes. Both height and width were accurate to within  $\pm 2$  cm (based on repeated measurements; unpub. data). When the width was greater than 40 cm (the limit of the calipers), I estimated the width by eye.

Plant volume was used as a measure of overall plant size. This was calculated using height and width measurements to determine the volume of a cylinder. For most plants this likely was an overestimate of the actual volume of the above-ground stem, because many plants are narrower at the base and crown than at the middle, where width was measured. However, the very compact and relatively symmetrical shape of these plants makes their overall volume much easier to measure than that of other plants. Moreover, plant volume is a good surrogate for plant biomass for these plants, because the vast majority of biomass occurs above-ground.

In testing the effects of plant size on growth rate I converted size to categorical data by classing plants according to the natural log ( $\ln$ ) of their volume. Size classes ranged from class 1, with  $\ln$  volume = 8.00 to 8.49, to class 8, for plants with  $\ln$  volume  $\geq 11.50$ . Classes spanned 0.5 of the  $\ln$  of volume (i.e., the bins were equal in size), and hence there were unequal numbers of plants in each size class. This method (as opposed to structuring size classes to contain equal numbers of plants) was used to facilitate comparisons of growth-size relationships across years.

### *Data analyses*

For the hand-pollination experiments, to test the hypothesis that treatment affected fruit set, I used nominal logistic regressions (log-likelihood ratios), with each data point being a single flower. The dependent variable was fruit set ("yes" or "no"), and the independent variables were treatment, year (or site) and individual plant (nested within site for *F. wislizeni*). To test the hypothesis that treatment affected seeds per fruit, I used multiway ANOVA tests, with seeds per fruit as the dependent variable, and treatment, year (or site) and individual plant (nested within site for *F. wislizeni*) as the independent variables. For the seeds per fruit data, I excluded aborted fruits (those with seeds = 0). In some analyses, a significant "lack of fit" (SAS Institute Inc., 1989-99b) was found, indicating the presence of an untested interaction between factors. However, because of unbalanced data I was not able to test for interactions (lost degrees of freedom). When a significant lack of fit was reported, I record it in the results. In some analyses, the residuals from the model were found to have a non-normal distribution, even after data transformation, and I also report this. However, ANOVA is generally robust to this violation of assumptions (Zar 1996). To test which treatments were significantly different, I used Tukey-Kramer HSD tests, with individual plant as the experimental unit.

For the female reproductive output tests, numbers of flowers, and seeds per fruit were square-root transformed, and plant volume was log-transformed (ln), for normality. To test the hypothesis that plant size affects flower production, seeds per fruit, or mean seed mass per seed per fruit (each data point = 1 plant), I used regression analyses with plant size as the independent variable, and tested the significance with a linear fit. All statistical tests employed JMP IN® software (SAS Institute Inc., 1989-99a).

## Results

### *Breeding systems*

In the hand-pollination experiments, treatment (bagged, selfed, outcrossed or open-pollinated) had a significant effect on both fruit set and seeds per fruit, for both *F. cylindraceus* and *F. wislizeni* (Tables 1 and 2). In addition, for *F. wislizeni*, individual plant had a significant effect on both fruit set and seeds per fruit. It is likely that individual plant also affected fruit set and seeds per fruit for *F. cylindraceus*, but that this result is masked by unbalanced data. Hence, in examining the effects of treatment alone, I used individual plant means.

Fruit set was much higher for the outcrossed and open-pollinated treatments than for the bagged and selfed treatments, for both species (Fig. 1). Seeds per fruit showed a similar pattern (Fig. 1). In all cases, the bagged and selfed treatments were not significantly different from each other, and the hand out-crossed and open-pollinated treatments were not significantly different from each other (unpub. data).

Flowers that were hand-pollinated on the first vs. second day of anthesis did not differ significantly in either fruit set or seeds per fruit (both species, 1995, unpub. data).

As is the case with many cacti (Gibson and Nobel 1986), these two species appear to be capable of hybridizing. Within each species, fruit set did not differ significantly between hybrid crosses and within-species crosses (Kruskal-Wallis tests, all *P*-values not significant). Seeds per fruit also did not differ significantly between hybrid crosses and within-species crosses (Kruskal-Wallis tests, all *P*-values not significant).

### *Female reproductive output*

The mean number of flowers per plant was similar for *F. cylindraceus* and *F. wislizeni* (Table 3). The maximum number of flowers produced during the study by an individual of *F. cylindraceus* was 98 flowers, and for *F. wislizeni*, 89 flowers. Mean number of flowers per individual per year increased each year of the study for both species. Weekly production of flowers per plant ranged from 1 to 21 for *F. cylindraceus*, and from 1 to 31 for *F. wislizeni*; the mean was 3 to 5 flowers per plant per week for both species (Table 3).

Three kinds of abortion were observed in both species: flowers aborted in the bud stage (bud < 1 cm in diameter), flowers aborted following anthesis, and flowers or buds aborted after damage by insects (in most cases, larvae of *Pseudoschinia elautalis*; Lepidoptera: Crambidae). Bud abortions were more common in *F. cylindraceus* (7 to 14%) than in *F. wislizeni* (1 to 2%; Table 3), flower abortions were similar in the two species (3 to 6% for *F. cylindraceus* versus 1 to 2% for *F. wislizeni*; Table 3), and lepidopteran-caused abortions were much higher in *F. cylindraceus* than in *F. wislizeni* (23 to 29% versus 1%). These differences in abortion rate resulted in lower overall fruit set in *F. cylindraceus* than in *F. wislizeni* (54 to 65% versus 94 to 96%; Table 3).

Because the overall fruit set rate was so high in *F. wislizeni*, I did not test the effects of different factors (such as individual plant, year, etc.) on fruit set in this species. For *F. cylindraceus*, 1997-1998 pooled, I tested year and plant effects on all three components plus the overall fruit set rate. The only significant effects were that of individual plant on bud abortion rate, and year on florivore abortions (Table 4).

Plants of both species produced large numbers of seeds per fruit (Table 3). In 1998 (the only year in which seeds were counted for both species), mean seeds per fruit

per plant were significantly higher for *F. wislizeni* than for *F. cylindraceus* (759 versus 575 seeds per fruit per plant: t-test  $P = 0.0231$ ,  $n = 51$  plants). The number of seeds per fruit was highly variable, both within and among individuals, for both species (see standard deviations in Table 3).

Individual plant was a significant factor in mean seeds per fruit for both species (Table 5). In addition, year had a significant effect on seeds per fruit for *F. wislizeni* (*F. cylindraceus* seeds were counted in only one year). Extrapolating from average fruit set and seeds per fruit per plant figures, the average seeds produced by an individual plant in one year was about 9,000 (1998) for *F. cylindraceus*, and 17,000 to 26,000 (1996 to 1998) for *F. wislizeni* (or about twice as high as for *F. cylindraceus*).

Seed mass was not as variable as seeds per fruit, for both species. Mean seed mass per fruit per plant was significantly higher for *F. wislizeni* (2.59 mg) than for *F. cylindraceus* (1.67 mg; t-test,  $P < 0.0001$ ,  $n = 52$  plants). For both species, number of seeds per fruit was significantly correlated with mean seed mass per fruit. For *F. cylindraceus*, however, the relationship was positive (Fig. 2a), whereas for *F. wislizeni*, the relationship was negative (Fig. 2b).

#### *Plant size, growth, and demography*

Plant height appears to increase throughout the life of the individual, whereas plant width tends to asymptote (at ~ 35 cm for *F. cylindraceus*, 45 cm for *F. wislizeni*; Fig. 3). Widths of up to 83 cm have been documented for *F. wislizeni*, but the maximum reported for *F. cylindraceus* is only 40 cm (Turner et al. 1995). The vast majority (92%) of *F. cylindraceus* plants were higher than wide, whereas for *F. wislizeni* only 30% were

higher than wide (Fig. 3). In other words, most *F. cylindraceus* plants were tall and slender, whereas most *F. wislizeni* plants were short and squat.

Plants of *F. cylindraceus* had a mean height of 70 cm in 1998 (Table 6), with a platykurtic distribution of heights (Fig. 4). Because height is correlated with age, this size structure, showing several clumps with gaps between, suggests that the population is made up of several cohorts. The widths were distributed around a mean of 29 cm (Fig. 4).

In the same year, plants of *F. wislizeni* were on average shorter (mean height = 38 cm) and wider (mean width = 35 cm) than those of *F. cylindraceus* (Table 6). The heights were tightly clustered around the mean, and strongly left-skewed, with two outliers (heights of 140 cm and 218 cm) leading a long right tail (Fig. 4). This population thus apparently has less age structure than the *F. cylindraceus* population, or growth rates vary among individuals or years in such a way as to mask age structure. The widths were more platykurtic and shifted to the right compared to those of *F. cylindraceus*.

The density of reproductive-age *F. cylindraceus* plants was about twice that of *F. wislizeni* (89 plants/ha for *F. cylindraceus*, 38/ha for *F. wislizeni*), but it must be remembered that the plots were not designed to sample density, but to enclose a large number of plants for ease of surveying.

The smallest *F. cylindraceus* individual that reproduced in 1998 was 17 cm tall and 17 cm wide. The smallest *F. wislizeni* individual that reproduced in 1997, 1998 or 1999 was 11 cm high and 20 cm wide.

Of 9 *F. wislizeni* plants added to the study in 1998, 6 flowered for the first time (3 had been overlooked during initial surveys). Thus recruitment to the reproductive class was 6%. In 1999, 7 plants were added, all of which were flowering for the first

time (recruitment of 7%). For *F. cylindraceus*, in contrast, 2 plants reproduced for the first time in 1998 (recruitment of 1.5%).

In each of the paired study years, two plants of *F. wislizeni* plants died (2%). Deaths averaged 3% per year from 1995 to 1998 for *F. cylindraceus*. Thus *F. cylindraceus* had a lower recruitment to reproductive class and higher death rate than *F. wislizeni*. Observed deaths had two apparent causes: 1) the plant fell and was uprooted (if their roots remain in the ground, plants that have fallen over can continue to grow and reproduce), and 2) the plant appeared to rot from within, possibly due to damage from insects boring within the stem. For *F. cylindraceus*, more plants died by falling over than by insect damage (6 versus 1, 1995 to 1998), while deaths of *F. wislizeni* were equal in each category (2 versus 2, 1997 to 1999). No data on germination or seedling recruitment were collected.

In 1997 to 1998, following a wet El Niño winter, growth of individuals of *F. wislizeni* was greater than in 1998 to 1999, following a dry La Niña winter (Table 6). This difference was significant for plant volume (t-Test = 7.479,  $P < 0.0001$ ,  $n = 103$  plants), and for height and width (unpub. data). Size did not always increase: in some cases, plants shrank in size (Table 6).

Size was correlated with absolute growth, with large plants growing more than small plants (significant only in 1997 to 1998; Fig. 5). In terms of percent change in volume, the relationship was the opposite: large plants grew less as a percent of size than small plants (Fig. 5), and this relationship was significant in both years.

For both species, the number of flowers produced by a plant was significantly and positively correlated with the size of that plant, although the relationship was much stronger for *F. wislizeni* than for *F. cylindraceus* (Fig. 6). Plant size was not a significant factor in any of the abortion rates for *F. cylindraceus* in 1998 (unpub. data).

hence the plant effect on bud abortions observed (Table 4) is not likely to be a size effect.

Plant size did not affect mean seeds per fruit per plant (linear regressions of ln plant volume and mean seed per fruit per plant: *F. cylindraceus* 1998.  $R^2 = 0.027$ .  $P = 0.4013$ .  $n = 28$ ; *F. wislizeni* 1997.  $R^2 = 0.027$ .  $P = 0.44766$ .  $n = 21$ ; *F. wislizeni* 1998.  $R^2 = 0.005$ .  $P = 0.7579$ .  $n = 23$ ), so the individual plant effects observed are not due to plant size. Individual plant also had a significant effect on seed mass (Table 7): however, plant size did not (linear regressions of ln plant volume and mean seed mass per seed: *F. cylindraceus*.  $R^2 = 0.046$ .  $P = 0.2753$ .  $n = 28$ ; *F. wislizeni*.  $R^2 = 0.000$ .  $P = 0.9302$ .  $n = 24$ ), indicating again that the plant effect is not a plant size effect.

## Discussion

### *Breeding systems*

The hand-pollination experiments show that, for both species, only a tiny fraction of seeds result from selfing. If you include the fruits that were aborted (with no seeds), thereby combining the fruit set and seeds per fruit effects, for *F. cylindraceus* the mean seeds per fruit for the bagged and selfed treatments combined were 8 seeds, as opposed to a mean of 388 seeds per fruit for the outcrossed and open-pollinated treatments combined. For *F. wislizeni*, it was 15 seeds per fruit for bagged plus selfed treatments, versus 619 seeds for outcrossed plus open-pollinated treatments. This means that of all seeds produced, only 2% were from selfing (both species). This accords well with previous work on *Ferocactus* showing very little seed set from selfing (McGregor and Alcorn 1959).

Are these two species of *Ferocactus* self-incompatible? Many cacti are completely self-incompatible (Burd 1994, Boyle 1997), and in the one case in which the mechanism was investigated, it was found to be a one-locus, gametophytic SI system (Boyle 1997). Interestingly, in this study some individual plants nearly always set a few seeds from the selfing or bagging treatments, whereas others never did (29% of *F. cylindraceus* plants and 32% of *F. wislizeni* plants set some seeds from bagged or selfed treatments). This pattern is more consistent with inbreeding depression, with the severity varying among individuals, than it is with a compatibility system (Husband and Schemske 1996). The flat (unfilled) seeds that were occasionally found in mature fruits could be the result of self-incompatibility, selective abortion of lower quality seeds, resource limitation, or inbreeding depression (Wiens et al. 1987). It may also be that my methods did not completely prevent outside pollen (such as from bees crawling on top of the mesh covers) from reaching covered flowers. However, strong inbreeding depression is probably the most likely mechanism for the very low but measurable amounts of selfing in these two species of *Ferocactus*.

Comparison of hand-outcrossed with open-pollinated fruit set and seeds per fruit indicate that neither species is pollen-limited.

For both species, anthers dehisce before flowers open (pers. obs), and flowers of *F. wislizeni* have been described as protandrous (Grant and Grant 1979). If the stigmas did not become receptive until the second day of anthesis, then one would expect to see a difference (in fruit set or in seeds per fruit) between flowers pollinated on the first versus the second day. For both species, I found no such difference. However, it could be that the stigma becomes receptive later on the first day, or it could be that the pollen remains viable until the second day. Hence, the degree of protandry (if any) is still unknown.

The two species appear to be interfertile, at least to the extent of producing fruits and seeds. There were no significant differences in fruit set and seeds per fruit between interspecies crosses and intraspecies crosses. At Desert Peak, there is a considerable overlap in the blooming periods of the two species (Appendix B). I observed several individual plants at Desert Peak that were intermediate between the two species in both appearance and in onset of flowering date (pers. obs.), suggesting the possibility that these two species do hybridize at this location. Hybridization between *F. cylindraceus* and *F. wislizeni* has been reported (Unger 1992), but not thoroughly documented. Specific tests of parentals and offspring with allozymes or other molecular methods would be required to document hybridization, and to rule out the possibility that the fruit set and seeds observed here were actually the result of selfing.

#### *Female reproductive output*

In this study, several components of female reproductive effort and success were measured (flowers produced, fruit set, seeds per fruit per fruit, and seed mass). Germination, establishment, and survival to reproduction were not examined, thus only pre-dispersal female reproductive success is evaluated here (Wiens et al. 1987).

Because these plants have determinate flowering, female reproductive output, as measured by numbers of flowers produced, has an upper limit imposed by the number of new areoles produced since the last flowering episode. However, for *F. wislizeni* in 1998, plant size explained more of the variation in flower production per plant than growth in volume during the previous year (unpub. data). This indicates that growth in volume does not necessarily correlate very well with production of new areoles, or that areoles may be held in reserve longer than a year.

For both species, the mean number of flowers produced per individual increased each year of the study. This is likely related to the fact that flower production is highly correlated with plant size. As the average size per plant in a population increases each year (assuming few small newly-flowering individuals join the reproductive class each year, as appears to be the case in this study), so does the average number of flowers produced. Bowers (1998) also found that plant size was highly correlated with numbers of flowers and fruits in *F. wislizeni*, and flower production for *F. wislizeni* reported here is similar to that reported by Bowers (1998) (here: mean flowers per *F. wislizeni* plant ranged from 23 in 1996 to 36 in 1998; in the Bowers study, the mean was 25 flowers per plant). Johnson (1992) also found a strong relationship between plant size and flower production in another cactus (*Echinomastus*).

Overall fruit set was 54 to 65% for *F. cylindraceus*, and 94 to 96% (very high) for *F. wislizeni* (Table 3). *F. cylindraceus* plants aborted more buds than *F. wislizeni* plants (7 to 14% versus 1 to 2%). Bowers (1996) found 14% bud abortion in another cactus (prickly-pear). The mechanism underlying bud abortion is not known for these plants, but it may be due to insufficient resources to produce a flower. However, the rate of bud abortions was higher for *F. cylindraceus* in 1998, after a wet winter, than in 1997, following a dry winter. Individual plant was the only significant factor affecting bud abortion rates in *F. cylindraceus*, indicating that there may be a genetic component. Rates of flower abortion were similar between the species (3 to 6% for *F. cylindraceus*, 0.1 to 4% for *F. wislizeni*). Flower abortion may be due to insufficient resources to mature a fruit, or to pollination failure. Temporal patterns of bud and flower abortion are examined separately (Appendix B).

*F. cylindraceus* was also very heavily impacted by a flower-eating caterpillar, *Pseudoschinia elautalis* (Crambidae) (23 to 29% of flowers and buds destroyed, versus

1% for *F. wislizeni*). Of the fruits resulting from hand-pollination treatments, 46% were destroyed in 1995, and 35% in 1996 (when insecticide was used). Because this florivore attacks many different cacti (Mann 1969), it is probably not the host-specificity of the florivore that accounts for this difference. Instead, it is more likely that the florivore does not occur in high numbers at the SRER, or is not active at the time of year that individuals of *F. wislizeni* flower. Florivores can significantly affect both male and female reproductive success through effects on pollinator behavior (Krupnick and Weis 1999), although such effects were not examined in this study.

The extremely high fruit set in *F. wislizeni* is unusual for plants in general, but not for Cactaceae (summarized in Bowers 1998). Bowers found 93% fruit set in another population of *F. wislizeni* (Bowers 1998), and fruit set rates of 53 to 89% for a prickly-pear (Bowers 1996). An entire body of theory has been developed to account for the phenomenon of "excess flowers" (see especially Stephenson 1981, Burd 1998), with explanations including resource limitation (Stephenson 1981; Casper and Niesenbaum 1993), pollen limitation (Casper and Niesenbaum 1993), increased pollen export (reviewed in Torres and Galetto 1999), architectural constraint (Diggle 1995), an overall "wider choice" model (Burd 1998), or some combination of the above (e.g., Haig and Westoby 1988; Casper and Niesenbaum 1993). The high fruit set observed in *F. wislizeni* does not mean that this plant is free from any of these constraints, but rather that meristem limitation (references summarized in Geber 1990) is the first constraint to come into play. The production of new areoles is likely affected by resource limitation, however, so that even though the very high fruit set observed would at first suggest that there was no resource limitation, resources may indirectly limit the number of flowers produced in a year. Fruit set in *F. wislizeni* was high in all years, perhaps because the large water storage capacity of these plants buffers them from variability in rainfall. In

cacti with less water storage capacity (such as prickly-pears), fruit set can be affected by rainfall (Bowers 1996).

*F. wislizeni* plants produce significantly more seeds per fruit than *F. cylindraceus* plants, and their seeds weigh significantly more (Table 3). Individual plant was a significant factor in both seeds per fruit and seed mass for both species (Tables 5 and 7), but plant size was not. With regard to seeds per fruit, however, the lack of correlation with plant size may be partly due to the huge variance in the number of seeds per fruit (Table 3). When plant size was compared to "maximum seeds" (the largest seeds per fruit produced by that individual over the whole flowering period), significant or nearly significant ( $P = 0.05$  to  $0.08$ ) positive correlations were found for *F. cylindraceus* in 1998 (maximum seeds versus plant width), and for *F. wislizeni* in both 1997 (plant width, height and volume) and 1998 (plant width: unpubl. data). To factor out the variability in seeds per fruit it would be necessary to count ovules to determine whether larger plants consistently produce more ovules.

Seeds per fruit for *F. wislizeni* in this study was similar to that found by Bowers (1996):  $665 \pm 381$  seeds (1997) and  $750 \pm 302$  seeds (1998) in this study, versus  $671 \pm 378$  seeds (October) and  $971 \pm 382$  seeds (November; Bowers 1996). Mean seed mass for *F. wislizeni* in this study (2.6 mg) was also similar to that found by Bowers (2 to 3 mg; Bowers 1996).

A striking difference between the two species is that, on a fruit-by-fruit basis, seed mass is significantly positively correlated with seeds per fruit in *F. cylindraceus*, but significantly *negatively* correlated in *F. wislizeni* (Table 7; Fig. 2). This hints that *F. wislizeni* is experiencing a size/number tradeoff in seeds per fruit, whereas *F. cylindraceus* exhibits a "general vigor" (Begon et al. 1990) pattern. Because these two species share a common ancestor (Cota and Wallace 1996), this difference cannot be

due to different ancestry. However, the relationship between seed mass and seed number would have to be studied over several years and in different populations to determine if this is indeed a species-level difference.

Another difference is in individual annual fecundities, which were nearly twice as great for *F. wislizeni* in 1998 as for *F. cylindraceus* in the same year. *F. cylindraceus* had slightly more flowers per plant than *F. wislizeni*, but this was more than offset by the differences in fruit set and seeds per fruit. Relative measures of reproductive success such as fruit set may have little meaning for lifetime individual reproductive success, if such success is controlled by absolute factors such as numbers of flowers or numbers of seeds per fruit (Herrera 1991).

#### *Plant size, growth, and demography*

Growth in height occurs at the apical meristem, which consists of an unusually large (for angiosperms; Boke 1980) apical dome. Growth in width results from the widening of the peripheral zone that gives rise to the vascular cylinder, and from growth at the vascular meristem. All water is stored in the single stem, and thus plant size is correlated with stored water resources. Barrel cacti can shrink in both height and width during times of drought, and hence size and growth measurements are influenced by the water status of the plant (Nobel 1986).

Because growth in height continues indefinitely in these and many other cacti, plant height is correlated with plant age. Thus the distribution of plant heights in a population can be used to estimate the age structure of the population (Nobel 1977, Jordan and Nobel 1981, Jordan and Nobel 1982, Steenbergh and Lowe 1983, Nobel 1988, Bowers et al. 1995, Bowers et al. 1997, Pierson and Turner 1998). In this study,

the growth rates of *F. wislizeni* were not measured in enough years to construct an accurate model for this population. Because only reproductive plants were measured, furthermore, height distributions do not represent the entire population. It does appear however that the *F. cylindraceus* population consists of several cohorts (Fig. 4), whereas the *F. wislizeni* population seems to have mainly one large cohort, with a scattering of much older plants (Fig. 4). Based on other estimates of size/age relationships in *F. cylindraceus*, the ages of the reproductive plants in the Desert Peak plot probably vary from about 20 to about 90 years old (Nobel 1977; Jordan and Nobel 1981; Jordan and Nobel 1982; Nobel 1988; Bowers et al. 1995; Bowers et al. 1997). The one outlier (height = 166 cm) may be over 100 years old.

The growth rates achieved by *F. wislizeni* in 1997 to 1998 (mean = 7.2 cm growth in height) seem quite high compared to those documented for *F. cylindraceus* (see below). The only other study in which growth rates were measured in *F. wislizeni* found a growth rate of about 2 cm in height per year (MacDougal and Spalding, 1910, as reported in Nobel 1977). The abundant rains of the 1996 to 1997 El Niño winter undoubtedly contributed to this high growth rate, and in fact part of the apparent growth may be due to increased turgidity (rather than actual new tissue). Growth rates in 1998 to 1999 were slower (mean of 3.1 cm growth in height). Production of new areoles, a better measure of actual growth (Nobel 1986), was not measured here. Growth in overall plant volume increased with plant size (age), but relative growth was negatively correlated with plant size.

Growth was not measured for *F. cylindraceus* in this study; however, other studies have estimated growth in height rates as 0.9 cm per year (Jordan and Nobel 1982), 1.4 cm per year (Nobel 1977), and 3.1 cm per year (Bowers et al. 1997). In comparison, saguaro growth in height rates rise from about 2 cm per year for very small

plants, to a maximum of about 14 cm per year before the plant begins flowering (at a height of about 250 to 350 cm), then declines to about 7 to 8 cm per year (Pierson and Turner 1998). Minimum reproductive size for *F. wislizeni* in this study (~20cm width) was similar to that found by Bowers (1996): 15 cm width. Bowers estimates that individuals are about 10 years of age when they achieve reproductive status (Bowers et al. 1997).

As in many long-lived, non-clonal woody desert perennials (Shreve 1917, Bowers et al. 1995), the proportion of seeds produced by these cacti that germinate, establish, and achieve reproductive size is vanishingly small. The population of *F. cylindraceus* at Desert Peak was producing roughly 387.245 seeds per season, but only 2 plants joined the reproductive class that year (1998). The *F. wislizeni* population produced roughly 112 million seeds annually, with 6 individuals becoming reproductive in 1998. The high fecundity and low recruitment to the reproductive class observed in these cacti are consistent with elasticities reported for saguaros (based on data in Steenbergh and Lowe 1977), in common with other large, long-lived perennials (such as sequoias: Silvertown et al. 1993).

### *Conclusions*

For *F. cylindraceus*, both extrinsic (florivore damage) and intrinsic (spontaneous abortions of flowers and buds; meristem limitation) factors were important in limiting reproductive output. For *F. wislizeni*, meristem limitation was the major factor affecting reproductive output. This study demonstrates that these two sister species share many similarities in their reproductive biology: both are functionally outcrossers, and for both, plant size has a considerable impact on reproductive output, primarily through flower

production. Because these plants characteristically have high fruit set rates, and because of the large number of seeds per fruit, each flower can account for a high proportion of a plant's annual seed production – i.e., every flower counts. This is in contrast to many other plants that produce large numbers of “excess” flowers. Comparisons between this study and other studies of the reproductive ecology of cacti will help establish how many of these characteristics are common to cacti as a group.

### **Acknowledgements**

I thank J. L. Bronstein, J. S. Miller, P. E. Scott, R. S. Wallace, and especially L. A. McDade, for critical comments that greatly improved the manuscript. The Agricultural Experimental Station at the University of Arizona kindly granted me permission to do field work on the Santa Rita Experimental Range. The main source of funding for this research was a Graduate Research Fellowship from the National Science Foundation. Further support was provided by the Department of Ecology and Evolution at the University of Arizona, a fellowship from the Graduate College of the University of Arizona, the Flinn Foundation, and the University of Arizona Research Training Group in the Analysis of Biological Diversification. This paper is part of a dissertation by M. E. McIntosh in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Arizona.

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*Table 1.* Likelihood ratio tests for the effects of treatment, year and plant on fruit set (unit = flower) in breeding system experiments, 1995 & 1996 pooled. "Plant" is nested within "site" for *F. wislizeni*.

Species	Effect in model	df	$\chi^2$	<i>P</i>
<i>F. cylindraceus</i> <i>n</i> = 361 fls	Treatment	3	32.57	0.0000
	Year	1	0.66	0.4179
	Plant	32	37.02	0.2485
<i>F. wislizeni</i> <i>n</i> = 399 fls	Treatment	3	73.80	0.0000
	Site	1	0.03	0.8754
	Plant[Site]	29	49.56	0.0101

Table 2. ANOVA results for the effects of treatment, year and plant on number of seeds per fruit in breeding system experiments. 1995 & 1996 pooled. Aborted fruits ( $n = 0$  seeds) were excluded. "Lack of fit" was significant for both tests (see Methods).

Species	Effect in model	df	F ratio	P
<i>F. cylindraceus</i> ( $n = 135$ fruits)	Treatment	3	25.1215	<.0001
	Year	1	2.3025	0.1323
	Plant	29	2.9929	<.0001
<i>F. wislizeni</i> ( $n = 181$ fruits) (residuals non-normal)	Treatment	3	21.4471	<.0001
	Site	1	2.2302	0.1375
	Plant[Site]	28	6.2116	<.0001

*Table 3.* Summary statistics (mean  $\pm$  1 standard deviation; range of values) for flower production, fruit set, and seeds per fruit. Unless otherwise specified, the means of individual plant means are shown.

	F.cyl 1997 <i>n</i> = 24 plants	F.cyl 1998 <i>n</i> = 33 plants	F.wis 1996 <i>n</i> = 54 plants	F.wis 1997 <i>n</i> = 24 plants	F.wis 1998 <i>n</i> = 24 plants
Mean flowers per plant	29.0 $\pm$ 17.0 fls (1-66 fls)	31.8 $\pm$ 21.7 fls (1-98 fls)	23.1 $\pm$ 13.2 fls (1-74 fls)	28.8 $\pm$ 18.8 fls (6-89 fls)	36.3 $\pm$ 17.8 fls (14-84 fls)
Abortion rates (unit=flower)					
aborted buds	buds = 6.5%	buds = 14.1%	buds = 1.0%	buds = 1.9%	buds = 2.0%
aborted flowers	flowers = 6.2%	flowers = 2.9%	flowers = 4.1%	flowers = 1.3%	flowers = 0.1%
florivore damage abortions	floriv. = 22.5%	floriv. = 28.8%	floriv. = 1.2%	floriv. = 0.9%	floriv. = 1.4%
Overall fruit set rate (population pooled)	65%	54%	94%	96%	96%
Mean seeds per fruit (range of plant means)	not available	575 $\pm$ 255 seeds (155-1277)	748 $\pm$ 500 seeds (182-2949)	665 $\pm$ 381 seeds (107-1559)	759 $\pm$ 305 seeds (254-1587)
actual range = range of individual fruits		actual range = 14-1727	actual range = 17-3064	actual range = 38-2001	actual range = 126-1941

Table 4. ANOVA results for the effects of individual plant and year on fruit set rates for *F. cylindraceus* in 1997 and 1998 (pooled). Unit = plant.

Dependent variable	Source of variation	df	F ratio	P
Overall fruit set rate	Plant	32	1.49	0.1693
	Year	1	4.13	0.0550
Florivore abortion rate (buds+fls+fruits)	Plant	32	1.32	0.2546
	Year	1	4.72	0.0415
Bud abortion rate (residuals non-normal)	Plant	32	2.81	0.0079
	Year	1	2.55	0.1252
Flower abortion rate	Plant	32	1.55	0.1486
	Year	1	3.21	0.0875

*Table 5.* ANOVA results for the effects of individual plant and year on number of seeds per fruit.

Species & year	Source of variation	df	<i>F</i> ratio	<i>P</i>
<i>F.cyl</i> 98 ( <i>n</i> = 122 fruits)	Plant	27	5.57	<.0001
<i>F.wis</i> 96-98 ( <i>n</i> = 384 fruits)	Plant	55	12.49	<.0001
	Year	2	9.45	0.0001

Table 6. Summary statistics (mean  $\pm$  1 standard deviation; range of values) of plant size, *F. cylindraceus* 1998, and *F. wislizeni* 1997-1999.

	F.cyl 1998 <i>n</i> = 128 plants	F.wis 1997 <i>n</i> = 97 plants	F.wis 1998 <i>n</i> = 104 plants	F.wis 1999 <i>n</i> = 109 plants
Height (cm)	70.3 $\pm$ 34.7 (17-166)	31.9 $\pm$ 25.7 (11-212)	37.8 $\pm$ 26.1 (15-218)	38.2 $\pm$ 19.1 (18-148)
Width (cm)	29.2 $\pm$ 5.2 (17-43)	28.6 $\pm$ 6.6 (17-45)	35.3 $\pm$ 5.3 (22-45)	37.0 $\pm$ 5.4 (25-50)
Volume (cm <sup>3</sup> )	54,665 $\pm$ 38,484 (3,859-174,264)	26,116 $\pm$ 40,718 (3,405-337,171)	42,740 $\pm$ 44,254 (5,702-346,714)	46,240 $\pm$ 37,464 (10,088-267,814)
Change in height (cm)			1997-1998 ( <i>n</i> = 95) 7.2 $\pm$ 4.6 (-1 to +21)	1998-1999 ( <i>n</i> = 102) 3.1 $\pm$ 3.1 (-5 to +8)
Change in width (cm)			7.3 $\pm$ 3.0 (-1 to +14)	2.3 $\pm$ 1.9 (-3 to +8)
Change in volume (cm <sup>3</sup> )			17,648 $\pm$ 9,945 (-4,337 to + 65,581)	8,350 $\pm$ 9,201 (-17,598 to +49,617)

Table 7. ANOVA results for the effect of individual plant on mean seed mass per fruit.  
*F.cylindraceus* and *F.wislizeni* 1998.

Species	df	F ratio	P
<i>F.cylindraceus</i> (n = 122 fruits)	27	4.2567	<.0001
<i>F.wislizeni</i> (n = 147 fruits)	23	14.8695	<.0001

## Figure Legends

*Figure 1.* Effects of hand-pollination treatments on (a) mean fruit set per plant and (b) on mean seeds per fruit per plant. Shown are means with one standard error. *F. cyl* = *F. cylindraceus*. *F. wis* = *F. wislizeni*. "Out." = outcrossed. "Open" = open-pollinated.

*Figure 2.* Seed number per fruit versus mean seed mass per fruit. Each point represents one fruit. . Statistics are from regression analyses using a linear fitting. (a) *Ferocactus cylindraceus* 1998. (b) *Ferocactus wislizeni* 1998.

*Figure 3.* Relationships between plant heights and widths. Each point represents one plant. (a) *Ferocactus cylindraceus*, 1998. (b) *Ferocactus wislizeni*, 1998.

*Figure 4.* Size structure of the populations. (a) *F. cylindraceus*, distribution of plant heights, 1998. (b) *F. wislizeni*, distribution of plant heights, 1998. (c) *F. cylindraceus*, distribution of plant widths, 1998. (d) *F. wislizeni*, distribution of plant widths, 1998.

*Figure 5.* Growth in volume as a function of plant size. *Ferocactus wislizeni*. Sizes classes are based on the ln of the plant volume (see Methods). (a) absolute growth 1997-1998  $P = 0.0002$  (Kruskal-Wallis) (b) absolute growth 1998-1999  $P = 0.1638$  (Kruskal-Wallis) (c) percent growth 1997-1998  $P < 0.0001$  (Kruskal-Wallis) (d) percent growth 1998-1999  $P = 0.0002$  (Kruskal-Wallis).

*Figure 6.* Relationship between plant size (ln plant volume) and number of flowers produced in a year. *P* - values are from regression analyses using a linear fitting. (a) *F. cylindraceus* 1998; (b) *F. wislizeni* 1997; (c) *F. wislizeni* 1998.

Figure 1.

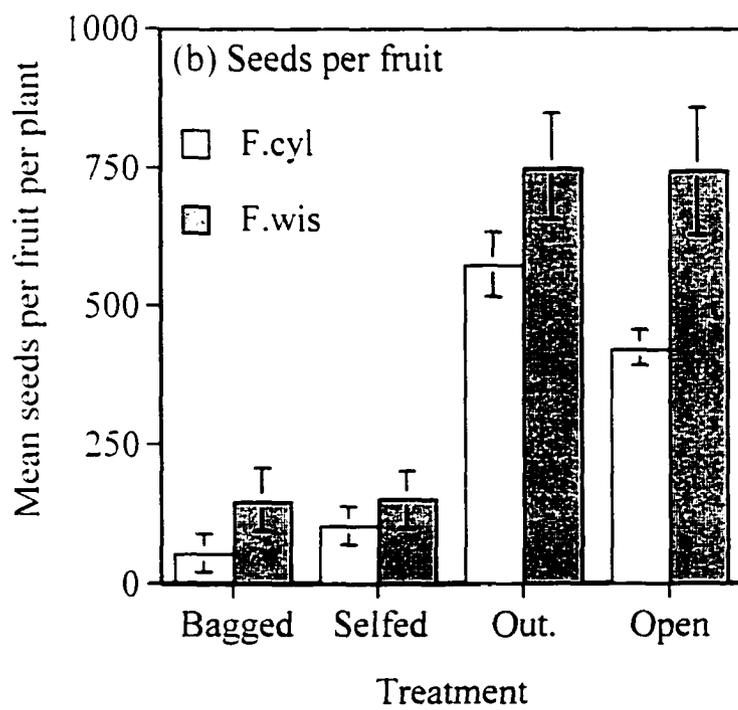
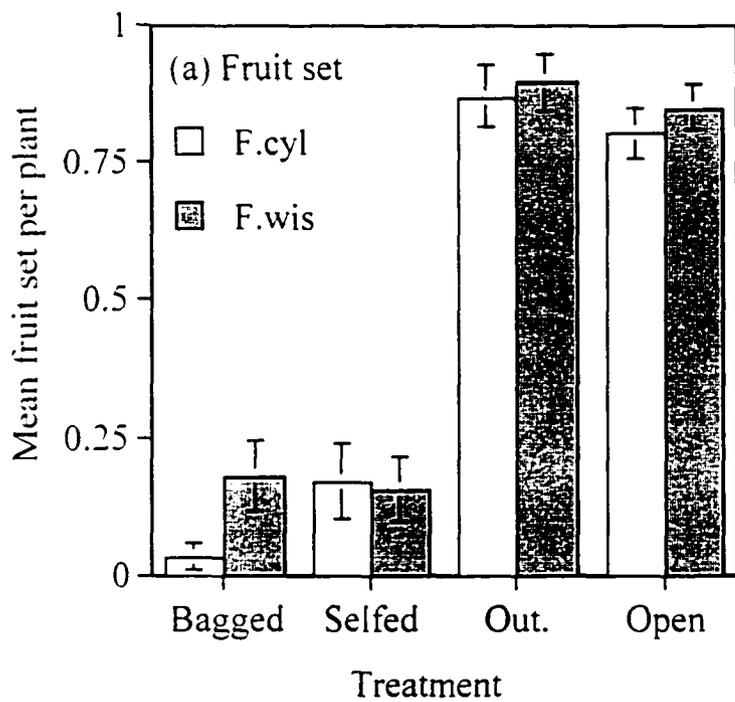


Figure 2.

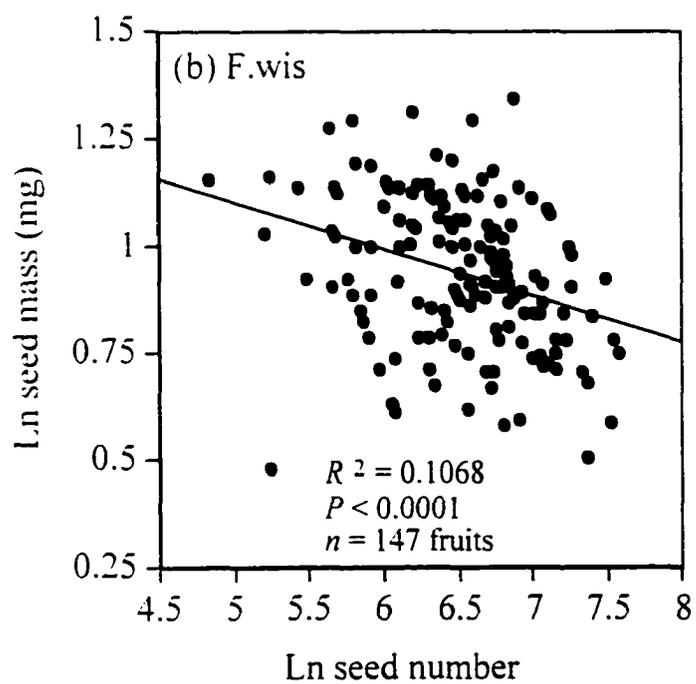
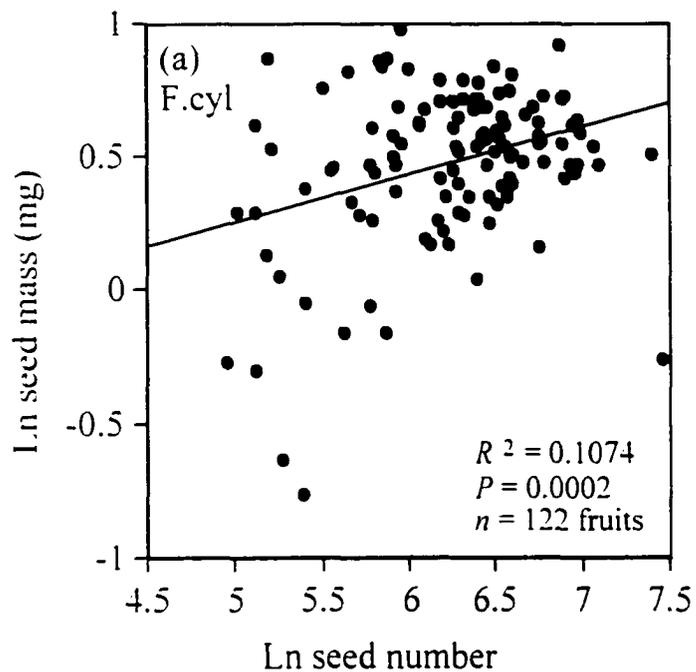
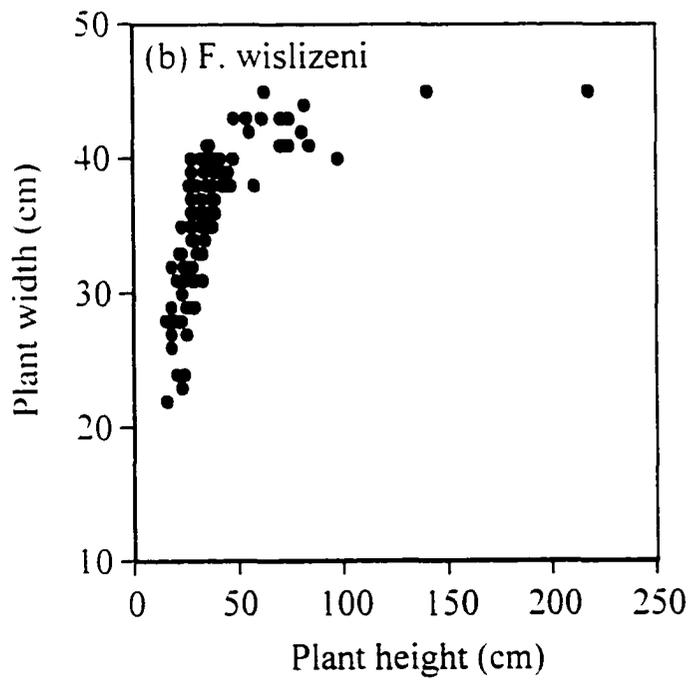
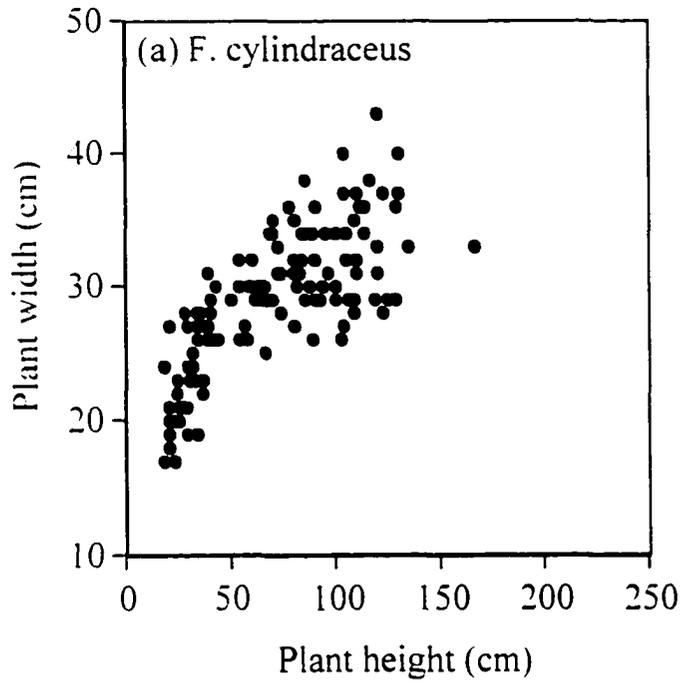


Figure 3.



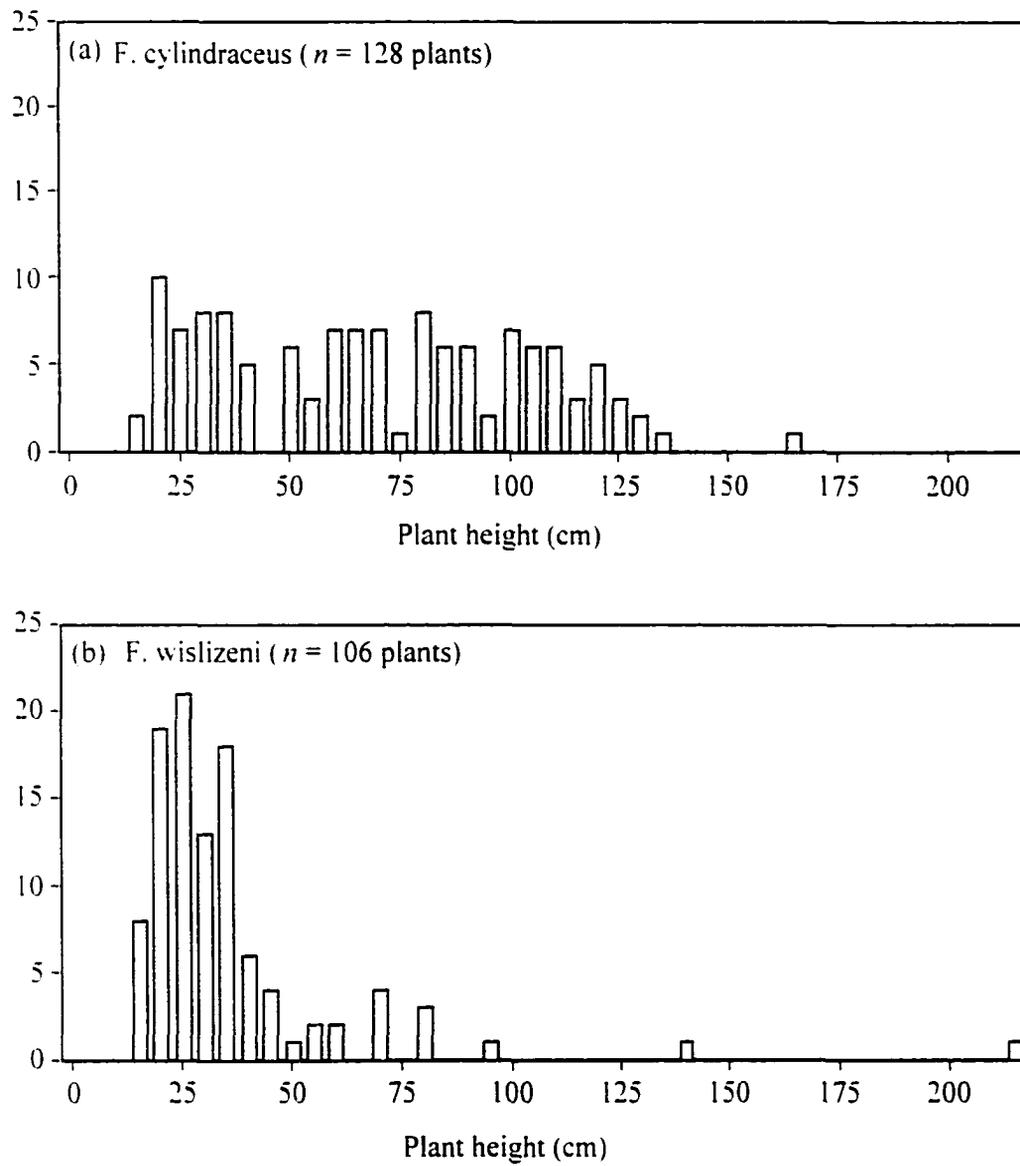
**Figure 4.**

Figure 4, continued

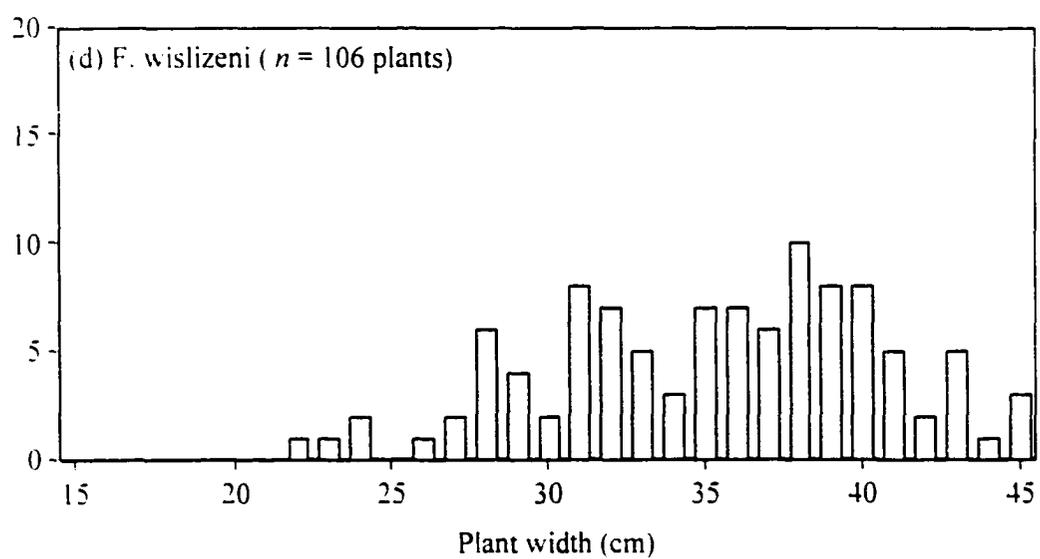
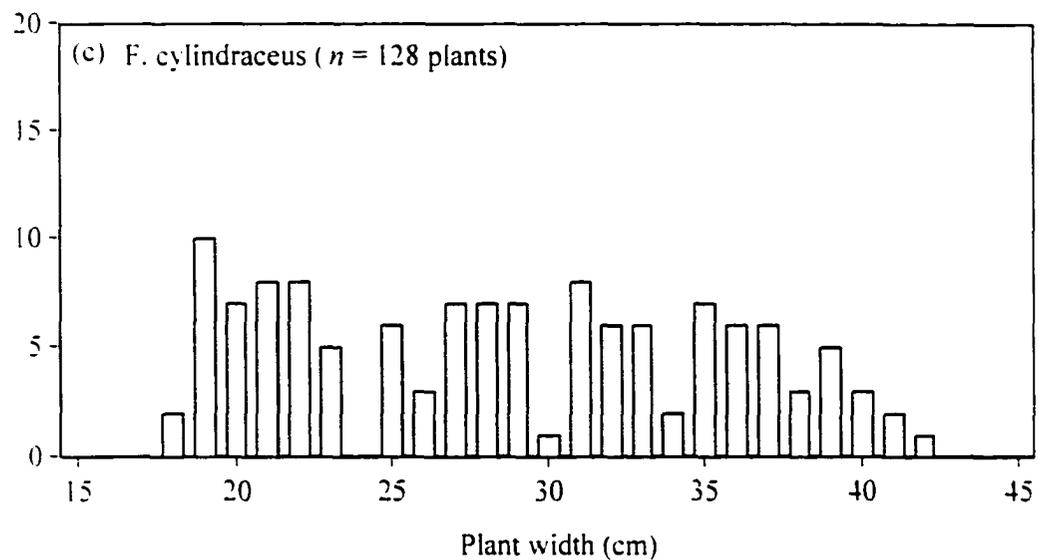


Figure 5.

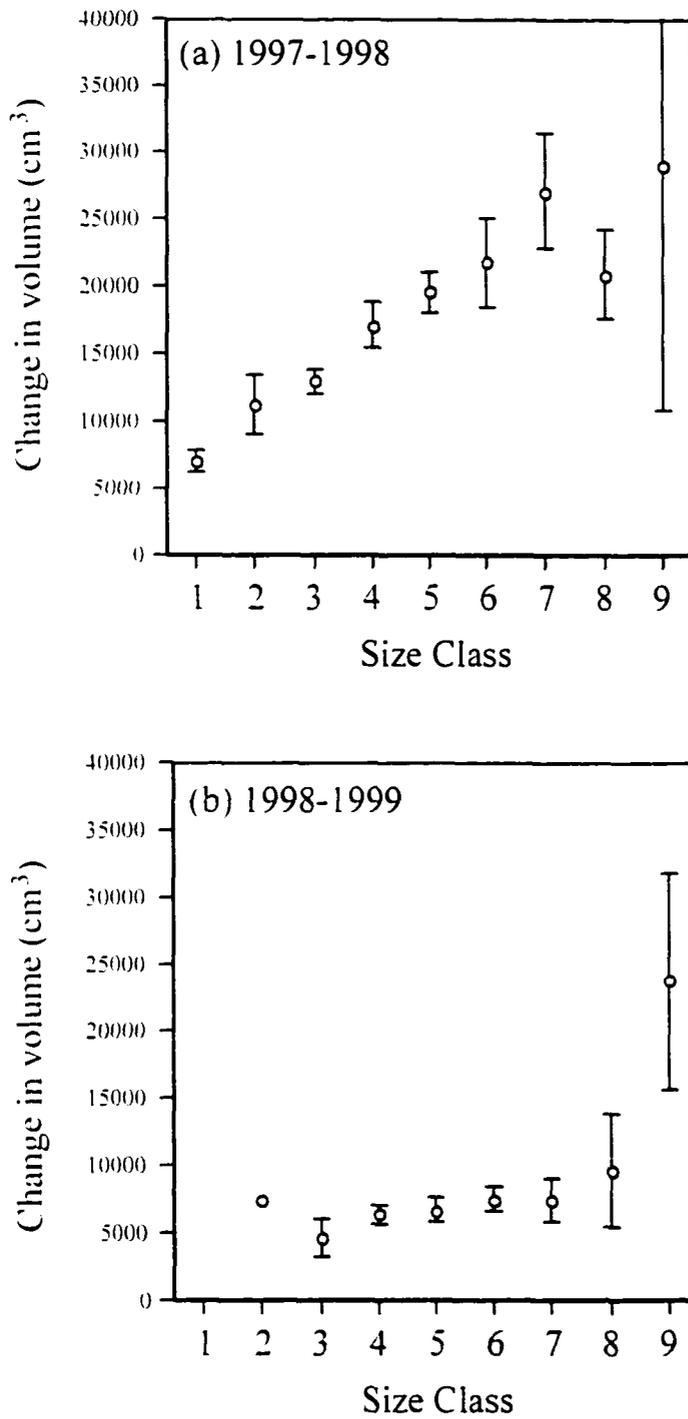


Figure 5, continued

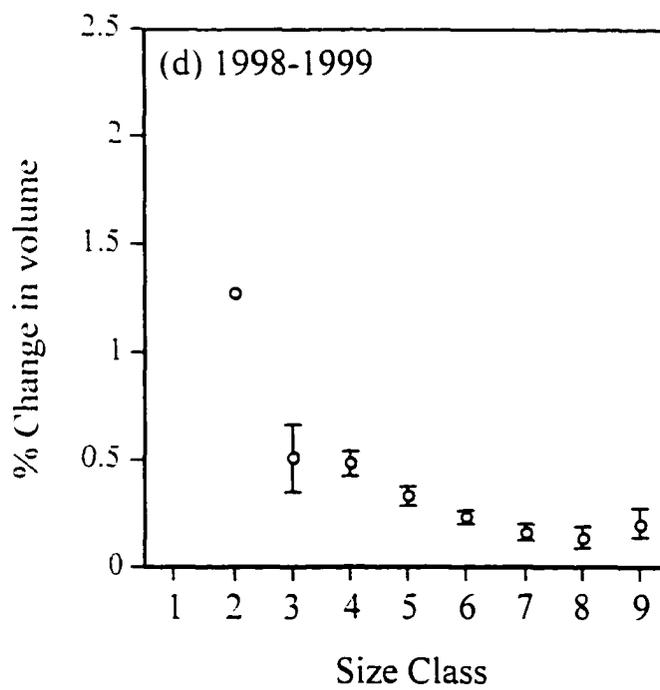
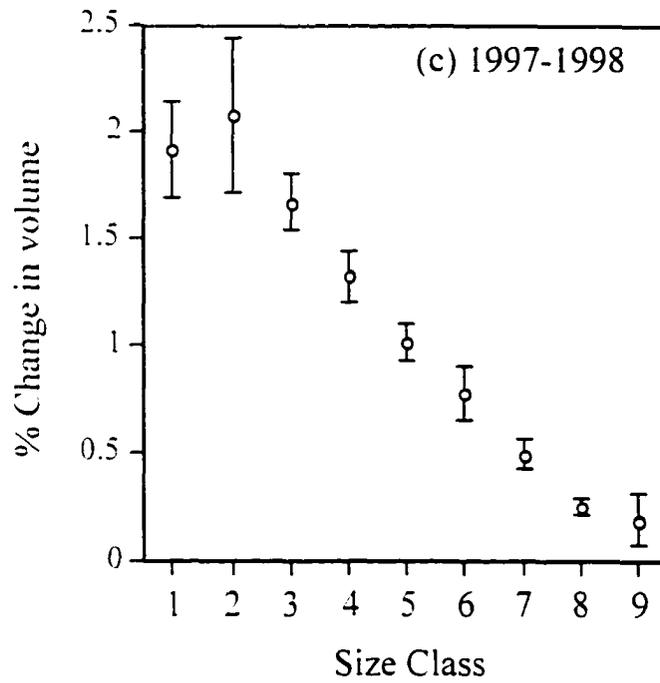


Figure 6.

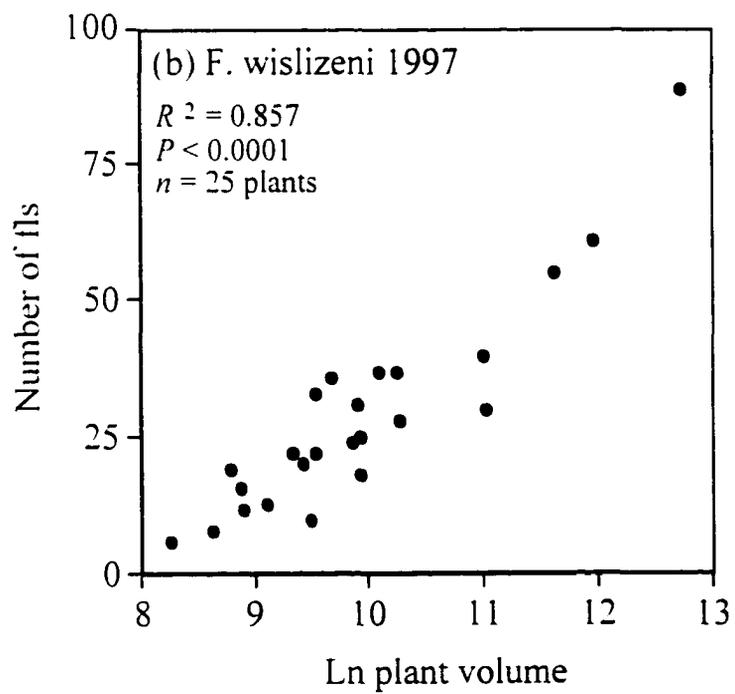
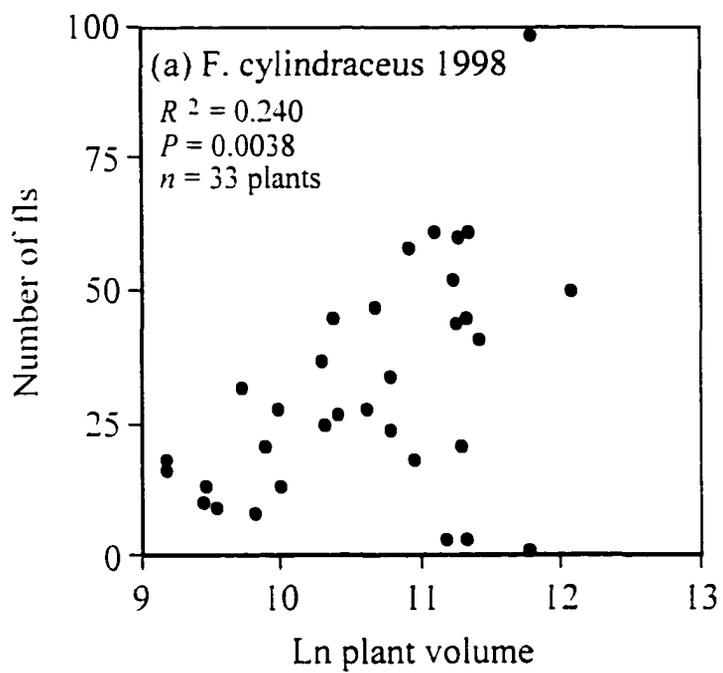
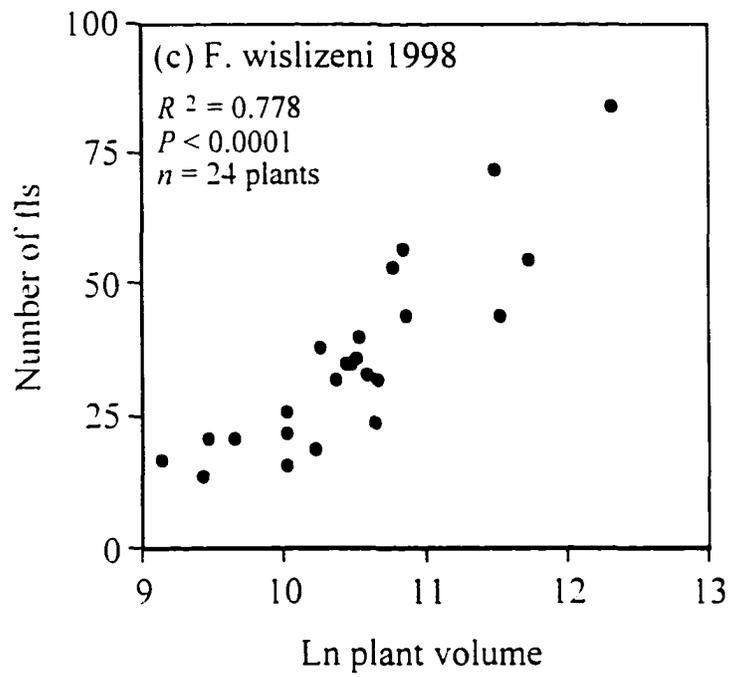


Figure 6, continued



**APPENDIX B****FLOWERING PHENOLOGY AND REPRODUCTIVE OUTPUT IN TWO  
SISTER SPECIES OF *FEROCACTUS* (CACTACEAE)**

Flowering phenology and reproductive output  
in two sister species of *Ferocactus* (Cactaceae)

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## Abstract

Flowering phenology is often strongly constrained by phylogenetic history: many closely-related plants have very similar phenologies. On the other hand, divergent flowering phenologies can function as isolating mechanisms, which may be reinforced if related plants occur sympatrically. I investigated flowering phenology and reproductive output of sister species of barrel cacti, *Ferocactus cylindraceus* and *F. wislizeni*, where they occur sympatrically in the Sonoran Desert surrounding Tucson, Arizona.

*Ferocactus cylindraceus* began blooming in May, and continued until early or mid-October, with a bimodal pattern of flowering amplitude. Individuals in the study population were moderately well-synchronized phenologically. *Ferocactus wislizeni* began blooming in July, and also continued until early or mid-October, with a single peak of intensity: individuals in the study population were well-synchronized phenologically. In both species, the vast majority of individuals bloom every year. Plant size was positively correlated with flowering amplitude in both species, and with flowering onset in *F. wislizeni*. The study population of *F. cylindraceus* was strongly affected by a flower-eating caterpillar in all years, with the earliest flowers most likely to be destroyed. For *F. wislizeni*, seed number per fruit was highest for flowers open in the middle of the blooming season in 1998. Other components of individual plant phenology, including among-plant synchrony, had little influence on reproductive output.

## Introduction

The timing of flowering can strongly influence the reproductive success of a plant in several ways (Rathcke and Lacey 1985). Such effects may be mediated by

factors operating within plants (for instance, plants that flower too young may not have sufficient resources stored to mature fruits), within populations (asynchronously flowering plants may not find mates), between species (plants flowering at the "wrong" time might not be visited by pollinators, or may be disproportionately affected by seed predators), or abiotic factors (plants flowering too late in the season may be killed by frost before they can mature fruits). Thus, flowering phenology can affect the ecology of a plant at multiple levels, including individual plant reproductive success, interactions of the plant with other organisms, plant population dynamics, and ecosystem functioning (e.g., the plant-pollinator landscape: Bronstein 1995). Examining the relationships between flowering phenology, fruit and seed production, plant size and growth, and the degree of spatial and temporal variability in these relationships can provide insight into the selective forces affecting the evolution of flowering time. Comparing these factors across closely related species can indicate the extent to which the evolution of flowering phenology is constrained by the history of the lineage to which they belong.

I studied the flowering phenology of two species of short-columnar unbranched cacti, *Ferocactus cylindraceus* (Engelm.) Orcutt and *F. wislizeni* (Engelm.) Britt. and Rose during 1996-1998 in the Sonoran Desert near Tucson, Arizona. The genus *Ferocactus* (Cactaceae) contains 25-30 species (Cota and Wallace 1997), most of which occur in México and the southwestern U.S. The species I studied are largely outcrossing (Appendix A), and are pollinated primarily by large solitary bees that specialize on cactus flowers (Grant and Grant 1979; McIntosh, unpub. data). The seeds are thought to be animal-dispersed; seeds probably germinate in the late summer or early fall (Jordan and Nobel 1981). The flowering periods of populations of *Ferocactus* are generally long (2-3 months), and in those species for which flowering times have been recorded,

flowering ranges from spring to late summer and fall (see Discussion). *F. wislizeni* has a more southerly and eastern distribution than *F. cylindraceus* (Turner et al. 1995), but their ranges overlap broadly in Arizona. *Ferocactus wislizeni* and *F. cylindraceus* have recently been found to be sister species, in a phylogenetic analysis of chloroplast DNA (Cota and Wallace 1997). Hence, differences in their phenologies are likely to be the result of recent evolutionary processes in their lineages rather than differing phylogenetic histories.

I quantified and compared the timing and duration of flowering at the level of the plant and the population for these two species. The goals of this study were (1) to compare the flowering phenology of the two species, (2) to determine how flowering time affects the fate of individual flowers, (3) to examine how plant size affects flowering phenology (Schmitt 1983, O'Neil 1997, Bishop and Schemske 1998, Ollerton and Lack 1998), and (4) to determine how flowering phenology affects the reproductive success of individual plants.

## **Methods**

### *Study organisms and study sites*

Study organisms and study sites are described in detail in Appendix A. In brief, I studied the flowering phenology of a population of *Ferocactus cylindraceus* 45 km NW of Tucson, Arizona in 1997 and 1998 (hereafter: Desert Peak). I marked 128 plants (all the reproducing plants within a 1.5 hectare plot), and studied flowering phenology in detail of a subsample of 22 (1997) or 33 (1998) plants. I also studied a population of *F. wislizeni* at the Santa Rita Experimental Range (hereafter: SRER) 40 km south of

Tucson, Arizona in 1996-1998. I marked 55 plants in 1996, and added 50 more plants in 1997 (comprising all reproducing plants in a 3 hectare plot). In 1996, a subsample of 54 plants was studied in detail, and a subsample of 24 plants was studied in detail in 1997 and 1998. Plants were selected for detailed study using a random stratified sampling method that ensured that all size classes were sampled.

### *Data collection*

To document flowering phenology, I censused selected plants every two weeks (*F. cylindraceus* in 1997, *F. wislizeni* in 1996) or once a week (*F. cylindraceus* in 1998, *F. wislizeni* in 1997-1998) during the flowering period. In October the census interval was usually expanded to two weeks. New flowers were marked at each survey. Fruits were collected when ripe and the seeds were counted. Seed counts were accurate to within  $\pm 1-2$  seeds for every 100 seeds counted, based on repeated counts. In 1998, seeds from a single fruit were weighed as a group; this was used to calculate mean seed weight per seed per fruit. Three components of female reproductive success were used in data analyses: fruit set, seeds per fruit, and mean seed weight per seed per fruit.

From flowering data I derived six phenology parameters: **onset** (date first flower opened); **end date** (date last flower opened); **duration** (difference between date of first and last flower); **mean flowering date** (peak of flowering: the mean of the census dates during which that individual was flowering, with each census date weighted by the number of flowers in that period: Bishop and Schemske 1998); **mean flowering amplitude** (number of flowers produced per unit time, terminology of Newstrom et al. 1994); and **synchrony** (flowering overlap among individuals). Of these six, all but synchrony were derived for both individual plants and the population as a whole.

Although most data were taken from the population subsamples, the onsets were based on all individuals in the plots (both species, each year). The peak flowering date of an individual plant can be difficult to quantify in individual plants whose flowering intensity over time lacks a clear mode. Mean flowering date (Bishop and Schemske 1998) combines the timing of flowering of an individual with the timing of the peak flowering intensity of that plant. Two plants that share the same beginning and end dates may thus have different mean flowering dates. However, this measure is only meaningful for plants with a roughly unimodal flowering pattern.

Because flowering of many *F. cylindraceus* plants was interrupted by a period of days or weeks during which no flowers were produced, in 1998 I also calculated the number of flowering days for each plant, defined as the flowering duration (in weeks) minus the weeks when they skipped flowering. In 1997, censuses occurred at 2-week intervals, and thus the level of resolution was not fine enough to accurately measure number of flowering days.

The flowering synchrony of an individual plant, as defined here, is the degree to which that plant's blooming period overlapped the blooming periods of all the other plants in the population. Synchrony was calculated using the method of Augspurger (1983, modified from Primack 1980). For each individual, the number of weeks that its flowering overlapped with that of other individuals was determined. The index of synchrony ( $X$ ) for an individual plant ( $i$ ) is given by

$$X_i = \left( \frac{1}{n-1} \right) \left( \frac{1}{f_i} \right) \sum_{j \neq i}^n e_{j \neq i}$$

where  $e_j$  is the number of weeks individual  $i$  and  $j$  overlapped in their flowering;  $f_i$  is the total number of weeks individual  $i$  was in flower, and  $n$  is the number of individuals in the sample.  $X$  varies from 1 (plant flowering overlaps with that of all other individuals) to 0 (no overlap with any other individuals).

### *Data analyses*

All statistical tests were performed with JMP IN® software (SAS Institute Inc., 1989-99a).

I examined three types of relationships: (1) the effect of flowering time on the reproductive success of individual flowers; (2) the effect of plant size on plant phenological parameters; and (3) the effects of plant phenological parameters on plant reproductive success.

To test the hypothesis that time of flowering affects the fate of a flower (i.e. whether or not that flower is aborted), and the seed number and seed mass resulting from that flower, I tested the effect of time of flowering (independent variable) on fruit set, seeds per fruit and mean seed mass per seed per fruit (dependent variables). Time of flowering, a categorical factor, was defined both for the flowering period of the individual plant, and for the flowering duration of the entire population. So, for example, a flower might open during the "middle" period of that individual plant, but during the "early" period of the population. For *F. cylindraceus* plants, whose mean duration was 13 weeks, "early" flowers were produced during the first two weeks of that plant's bloom, "late" flowers during the last two weeks, and "middle" as the intermediate

weeks. For *F. wislizeni* plants, whose mean flowering duration was only 6 weeks, I defined the first and last weeks as "early" and "late." I assigned population periods based on the pattern of flowering amplitude over time for each species in each year (Figs. 1 and 2). The flowering duration of the population of *F. cylindraceus* was divided into "first peak," "lull," "second peak," and "late" periods (Fig. 1). The flowering duration of the population of *F. wislizeni* was divided into "early," "middle" and "late" periods (Fig. 2). Although plant periods and population periods are not necessarily congruent, they do tend to be correlated, and hence were tested separately. Because flowers from the same plant are likely to not be independent data points, I included individual plant as a factor in all tests. Thus for each dependent variable I performed two tests: one with "plant" and "plant period" as factors, and one with "plant" and "population period" as factors.

Fruit set was extremely high for plants of *F. wislizeni* in all years (range: 0%-49%; Appendix A), hence I did not test for effects of phenology on fruit set for this species. For *F. cylindraceus*, flower abortions were classified as "bud abortions" (flower bud aborted before anthesis), or "flower abortions" (flower aborted after anthesis, before forming a fruit). I used nominal logistic tests to test the effects of both individual plant and flowering period (either plant period or population period: independent variables) on abortion (yes or no: dependent variable), with the experimental unit being the individual flower.

I used two-way ANOVAs to test the effect of individual plant and of flowering period (either plant period or population period: independent variables) on seeds per fruit, and on mean seed mass per seed per fruit (dependent variables), with the individual flower as the experimental unit. In some analyses, a significant "lack of fit" (SAS Institute Inc., 1989-99b) was found, indicating the presence of an untested

interaction between factors. However, because of unbalanced data I was not able to test for interactions (lost degrees of freedom). When a significant lack of fit was reported, I record it in the results. In some analyses, the residuals from the model were found to have a non-normal distribution, and I also report this. However, ANOVA is generally robust to this violation of assumptions (Zar 1996).

To test the hypothesis that plant size affects plant phenological parameters, I used linear regressions of the natural log of plant volume (independent variable) against each of 5 plant phenological parameters: onset, duration, mean date, and mean flowering amplitude (dependent variables). To calculate plant volume, I measured the size of all *F. cylindraceus* plants within the plot at Desert Peak in April 1998, and all *F. wislizeni* plants within the plot at the SRER in July of 1997 and 1998. A detailed description of measuring technique is given in Appendix A. In brief, I measured the height and width of each plant, and used these to calculate plant volume. Volume is a good surrogate for plant biomass, because the vast majority of biomass occurs above-ground in these cacti.

To test whether individual plants showed between-year consistency in their flowering phenology, I calculated Spearman rank correlations of those parameters (onset, end date, mean date, duration) for individual plants across years.

To test the hypothesis that plant phenological parameters affect the reproductive output of individual plants, I tested 3 phenological parameters: onset, mean date, and synchrony (independent variables), against mean fruit set (*F. cylindraceus* only), mean seeds per fruit, and mean seed mass per seed per fruit (dependent variables). Individual plant was the experimental unit. Linear regressions were used to test for correlations between phenology and reproductive output. Because flowering onset, mean date, and synchrony are inter-related, each parameter was tested separately.

## Results

### *Flowering phenology*

The *F. cylindraceus* population had a bimodal amplitude of flowering in both years (Fig. 1). The rate of flower production rose quickly to the first peak in 1998 (in 1997, censuses did not begin until about two weeks after flowering onset). A lull followed the first peak in May-June, then the second smaller peak occurred in August to early September. Flowering continued sporadically into October. Flowering amplitude was greater in 1998 (following a wet winter) than in 1997 (following a dry winter; Table 1). Greater amplitude can also be the result of plant growth (Appendix A). The total flowering duration of the population was 23 weeks in 1997 and 20 weeks in 1998 (Table 1). Because flowering is bimodal in this population, the phenological parameter "mean date" does not apply.

The pattern of flowering amplitude was quite different for *F. wislizeni* (Fig. 2). In all three years there was a single peak of flower production. Time between onset and peak varied from 3 weeks (1998) to 7 weeks (1997). As in *F. cylindraceus*, flowering continued into October. Amplitude increased each year (Table 1). Population duration was much less than for *F. cylindraceus*: 13 to 15 weeks, as opposed to 20 to 23 weeks. Although flowering onset varied from early July to early August, mean flowering date was remarkably similar across years, ranging only from August 29 to August 31 (Table 1).

*F. cylindraceus* individual plants had longer flowering durations and more evenly distributed amplitudes than *F. wislizeni* individuals (Fig. 3). *F. cylindraceus*

plants also usually had at least one gap in flowering, whereas *F. wislizeni* plants did not (Fig. 3). As a result, for *F. cylindraceus* individuals, mean flowering duration (first flower to last flower) was 81 days, but number of days actually flowering was only 55 (1998: Table 1). Because of these gaps in blooming, mean synchrony of plants was much lower for *F. cylindraceus* (0.50) than for *F. wislizeni* (0.75 to 0.84: Table 1).

The vast majority of plants of both species flowered every year. In each population, only once (one plant, one year) did a plant that had flowered previously fail to flower in a subsequent year.

#### *Flowering time of individual flowers*

For *F. cylindraceus*, individual plant was a significant factor in bud abortions in 1998, but not in 1997 (Table 2). Plant period (whether the individual flower opened in the early, middle, or late portion of the flowering duration of the individual plant) was a significant factor in both years: bud abortions increased over time within a plant's bloom period. Population period (whether the flower opened during the first peak, lull, second peak, or late period of the population flowering duration) was also significant in both years: in 1997, bud abortions increased over time, whereas in 1998, although they were still lowest in the early population period, they were highest in the lull and second peak periods (Table 2). Patterns in abortions can be incongruent between plant periods and population periods, because plant periods and population periods are not exactly correlated (e.g., a flower can occur late in a plant's flowering duration, but early in the population period).

Individual plant was not a significant factor in flower abortions (Table 2), and plant period was also not significant. In other words, it is not the case that *F.*

*cylindraceus* plants routinely abort flowers early, or late, in their flowering duration, as do many other plants (Stephenson 1981). In 1997, population period was significant, with very low flower abortion rates during the lull, and flower abortions in the remaining three periods approximately equal, ranging from 10%-14% (Table 2).

Abortions resulting from damage caused by a florivorous lepidopteran larvae (Appendix A) were highest in the early population period, and declined over time, in both years (McIntosh, unpublished data).

Individual plant was always a significant factor in seeds per fruit and in mean seed mass per seed per fruit for both species (Table 3). For *F. cylindraceus*, the only other significant factor affecting seed number per fruit was population period, with seed number highest in the first peak and declining over time (Table 3a). Population period was also the only factor besides individual plant affecting seed mass, with seeds from flowers in the second blooming peak and late periods having the greatest mass (Table 3a).

For *F. wislizeni* in 1997, seed number increased over time, in terms of both plant period and population period (Table 3b). In 1998, seed number was highest in the middle periods at both the plant and the population levels. When seed number is plotted against time a clear temporal pattern is evident in 1998 (Fig. 4), with the most seeds being produced by flowers that bloomed in the middle of the population bloom period. In both years, seed number per fruit peaked at nearly the same date (1997: August 29, 1998: Sept. 2; Fig. 4) and these dates correspond closely to the mean population dates (1997: Sept. 1; 1998: August 31; Table 1). Seed mass per seed per fruit was not affected by flowering time (Table 3b).

### *Plant size and other plant effects*

Large *F. wislizeni* plants began flowering earlier in the season (Table 4, Fig. 3b), and flowered for a longer duration (Table 4) than small plants, in both years. However, plant size had no effect on flowering onset or duration for *F. cylindraceus*. Plant size was not correlated with mean flowering date (*F. wislizeni*), and large *F. wislizeni* plants were less synchronized with other plants in the population than small plants (1998 only), probably because of their longer flowering duration. In both species, plant size was positively correlated with mean flowering amplitude (Table 4).

The flowering onsets of individual plants were significantly correlated across years for both species (*F. cylindraceus* 1997-1998, Spearman  $r = 0.4705$ ,  $P < 0.0001$ ,  $n = 125$  plants; *F. wislizeni* 1996-1997, Spearman  $r = 0.6195$ ,  $P < 0.0001$ ,  $n = 53$  plants; *F. wislizeni* 1997-1998, Spearman  $r = 0.5927$ ,  $P < 0.0001$ ,  $n = 97$  plants). For *F. cylindraceus* in 1997-1998, duration and end date were not correlated across years ( $P = 0.0555$  and  $0.6198$  respectively). For *F. wislizeni*, mean date, duration, and end date were all significantly correlated for both year comparisons (mean date: 1996-1997, Spearman  $r = 0.7556$ ,  $P = 0.0003$ ; 1997-1998, Spearman  $r = 0.5402$ ,  $P = 0.0078$ ; duration: 1996-1997, Spearman  $r = 0.6009$ ,  $P < 0.0001$ ; 1997-1998, Spearman  $r = 0.5601$ ,  $P = 0.0054$ ; end date: 1996-1997, Spearman  $r = 0.4455$ ,  $P = 0.0009$ ; 1997-1998, Spearman  $r = 0.4445$ ,  $P = 0.0336$ ).

### *Flowering phenology and the reproductive success of individual plants*

For *F. cylindraceus* plants, neither their flowering onset nor their synchrony affected their overall flower abortion rates (onset: 1997,  $R^2=0.0177$ ,  $p=0.5454$ ,  $n=23$ ;

1998,  $R^2=0.0001$ ,  $p=0.9166$ ,  $n=27$ ; synchrony: 1998,  $R^2=0.0260$ ,  $p=0.4204$ ,  $n=27$ ). For individuals of both species, neither seeds per fruit nor mean seed mass per seed per fruit was affected by phenological parameters (Table 5).

## Discussion

### *Individual flowers*

The number of aborted flower buds increased over time in *F. cylindraceus*, both in the overall population bloom season, and within the blooming periods of individual plants. This suggests that bud abortions may be affected by climatic factors, and/or by plant factors (e.g., levels of stored resources) that change as the season progresses. Rates of flower abortion were unrelated to the timing within the flowering periods of individual *F. cylindraceus* plants, and individual plant was not a significant factor in flower abortions (Table 2). Thus these plants do not routinely abort early or late flowers preferentially (Stephenson 1981). It seems more likely that flowers are aborted when they fail to receive enough pollen to set seed (Appendix A). The lack of a temporal pattern in flower abortions suggests that the levels of pollination are not substantially different in different periods of the flowering duration of *F. cylindraceus*. The low levels of flower abortion in *F. wislizeni* suggest that these plants receive adequate pollination services throughout their flowering duration.

Seed production was affected by timing of flowering for both species. Seed production in *F. cylindraceus* was highest in the first peak of flowering, and decreased over time (Table 3). For *F. wislizeni*, in 1997 seed production was lowest early in the flowering period (in both plant periods and population period) and increased over time

(Table 3). In 1998 seed production was highest in the middle of flowering (both plant periods and population period: Table 3). Peak seed production (Fig. 4) coincided with peak flowering (Fig. 2) in both 1997 and 1998.

### *Plant effects*

In work reported elsewhere (Appendix A), plant size was found to be positively correlated with the number of flowers produced by a plant in a season for both species. Size was not found to impact fruit set rates or seed mass in that study, however. The overall effect of size on fecundity was strong (Appendix A), as is the case with many other plants (references summarized in Schmitt 1983, O'Neil 1997, Bishop and Schemske 1998, Ollerton and Lack 1998). In this study, I found that size had a strong positive correlation with flowering amplitude in both species. Because amplitude is defined as number of flowers divided by flowering duration, this relationship could result from a positive correlation between flowers and size (as found in Appendix A), from a negative relationship between duration and size, or from both. However, the relationship between duration and size was positive for *F. wislizeni* (Table 4), as it is for many other plants (Schmitt 1983, Ollerton and Lack 1998). Thus at least for this species, large plants make so many more flowers than small plants, that even though they also flower for a longer period, their mean flowering amplitude is still higher than that of small plants.

Larger *F. wislizeni* plants also begin blooming earlier than smaller ones (Table 4, Fig. 3b), but this was not the case for *F. cylindraceus* (Table 4, Fig. 3a). In other studies, correlation between plant size and flowering onset, when it occurs, was positive for some plants (large plants begin flowering earlier) and negative for others

(summarized in Ollerton and Lack 1998), and these effects were often variable in time and space. Plant effects other than those related to plant size were also found here: for *F. wislizeni*, mean date was correlated for individual plants across years, although mean date was not related to plant size (Table 4).

The peak flowering date of an individual plant has been linked to the relative reproductive success of that plant in other studies (Bishop and Schemske 1998, Ollerton and Diaz 1999), but that relationship was not found here: there was no correlation between mean date and seed production or seed mass for *F. wislizeni* (Table 5). In fact, none of the phenological parameters examined in this study (flowering onset, mean flowering date and synchrony) affected female reproductive success of individual plants. Because plants were surveyed only once a week, however, the level at which I calculated synchrony may be too coarse to detect actual correlations with reproductive output or success.

Producing large numbers of seeds may be one way for desert perennials to survive the difficult recruitment (germination and establishment: Clark et al. 1999) phase of their life history (Bowers 1994, Pierson and Turner 1998). With annual per-plant fecundities of 14,000 to 25,000 seeds (*F. cylindraceus* and *F. wislizeni*, respectively: Appendix A), these cacti may be able to beat the recruitment lottery by sheer number of entries. The magnitude of lifetime seed production, which is probably most strongly influenced by numbers of flowers produced (Appendix A) may thus affect lifetime reproductive success much more than, for example, the exact timing of individual flowers. In this study, although the timing of individual flowers did affect their reproductive success (whether or not the flower set a fruit, seeds per fruit, and mean seed mass per seed per fruit), the flowering phenology of individual plants had no effect on their overall fruit set or seed production. It may indeed be the case that in these

species. flowering phenology is a trait not under strong selection (Ollerton and Lack 1992).

#### *Comparison of the two species*

Although phylogeny plays a dominant role in the flowering phenologies of many plants (Kochmer and Handel 1986, Wright and Calderon 1995, Ollerton and Diaz 1999), in some cases members of the same genus or family can differ significantly in their phenologies (Haddock and Chaplin 1982, Proença and Gibbs 1994, Pickering 1995, Madeira and Fernandes 1999, Soliva and Widmer 1999), and these differences can function as isolating mechanisms for related plants occurring in sympatry. Despite the fact that *F. cylindraceus* and *F. wislizeni* are sister species, they exhibit strikingly different flowering phenologies. They begin blooming at different times (May for *F. cylindraceus*, July or August for *F. wislizeni*), and flowering exhibited two peaks in *F. cylindraceus* but only one in *F. wislizeni*. *F. cylindraceus* individuals flower for more than twice the time that *F. wislizeni* individuals do (12-15 weeks for *F. cylindraceus*, 6 weeks for *F. wislizeni*). The *F. cylindraceus* population was also in bloom much longer than the *F. wislizeni* population (20-23 weeks for *F. cylindraceus*, 12-15 weeks for *F. wislizeni*). Patterns at the individual level are different also: *F. cylindraceus* individuals usually have a lull in the middle of their blooming period.

The bimodal flowering pattern of *F. cylindraceus* was one of the more surprising findings of this study. However, because only one population of this species was studied, it is unknown whether this pattern is characteristic of the species as a whole, or just this population. This population is near the edge of the range for this species (Turner et al. 1995) and many plants are known to reproduce differently at the edge of

their range than they do in the center. It could also be that introgression from *F. wislizeni*, if such is occurring, is responsible for the second period of bloom (Appendix A).

Conceivably, the extended bloom period could also be a response to the loss of early flowers and fruits to caterpillars. Resources that would have been invested in early fruits may be redeployed to later fruits if early fruits are destroyed. In the first peak of flowering, 43-44% of all fruits were lost to caterpillar damage, whereas only 11-13% were lost in the second peak of flowering (unpublished data). Hence, plants may achieve higher fecundity during the second blooming peak, even though their flowering amplitude is lower than in the first peak. Thus the bimodal flowering pattern may be currently adaptive in this population, whatever its original causes or underlying mechanisms. However, the most likely explanation is that it is an adaptive response to the abundant summer rains that occur in southeastern Arizona. *F. cylindraceus* has been reported to bloom sporadically following summer rains where these occur (Benson 1982). Phenological data from other populations are needed to resolve this question.

The difference in the timing of flowering onset (as opposed to peak flowering) may also be related to the differing rainfall patterns that occur in different parts of the Desert Southwest. In California, the center of distribution for *F. cylindraceus*, most of the annual rain falls in winter, whereas in New Mexico and Texas (the center of the range of *F. wislizeni*) most rain falls in the summer. *F. wislizeni* has been described as occurring only in areas where summer rains occur, and where the summer rains are greater than the winter rains if there are two rainy periods (Benson 1982, Turner et al. 1995). Summer and winter rains are about equal at the Santa Rita Experimental Range. It seems plausible that the two species have evolved to flower partly in response to different rainfall periods, with *F. cylindraceus* responding to winter rains and *F.*

*wislizeni* to summer rains. The timing of flowering onset may be indirectly tied to resource allocation. Flower production is meristem limited (each areole flowers only once, hence flower production is limited by production of new areoles: Appendix A), and if meristem production is water-limited, this might account for the link between flowering onset and local rainfall patterns. However, these plants do not respond facultatively to significant rain regardless of the timing within the year, as does (e.g.) *Larrea tridentata* (Bowers and Dimmitt 1994).

The flowering periods of other species of *Ferocactus* span the range from March to September (Kearney and Peebles 1951, Shreve and Wiggins 1964, Benson 1982), and many cannot be simply categorized as spring-bloomers or summer-bloomers. In fact, a purported subspecies, *F. wislizeni tiburonensis*, is recorded as blooming in April and May on Tiburon Island in the Gulf of California (Shreve and Wiggins 1964). Further work is needed to determine the exact nature of the climatic triggers for flowering onset.

Flowering phenology can also be indirectly constrained if selection acts upon the timing of seed dispersal and germination (Primack 1987), rather than on the timing of flowering. The fruits of *F. cylindraceus* mature about 6 weeks after pollination, remain fleshy for a few weeks thereafter, and then become desiccated. *F. wislizeni* fruits mature about 80d (11-12 weeks) after pollination, and although they can remain fleshy for at least a year thereafter, the fruits do not normally remain on the plant that long. Seed dispersal has not been studied in *Ferocactus*, but it would appear that they are primarily animal-dispersed. Seed germination requirements, which are related to timing of seed dispersal, suggest that *Ferocactus* seeds germinate best at 29-30°C, and require light but not cold treatment (Jordan and Nobel 1981, Romero-Schmidt et al. 1992). Periods when *Ferocactus* seed germination near Tucson would seem likely are mid-April to late May, and from late Sept. to late October (both are periods when daily highs in Tucson average

27°C to 33°C). Late summer or early fall has in fact been proposed as the most likely germination time for *F. cylindraceus* (Jordan and Nobel 1981). If *Ferocactus* seeds remain viable for extended periods, however, it may be that timing of seed dispersal is not critical. Again, more work would be needed to determine if selection acting upon the timing of seed dispersal and germination is affecting flowering phenology.

### *Conclusions*

This study has shown that these two cacti possess markedly different flowering phenologies, despite their shared evolutionary history. These differing phenologies may in turn be related to climatic factors rather than to selection acting directly on the timing of flowering. However, their flowering periods do overlap considerably, and hence cannot function as isolating mechanisms, at least at Desert Peak, where both species occur. Although flowering time did affect the reproductive output of individual flowers, differences among individual plants in reproductive output are probably more closely linked to flower production (strongly correlated with plant size) and with pollinator-mediated seed production, than to the differing flowering phenologies of those plants.

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*Table 1.* Phenology data at the plant and population levels. For plant data, values shown are the mean of all plant values  $\pm$  1 standard deviation, and range of values.  $n$  = number of plants. For population data, values shown are for the whole population (i.e., the onset is the date of the first flower on the first plant in the population). Dates are given in Julian date format first, followed by the calendar date.

	F. cylind. 97	F. cylind.98	F. wis.96	F. wis.97	F. wis.98
onset: plants (mean of plant values)	145 $\pm$ 15 (May 25) 128-261 n = 131	162 $\pm$ 15 (June 11) 139-223 n = 140	213 $\pm$ 9 (August 1) 202-246 n = 54	225 $\pm$ 15 (August 13) 188-269 n = 99	225 $\pm$ 7.0 (August 13) 205-243 n = 103
onset: population	-128 (May 8)	139 (May 19)	202 (July 21)	188 (July 7)	205 (July 24)
duration: plants	107 $\pm$ 22 d 57-153 n = 23	81 $\pm$ 32 d 1-129 n = 33	42 $\pm$ 16 d 3-78 n = 54	41 $\pm$ 17 d 5-93 n = 98	41 $\pm$ 10.0 d 28-70 n = 24
duration: population	162 d (23 wk)	140 d (20wk)	87 d (13 wk)	105d (15 wk)	90 d (13 wk)
$\approx$ days blooming	not available	55 $\pm$ 22d 7-98 n = 33			
mean date: plants	186 $\pm$ 18 (July 5) 147-227 n = 23	200 $\pm$ 22 (July 19) 168-273 n = 32	235 $\pm$ 9 (August 23) 221-266 n = 54	244 $\pm$ 9.0 (Sept. 1) 228-260 n = 24	243 $\pm$ 5.0 (August 31) 237-253 n = 24
mean date: population	181 (June 30)	194 (July 13)	241 (Aug 29)	243 (Aug 31)	243 (Aug 31)
synchrony: plants	not available	0.50 $\pm$ 0.10 0.125-0.664 n = 33	0.77 $\pm$ 0.086 0.57-0.91 n = 53	0.755 $\pm$ 0.12 0.536-0.913 n = 24	0.84 $\pm$ 0.07 0.63-0.92 n = 24
amplitude (fls/plant/day): plants	0.28 $\pm$ 0.15 0.08-0.74 n = 23	0.41 $\pm$ 0.23 0.08-1.00 n = 33	0.54 $\pm$ 0.22 0.03-1.40 n = 54	0.625 $\pm$ 0.29 0.29-1.48 n = 24	0.88 $\pm$ 0.37 0.39-2.00 n = 24
amplitude: population	0.1871 fls./plant/day	0.2271 fls./plant/day	0.2629 fls./plant/day	0.2743 fls./plant/day	0.4029 fls./plant/day

Table 2. Effects of time of flowering on bud abortions and flower abortions (fruit set), *F. cylindraceus*. Experimental unit is the flower. Results from nominal logistic tests, with response (dependent variable) being abort, Y or N, weighted by counts. "Plant period" is the period within the flowering duration of the individual plant. "Population period" is the period within the flowering duration of the entire population. Plant period and population period are correlated to some extent, and hence were tested separately. If the flowering period was significant, I report the abortion percentages for the time periods. (a) bud abortions. (b) flower abortions

## (a) Bud abortions

Independent variable	1997			1998		
	df	$\chi^2$	<i>P</i>	df	$\chi^2$	<i>P</i>
Plant	22	32.76	0.0653	30	82.07	0.0000
Plant period	2	37.32	0.0000	2	16.70	0.0002
early	0% (217)			5% (405)		
middle	5% (443)			10% (607)		
late	29% (85)			17% (184)		
Plant	22	31.14	0.0933	30	65.13	0.0002
Population period	3	44.92	0.0000	3	31.88	0.0000
1st peak	2% (452)			3% (668)		
lull	4% (92)			16% (77)		
2nd peak	13% (165)			17% (430)		
late	31% (36)			10% (21)		

## (b) Flower abortions

Independent variable	1997			1998		
	df	$\chi^2$	<i>P</i>	df	$\chi^2$	<i>P</i>
Plant	22	18.99	0.6458	30	20.86	0.8921
Plant period	2	0.685	0.7099	2	1.52	0.4673
Plant	22	24.11	0.3413	30	19.36	0.9321
Population period	3	10.95	0.0120	3	2.15	0.5411
1st peak	10% (408)					
lull	13% (77)					
2nd peak	1% (142)					
late	14% (22)					

Table 3. Effects of phenology on seed number and seed mass. The experimental unit is the individual fruit.

(a) *F. cylindraceus*, 1998.

Dependent variables	Independent variables	df	F-ratio	P
seeds per fruit	Plant	27	7.9692	<.0001
	Plant period	2	1.8764	0.1579
seeds per fruit (significant lack of fit) 1st peak 629 = 285 (66) lull 532 = 369 (16) 2nd peak 536 = 312 (51) late 410 = 276 (11)	Plant	28	8.4259	<.0001
	Pop. period	3	4.3871	0.0059
	Plant	26	4.2736	<.0001
	Plant period	2	2.7933	0.0665
	Plant	27	5.1016	<.0001
mean seed mass per seed per fruit 1st peak 1.6 = 0.380 (54) lull 1.5 = 0.351 (15) 2nd peak 1.8 = 0.443 (45) late 1.8 = 0.328 (7)	Pop period	3	7.6043	0.0001

(b) *F. wislizeni*, 1997

Dependent var	Independent vars	df	F-ratio	P
seeds per fruit early 429 = 310 (15) middle 779 = 530 (51) late 848 = 447 (6)	Plant	21	10.4437	<.0001
	Plant period	2	13.5052	<.0001
	Plant	21	6.5882	<.0001
seeds per fruit early 355 = 339 (21) middle 826 = 501 (43) late 1033 = 377 (8)	Pop period	2	6.8936	0.0023

Table 3. continued

(c) *F. wislizeni*, 1998

Dependent var	Independent vars	df	F-ratio	P
seeds per fruit	Plant	23	10.7113	<.0001
(residuals non-normal)	Plant period	2	8.3018	0.0004
early 653 ± 325 (24)				
middle 820 ± 389 (107)				
late 624 ± 370 (22)				
seeds per fruit	Plant	23	11.0230	<.0001
early 640 ± 321 (24)	Pop period	2	9.9197	<.0001
middle 822 ± 386 (107)				
late 629 ± 386 (22)				
mean seed mass per seed per fruit.	Plant	23	15.1321	<.0001
(significant lack of fit;	Plant period	2	1.7918	0.1711
residuals non-normal.)				
mean seed mass per seed per fruit.	Plant	23	14.5691	<.0001
(significant lack of fit;	Pop period	2	0.6764	0.5104
residuals non-normal.)				

Table 4. Effect of plant size on phenological parameters. Correlations were tested using linear regressions. Independent variable is the natural log (ln) of plant volume. The sign of the trend, if significant, is in parentheses.  $n$  = number of plants.

Dependent variable	<i>F.wislizeni</i> 1997	<i>F.wislizeni</i> 1998	<i>F.cylindraceus</i> 1998
onset	$R^2 = 0.124$ , $P = 0.0005$ , $n = 94$ (negative)	$R^2 = 0.040$ , $P = 0.0427$ , $n = 103$ (negative)	$R^2 = 0.005$ , $P = 0.4311$ , $n = 133$
duration	$R^2 = 0.268$ , $P < 0.0001$ , $n = 93$ (positive)	$R^2 = 0.321$ , $P = 0.0039$ , $n = 24$ (positive)	$R^2 = 0.004$ , $P = 0.7247$ , $n = 33$
mean date	$R^2 = 0.013$ , $P = 0.5990$ , $n = 24$	$R^2 = 0.046$ , $P = 0.3136$ , $n = 24$	$R^2 = 0.015$ , $P = 0.5117$ , $n = 32$
synchrony	$R^2 = 0.124$ , $P = 0.0919$ , $n = 24$	$R^2 = 0.209$ , $P = 0.0248$ , $n = 24$ (negative)	$R^2 = 0.067$ , $P = 0.1445$ , $n = 33$
amplitude	$R^2 = 0.562$ , $P < 0.0001$ , $n = 24$ (positive)	$R^2 = 0.584$ , $P < 0.0001$ , $n = 24$ (positive)	$R^2 = 0.400$ , $P < 0.0001$ , $n = 33$ positive

*Table 5.* Effects of phenological parameters on (a) mean seeds per fruit and on (b) mean seed mass per seed per fruit. Shown are results of linear regressions. Because phenological parameters are inter-related, each was tested separately.

(a) Effects on mean seeds per fruit per plant

	F. cyl 98 $R^2$	( $n = 28$ ) $P$	F. wis 97 $R^2$	( $n = 21$ ) $P$	F. wis 98 $R^2$	( $n = 24$ ) $P$
onset	0.002	0.8043	0.007	0.7189	0.060	0.2862
mean date			0.017	0.5700	0.001	0.8916
synchrony	0.095	0.1115	0.000	0.9659	0.029	0.4596

(b) Effects on mean seed mass per seed per fruit per plant

	F. cyl 98 ( $n = 27$ ) $R^2$	$P$	F. wis 98 ( $n = 24$ ) $R^2$	$P$
onset	0.112	0.0884	0.152	0.0595
mean date			0.031	0.4094
synchrony	0.132	0.0626	0.004	0.7670

## Figure Legends

*Figure 1.* Flowering amplitudes for the *F. cylindraceus* population at Desert Peak, 1997-1998. Census intervals were 2 weeks (1997) or 1 week (1998). Amplitude shown is number of flowers opened during the interval ending on the census date, divided by the number of plants measured that year (to standardize for different sample sizes). In 1997 sampling began after flowering had commenced. Defined "population periods" (first peak, lull, second peak, end) are indicated.

*Figure 2.* Flowering amplitudes for the *F. wislizeni* population at the Santa Rita Experimental Range, 1996-1998. Amplitude shown is number of flowers opened during the interval ending on the census date, divided by the number of plants measured that year (to standardize for different sample sizes). Defined "population periods" (early, middle, late) are indicated.

*Figure 3.* Flowering amplitudes of individual plants, 1998. Each row of lines and rectangles represents one plant. The height of the rectangles indicates the number of flowers opened on the plant during that census interval. Plants are arranged along the y-axis in ascending order of plant size (based on plant volume). Dashed lines indicate periods when a plant did not bloom. (a) *F. cylindraceus* (b) *F. wislizeni*. Each tick-mark on the x-axis delimits one week. Each tick-mark on the y-axis represents (a) 15 flowers, or (b) 23 flowers.

*Figure 4.* Mean seeds per fruit over time. *F. wislizeni*. Error bars denote one standard error of the mean. Point with no error bars represents one fruit. (a) 1997:  $n = 72$  fruits from 22 plants. (b) 1998:  $n = 153$  fruits from 24 plants.



Figure 2.

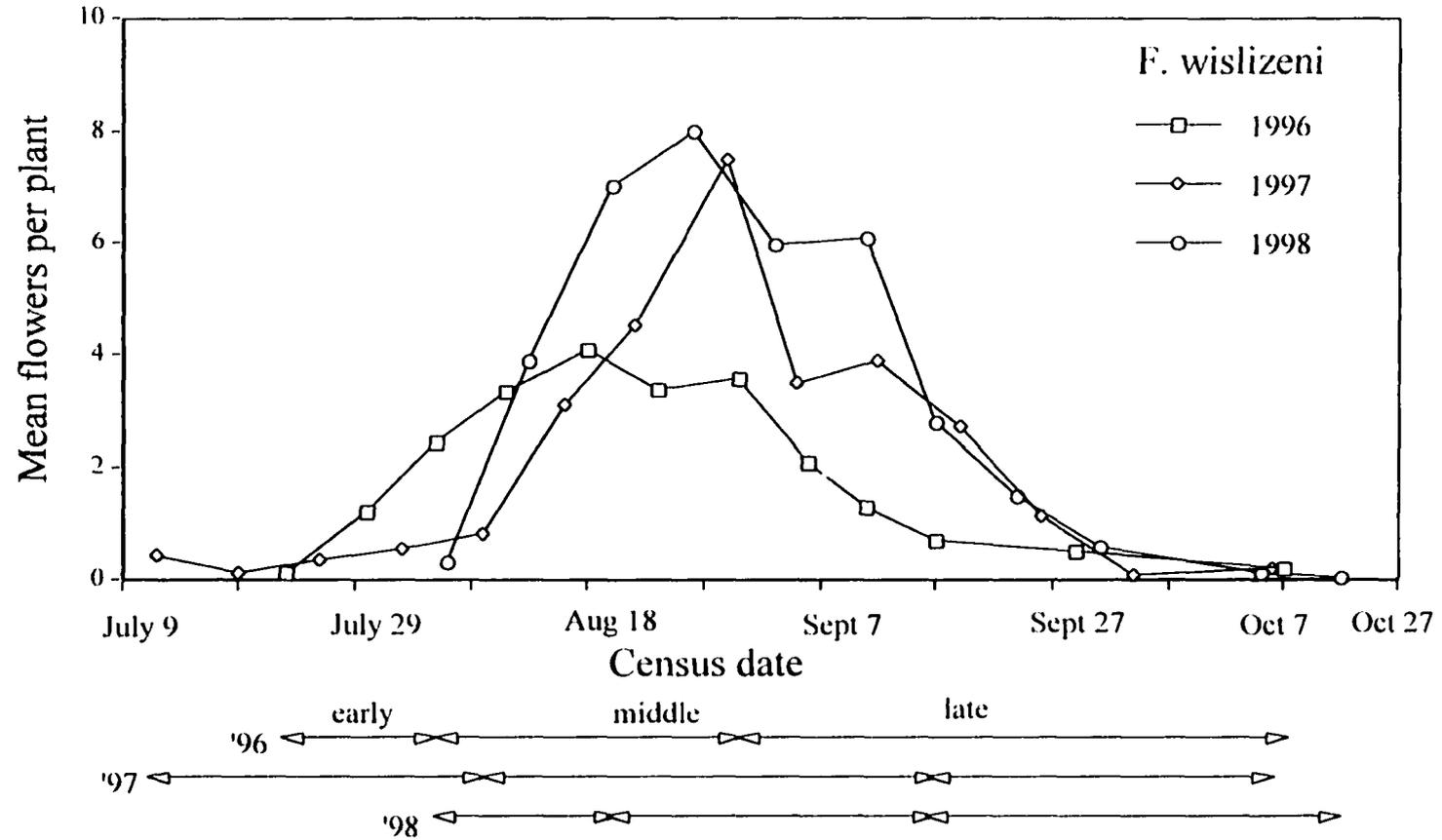


Figure 3a.

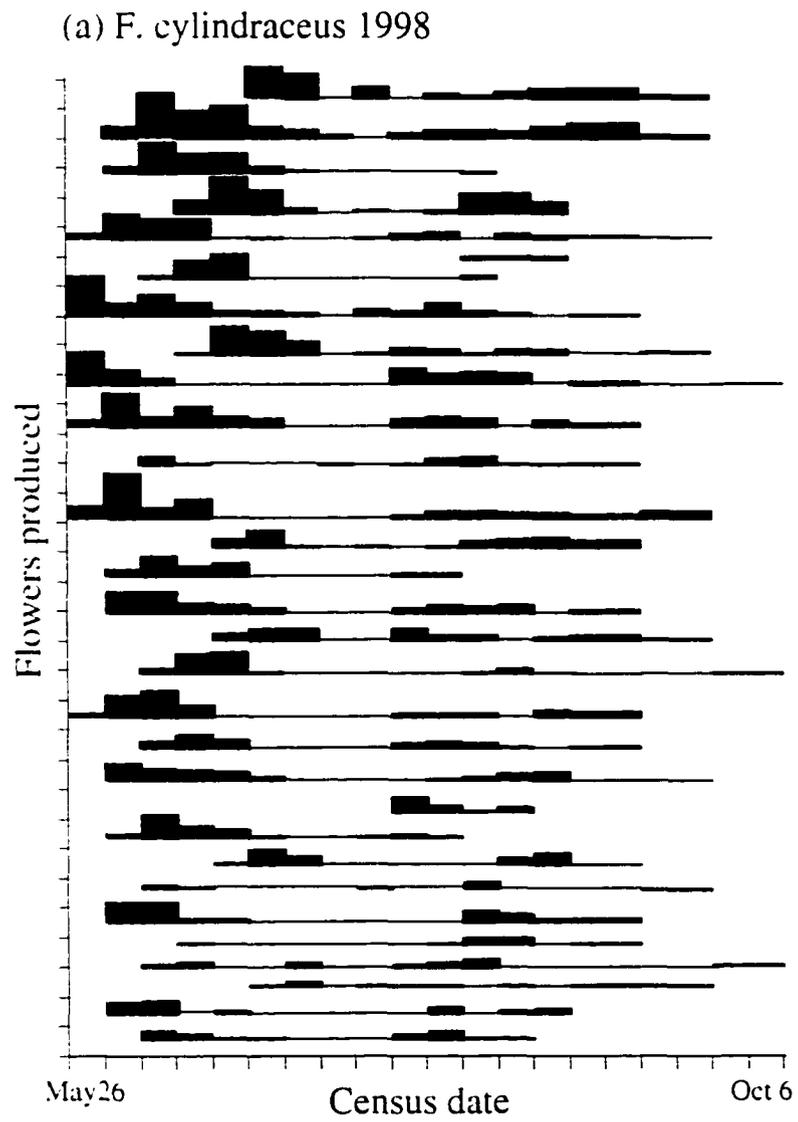


Figure 3b.

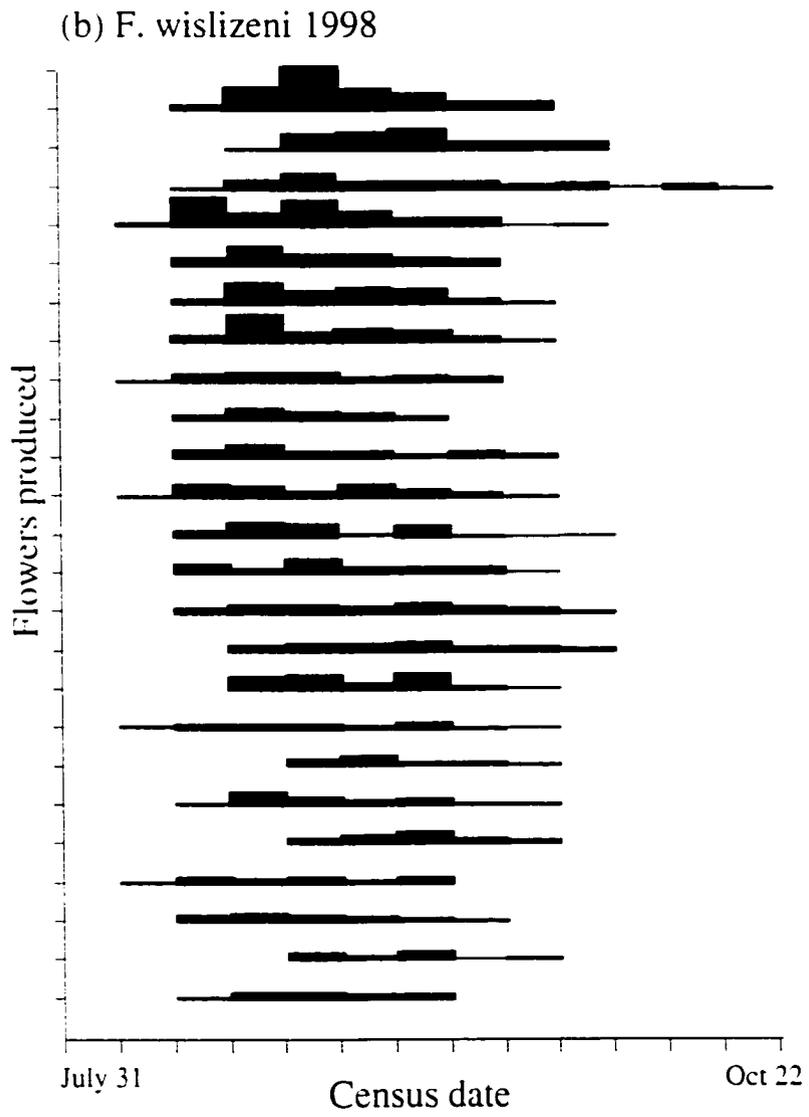
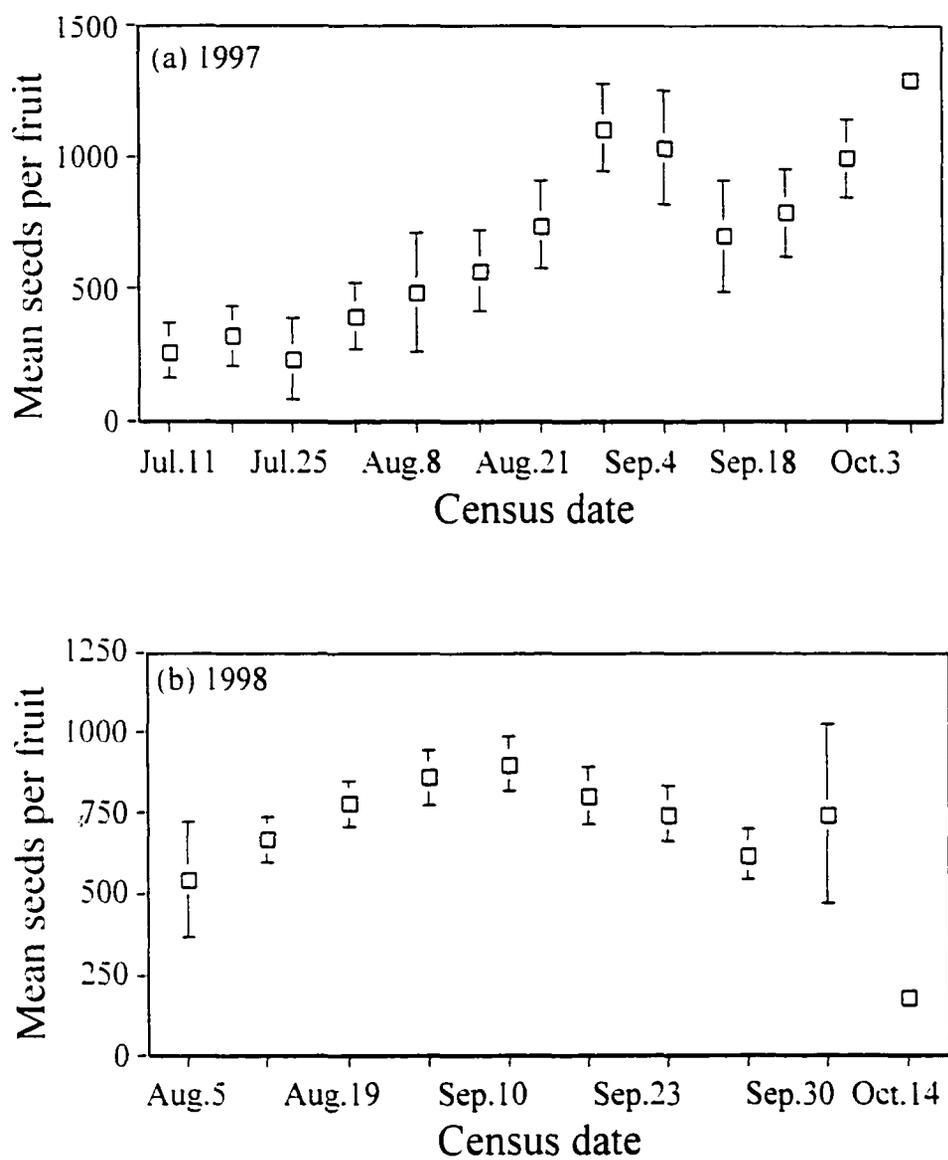


Figure 4.



**APPENDIX C****POLLINATION OF TWO SPECIES OF *FEROCACTUS*: INTERACTIONS  
BETWEEN CACTUS-SPECIALIST BEES AND THEIR HOST PLANTS**

Pollination of two species of *Ferocactus*:  
interactions between cactus-specialist bees and their host plants

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## Abstract

Resolving the controversy over the importance and prevalence of generalization in plant-pollinator interactions requires rigorous field studies that characterize the pollinator effectiveness of all members of a plant's visitor assemblage. I studied the floral visitor assemblages of two species of barrel cactus (*Ferocactus*), using the method of single pollinator visits to quantify the pollinator effectiveness of different assemblage members. Despite the fact that the large open flowers of barrel cacti do not physically exclude any type of visitor, flowers of both species were pollinated almost exclusively by cactus-specialist bees: 99% (*F. cylindraceus*) and 94% (*F. wislizeni*) of all seeds produced resulted from visits by cactus-specialist bees. For *F. cylindraceus*, the cactus-specialist bee *Diadasia rinconis* was by far the most abundant and effective visitor. For *F. wislizeni*, three cactus-specialist bees (including *D. rinconis*) plus generalists in the family Halictidae each accounted for a quarter of all visits. All three cactus bees acted as pollinators of *F. wislizeni*, whereas the small halictids never pollinated the flowers. *D. rinconis* visits to *F. wislizeni* flowers were more effective (on a per-visit basis) than visits by the other two cactus bees. Apart from the non-pollinating halictids, the flowers were not visited by generalist bees that are commonly found at the same locality and time on other flowers. Pollen-collecting visits and nectar-collecting visits did not differ in their per-visit pollination effectiveness. However, nectar-collecting visits were more abundant than any other visit type and consequently accounted for the majority of seeds produced for both species of cacti.

## Introduction

Recent studies of plant-pollinator interactions have stressed that many more of such interactions may be generalized than previously realized (Herrera 1996, Ollerton 1996, Wilson and Thomson 1996). In particular, it has been found that the visitor assemblages of many plants are taxonomically diverse and spatially and temporally variable (e.g., Herrera 1988, Thompson 1994, Fishbein and Venable 1996). However, in some cases only a tiny minority of a diverse assemblage of flower visitors actually pollinate the flowers (reviewed in Ollerton 1996, Johnson and Steiner 2000). Rigorous studies characterizing the pollinator effectiveness of each member of a visitor assemblage to a plant are necessary to address the question of whether generalized pollination systems do actually predominate, but such studies are labor-intensive and time-consuming, and hence for many angiosperms we have no direct information on the identity and relative importance of their pollinators. With the exception of comparatively well-studied large columnar cacti, for example, we know little of the pollination systems of many Cactaceae, plants that are widespread and ecologically important in arid and semi-arid lands of the New World.

We do know that the flowers of many cacti are visited by bees that specialize on cacti (Simpson and Neff 1987). Bees are a dominant taxonomic group within floral visitors, both in quantity and quality of pollinator service they provide (Proctor et al. 1996, Michener 2000). Most bees are generalized to their nectar sources, visiting many kinds of plants for nectar, but many if not most bees are pollen specialists: the females visit a restricted subset of the plants available for pollen, which they use as food for their larvae, and their preferences appear to be genetically based and unaffected by the

abundance of alternative floral resources available (Müller 1996, Wcislo and Cane 1996).

Pollen-specialist bees might be antagonists to the plants they visit, because they actively collect and sequester pollen for larval provisions, making that pollen unavailable for pollination (Vogel 1978, Harder and Thomson 1989, Westerkamp 1991, Harder and Barclay 1994, Westerkamp 1996). On the other hand, one might predict that pollen-specialist bees should be mutualists, because they might in general be morphologically adapted to the flowers they visit, and may be more abundant and predictable than other visitors within the visitor assemblage of a particular plant (Cane and Payne 1993). They can also be more efficient pollen harvesters than generalist bees (Strickler 1979). Evidence to date on the relative value of specialist versus generalist visitors to flowers has been equivocal (Motten et al. 1981, Neff and Simpson 1990, Keys et al. 1995).

Several recent studies of plant-pollinator interactions have found that characterizing visitors by the floral reward they seek (nectar vs. pollen) may be a more functionally significant way to group visitors than by taxonomic affiliation. In one case, patterns in the distribution of floral visitors among different plants were best explained by the composition of floral rewards offered by the plants (Bosch et al. 1997). In another, Gómez and Zamora (1999) found a functional pattern of plant-pollinator interactions based on the trophic habit (nectarivorous vs. pollinivorous) of the visitors. They postulated that unrelated floral visitors within a trophic guild may often be functionally equivalent in their effect on plant reproductive success.

If pollen-seeking visitors are intrinsically better (or worse) pollinators than nectar-seekers, then the pollen-seeking females of specialist bee taxa may be more (or less) likely to be mutualists of the plants they visit than nectarivores. This effect (if it

exists) should be enhanced by the fixed nature of the floral preferences of specialist bees: the preference is more likely to be consistent in time and space than short-term, density-dependent floral constancy (Cane and Payne 1993). Such an effect would be of interest because the relationship between the degree of a visitor's specialization to its host plant and the location of that visitor along the mutualism – antagonism continuum has been little explored in pollination mutualisms – interactions that are widespread and of great ecological import (Bronstein 1994).

I studied the floral visitor assemblages of two species of barrel cacti: *Ferocactus cylindraceus* and *F. wislizeni*. These plants are functionally outcrossers and have no form of vegetative reproduction, making them completely dependent on floral visitors for their reproduction (Appendix A). To determine the effectiveness of different visitors as pollinators of barrel cactus flowers, I used the single-pollinator-visit method (Motten et al. 1981, Pellmyr and Thompson 1996), in which a single visit is allowed to each observed flower, and the result of that visit (fruit set, seeds per fruit) is recorded. I determined the "quantity" and "quality" components of pollinator effectiveness in this system (Herrera 1987, 1989, Fishbein and Venable 1996), and evaluated their relative importance to the "total realized fecundity" of the plants. I measured fecundity as the total number of seeds produced by each group of visits or visitors (Herrera 1991, Fishbein and Venable 1996, Pellmyr and Thompson 1996). Evaluating pollinator effectiveness in terms of the total effect on fecundity is important because the effect of the "quality" components (e.g., fruit set or seeds per fruit) can easily be overwhelmed by the "quantity" component. For example, a visitor may lead to relatively low fruit set, but be an effective pollinator by virtue of the sheer number of visits. Conversely, a visitor whose visits always result in large numbers of seeds may be of negligible importance if its visits are very rare.

The questions I addressed in this study were: (1) what is the taxonomic composition and relative abundances of floral visitors to *F. cylindraceus* and *F. wislizeni* and how do these vary among years? (2) what is the pollinator effectiveness of different taxa? and (3) are nectar-collecting visits different in quality from pollen-collecting visits? Because of the generalized floral morphology of *Ferocactus* flowers (see Methods), I predicted that the visitor assemblage to these plants would be diverse in composition and variable in time. I also predicted that pollen-specialist bees would act as pollinators, but that they would comprise only a part of the pollinator assemblage. Finally, although I had no *a priori* prediction as to the relative effectiveness of pollen-collecting versus nectar-collecting visits, I predicted that the two visit types would be significantly different in per-visit quality.

## Methods

### *Study organisms*

The genus *Ferocactus* (Cactaceae), comprising 25-30 species (Cota and Wallace 1997), is one of several genera of short-columnar, unbranched cacti commonly called "barrel cacti." *Ferocactus* species occur only in North America, with most species in México. A phylogenetic analysis of the genus based on chloroplast DNA (Cota and Wallace 1997) has shown that *F. wislizeni* and *F. cylindraceus* are sister species. *Ferocactus cylindraceus* flowers in late spring and summer (late April through September in the study population), and *F. wislizeni* flowers in late summer and fall (mid-July through October in the study population). Flowering phenology, breeding

system and reproductive output have been recently studied in both species in the same populations used in this study (Appendices A, B).

In both species strong inbreeding depression limits the number of seeds produced by selfing to ca. 2% of all seeds produced (Appendix A). Pollinator visitation is necessary for fruit set (Appendix A). Unlike some branched cacti such as prickly-pears and chollas, these plants do not reproduce vegetatively. Therefore, pollinator visitation is required for reproduction. Levels of fruit set for open-pollinated flowers in the populations studied range from 93% to 98% for both species, although *F. cylindraceus* plants often lose an additional 20 to 30% of flowers to a florivorous caterpillar (Appendix A). Seeds per fruit for open-pollinated flowers ranged from 14 to 1727 seeds (mean = 575) for *F. cylindraceus*, and from 17 to 3064 seeds (mean = 724) for *F. wislizeni* (Appendix A).

The showy flowers are borne at the apex of the plant and are open for several days. Plants commonly open 2 to 3 new flowers per week (Appendix B) and produce 30 – 40 flowers during a flowering season (range: 1 to 98 flowers for *F. cylindraceus*, 1 to 89 for *F. wislizeni*; Appendix A). The number of flowers produced in a season is strongly and positively correlated with plant size (Appendix A). The flowers of both species are large (diameter 3.8 to 6 cm for *F. cylindraceus* and 4.5 to 6 cm for *F. wislizeni*; Benson 1982). They are bowl- or cup-shaped, but with a short funnel-form floral tube. The stigma is elevated above the anthers, and is often used as a landing site by visitors. The numerous stamens are tightly appressed to the style when the flower first opens, and gradually move away from the style over the 2 to 5 days that the flowers are open. Nectar is secreted near the base of the floral tube, which is recessed within stem tissue.

*Diadasia rinconis* (Hymenoptera: Apidae) is a ground-nesting solitary bee, one of several species of *Diadasia* that specialize on cacti (Ordway 1984, 1987, Neff and Simpson 1992, Sipes and Wolf 2001). Females are distinguishable from males because their abdomen is usually strongly banded, whereas abdomens of males are not. The scopae (pollen brushes) of females are also quite prominent. Its nesting and foraging biology is moderately well-known, and it is active on cactus flowers from late March through September. *Idiomelissodes duplocincta* (Hymenoptera: Apidae) is also a ground-nesting solitary bee, and has been reported to be a specialist on barrel cacti (Zavortink 1975). However, this apparent specialization probably reflects a phenological constraint (*F. wislizeni* is one of the few cacti blooming during this bee's flight period). Males are easily distinguished from females by their long antennae. *Ashmeadiella opuntiae* (Hymenoptera: Megachilidae) is a solitary cavity-nesting bee that is active through spring, summer and fall. It too is a specialist on cactus flowers. Differences between sexes are more subtle in this species, and I was not usually able to distinguish males from females in this species.

### *Methods*

To determine the pollinator effectiveness of different floral visitors, I conducted single-pollinator-visit experiments on *F. cylindraceus* plants at Desert Peak, 45 km NW of Tucson, Arizona (lat 32°36'N, long 111°14'W, 640 m elevation), and on *F. wislizeni* plants at the Santa Rita Experimental Range, 40 km south of Tucson (lat 31°54'N, long 110°53'W, 914 m elevation), in the summers of 1996-1998. I covered the tops of flowering plants with mesh-covered wire baskets to exclude pollinators. The treatment consisted of briefly removing a cover to allow a single pollinator visit to a previously

unvisited flower. I recorded the identity, gender, and behavior of the visitor, marked the visited flower, and replaced the cover. In 1998, I also recorded the duration of the visit with a stopwatch. I later recorded the fate of the flower (formed a fruit, or was aborted), collected fruits and counted seeds.

A particular visitor or taxonomic group of visitors was considered a "pollinator" if any fruits resulted from their visits, and a "non-pollinator" if no fruits ever resulted from a visit. Repeat visits by a particular visitor may result in fruit set, whereas a single visit may not. However, although I primarily recorded single visits, visits to a single flower by multiple visitors sometimes occurred inadvertently. The results of these multiple visits were considered when classifying taxa as pollinators or non-pollinators, although multiple visits were not included in data analyses of pollinator quality.

In this paper, "pollinator effectiveness" refers to the combined effects of "quantity" and "quality" components on realized plant fecundity (total seeds produced). "Quantity" was defined as number of visits recorded. I used two measures of "quality" (per-visit effects on the reproductive fate of a single flower): the probability of fruit set from a single visit, and the mean number of seeds per fruit resulting from a single visit (fruits with zero seeds were excluded). Only measures of female reproductive success were used.

The number of visits observed is not a direct measure of absolute pollinator abundance. My method was to remove a cover and leave it off until a single visitor arrived. The time this took ranged from a few seconds to 15-20 min. If no visitor arrived within 20 minutes, I usually replaced the cover and tried again later the same day or on another day. In addition, I conducted experiments between approximately 9 a.m. and 1 p.m., and hence did not formally record visitors after around 1 p.m. It is however likely that my observations recorded many if not most visitors (see Discussion). The "number

of visits" reported here measure the *relative* abundances of the different floral visitors during the experimental periods. Visitation rates (visits per unit time) were not measured.

Fruit set is percent of visits in a category (taxon, visit type, etc.) that resulted in a fruit. Plants of both species of *Ferocactus* have a very high average fruit set in open-pollinated flowers (Appendix A). Also, plants did not abort more fruits at the beginnings or ends of their flowering periods than in the middle (Appendix B). Therefore, it is reasonable to assume that when a flower was aborted following a single pollinator visit, it was because the visitor failed to deposit pollen that was sufficient in quality and/or quantity to set fruit.

For calculating mean seeds per fruit resulting from a visit, only flowers that set fruit were included (aborted fruits with no seeds were excluded from the dataset). Because the reproductive success or output of individual flowers on the same plant (fruit set and seeds per fruit) are not likely to be independent of each other, data were summarized for each individual plant before analysis, so that plant (not flower) was the experimental unit. The only exceptions were in analyses relating to visit duration, because individual plant should not affect visit duration.

To clarify the relationship between quantity and quality, I compared the percent of all visits in a category to the percent of all fruits matured (which equals number of visits multiplied by mean fruit set), and to the percent of all seeds matured (number of fruits multiplied by mean seeds per fruit). If visits do not differ in quality (in terms of fruit set or seeds per fruit), realized fecundity is completely determined by the number of visits. If fruit set is higher than average in a category, then the percent of all fruits in that category will be greater than the percent of all visits in that category. If mean seeds per fruit is higher than average for that category, then the percent of all seeds will be

greater than the percent of all fruits in that category. Total seeds produced is the product of the preceding three measures, and thus translates the preceding three measures into the "bottom line" impact on the plant's overall female reproductive success.

Three flower visit variables were examined as independent factors: the taxon of the visitor, the gender of the visitor, and the type of visit. Visitors other than the three cactus-specialist bees were identified by sight to genus or tribe, if bees, and to family for other insects. Field identifications were corroborated by collecting and keying out floral visitors both before and during the experiments. However, visitors could not always be identified to the species level (for example, there is currently no key to the estimated 480 North American species of the halictid bee genus *Lasioglossum*). In some cases (e.g., *Augochlorella*), a plausible identification by sight to genus was possible based on my own collection records; in other cases (e.g. bees in the tribe Anthidiini), field expertise did not permit identification to genus.

In the analyses of effects of visits on fruit set and seeds per fruit, only visits by the three cactus-specialist bees were used, because of the small sample sizes for the other visitors. In 1996-1997, all observed visits were recorded. In 1998, visits by *Augochlorella* spp., *Lasioglossum* spp., small flies or other small to tiny bees were not recorded. This was because out of 55 such visits in 1996 to 1997, none resulted in a fruit.

Because of the structure of the flower, pollen-collecting visits were easily distinguished from nectar-gathering visits. Visitors collecting pollen walk along the tops of the stamens, but because the nectar is at the base of the floral tube, nectar-seeking visitors are forced to burrow down through the stamens headfirst until their tongue can reach the nectar. Four visit types were recorded: (1) *Brief visits* were those in which the visitor did not collect any floral rewards. These included split-second landings.

motionless perching, and walking in the flower. (2) *Nectar-collecting visits* were distinguishable from other visits, because the floral nectar is recessed inside the floral tube, and the visitor must burrow down between the stamens to access it. (3) *Pollen-collecting visits* were identifiable by the visitor "swimming" along the tops of the stamens, passing pollen to the scopae. (4) *Nectar- & pollen-collecting visits* were those in which a single visitor collected both nectar and pollen in a single visit. In a few cases visits were not easily categorized and were not analyzed.

#### *Data analyses*

All statistical tests were performed with JMP IN® software (SAS Institute Inc., 1989-98).

To test the hypotheses that visitor taxon, visitor gender, or visit type (independent factors) affected fruit set or seeds per fruit (dependent factors), I first summarized the data in each category for each individual plant as described above. I then tested each independent factor against either fruit set or seeds per fruit, using ANOVAs, or Kruskal-Wallis tests if the variances were unequal. Only data from *D. rinconis* visits were used for tests of effects on seeds per fruit, because sample sizes for the other two cactus bees were too small. For effects of taxon I used data from *F. wislizeni* plants (only one visitor taxon made significant numbers of visits to *F. cylindraceus*). For effects of visitor gender, for *D. rinconis* bees I pooled data across the two plant species, because a separate test of plant species (independent factor) versus fruit set rate (dependent factor) was not significant in *D. rinconis* visits (data not shown). The same held true for plant species versus seeds per plant in *D. rinconis* visits. For visits by male versus female *I. duplocincta* bees, I used data from *F. wislizeni*

plants. Similarly, for tests involving visit type, I used data from both plant species for *D. rinconis* visits, and from *F. wislizeni* only for *I. duplocincta* and *A. opuntiae* visits. In the case of a significant effect, I used a post-hoc Tukey-Kramer HSD tests for multiple comparisons to determine the significantly different categories.

To test the hypothesis that visit duration (independent factor) affects fruit set (dependent factor), I used a nominal logistic regression. To test the hypothesis that visit duration affects seeds per fruit, I used a linear regression test. In these tests the experimental unit was the flower, not the plant. Hence the fruit set data were categorical (abort = Y or N) instead of continuous (fruit set).

## Results

### *Taxonomic composition, relative abundances, and pollinator effectiveness of floral visitors*

For both species of *Ferocactus*, the only taxa whose visits resulted in fruit set were the three cactus bees (*D. rinconis*, *I. duplocincta* and *A. opuntiae*), plus two other bees: *Megachile* spp. and *Halictus* spp. (Fig. 1; Table 1). Visits by small halictids (*Augochlorella* spp.,  $n = 41$ , and *Lasioglossum* spp.,  $n = 14$ ), the non-native honeybee (*Apis mellifera*  $n = 5$ ), and bees of the tribe Anthidiini ( $n = 2$ ), never resulted in fruit set (Fig. 1). For *Augochlorella* species, thirty-five single visits, one double visit by two *Augochlorella* individuals, and five visits by both *Augochlorella* species and *Lasioglossum* species were recorded; none resulted in fruit set. For *Lasioglossum* species, nine single visits and five visits with *Augochlorella* spp. were recorded; again,

no fruit set resulted. Nor did visits by flies (Diptera:  $n = 10$ ) or butterflies (Lepidoptera:  $n = 1$ ) result in fruit set (Fig. 1).

For *F. cylindraceus*, the majority (72 to 88%) of all visits were made by the three cactus bees (Table 1). Among these, visits by *D. rinconis* were by far the most abundant. Other pollinating visitors accounted for 1 to 3% of visits recorded, and non-pollinating visitors made up 11 to 25% of all visits (Table 1). For *F. wislizeni*, 65 to 80% of visits were made by cactus bees (Table 1). Other pollinating visitors accounted for 3% of visits, and non-pollinating visitors made up 20 to 32% of all visits (Table 1).

For the three cactus bees alone, on *F. cylindraceus* 88 to 96% of cactus bee visits were by *D. rinconis*, 4 to 11% by *I. duplocincta*, and 0 to 7% by *A. opuntiae* (Table 2). On *F. wislizeni*, in 1996 visits by *I. duplocincta* were the most abundant (64%), whereas in 1997 and 1998, visits were almost exactly evenly divided among the three cactus bees: *D. rinconis* 34 to 39%, *I. duplocincta* 31 to 32%, and *A. opuntiae* 30 to 34% (Table 2).

Visits by *D. rinconis* to *F. wislizeni* resulted in significantly higher mean fruit set than visits by either *I. duplocincta* or *A. opuntiae* (Fig. 2a). Visits by *D. rinconis* and *A. opuntiae* resulted in significantly more seeds per fruit than visits by *I. duplocincta* (Fig. 2b). Although visits to *F. cylindraceus* by *I. duplocincta* and *A. opuntiae* were too few to allow comparison among the three cactus bees on this plant species, for *D. rinconis*, fruit set rates were similar between the two plant species (50% *F. cylindraceus*, 47% *F. wislizeni*). Mean seeds per fruit per *D. rinconis* visit were fewer on *F. cylindraceus* flowers (157) than on *F. wislizeni* (243; Table 3), probably because *F. cylindraceus* plants habitually produce fewer seeds per fruit than *F. wislizeni* plants (Appendix A).

Visit abundance (quantity) and per-visit pollination effectiveness (quality) can differ in the strength of their effect on total fecundity (percent of all seeds produced by observed visits). Differences among the three cactus bees in visit abundance had a much

greater effect on *F. cylindraceus* fecundity than did differences in per-visit effectiveness (Fig. 3a, Table 3). In contrast, on *F. wislizeni*, visit abundances were almost exactly equal among the three cactus bees (Fig. 3b, white bars), but the greater per-visit effectiveness of *D. rinconis* visits, in terms of both fruit set and seeds per fruit, had a large impact on fecundity (Fig. 3b). Thus, although visits by *D. rinconis* accounted for only 33% of all visits to *F. wislizeni*, they led to 79% of all seeds resulting from visits by the three cactus bees (Table 3).

#### *Visit type, visitor gender, and visit duration*

There were no significant differences in fruit set among the four visit types (brief, nectar, pollen, nectar&pollen) for any of the three cactus bees (*A. opuntiae* on *F. wislizeni*,  $P = 0.3546$ ; *I. duplocincta* on *F. wislizeni*,  $P = 0.5311$ ; *D. rinconis* on both plants species pooled,  $P = 0.4852$ ). For *D. rinconis*, the only one of the three cactus bees whose foraging produced enough fruits with seeds to test differences among the visit types, seeds per fruit too did not differ significantly among visit types ( $P = 0.4709$ , both plant species pooled). Nectar visits were the most abundant visit type for all three cactus bees, and also resulted in the most total seeds (Fig. 4).

Overall, visits by female bees were more abundant than visits by male bees, for both *D. rinconis* and *I. duplocincta* (Table 4). Female to male ratios ranged from 2.5:1 to 3.5:1 for *D. rinconis*, and from 2.2:1 to 3:1 for *I. duplocincta*. There was considerable among-year variation in these proportions. On *F. cylindraceus*, in 1996-1997 only male *I. duplocincta* were observed, whereas in 1998, only female *I. duplocincta* appeared. On *F. wislizeni*, female:male ratios for *D. rinconis* were 0.56 to 1 in 1997, but 14:1 in 1998.

Also on *F. wislizeni*, female and male *I. duplocincta* visits were about equal in 1996 and 1997, but the female:male visit ratio was 4.5 to 1 in 1998.

There were no differences in per-visit pollinator effectiveness between visits by female bees and male bees, for either *D. rinconis* (fruit set:  $P = 0.9911$ , seeds per fruit:  $P = 0.5430$ ; both plant species pooled) or *I. duplocincta* (fruit set on *F. wislizeni*:  $P = 0.3912$ ). However, because visits by female bees were more abundant, female visits were responsible for a higher proportion of total seeds produced (Fig. 5).

Visit duration was positively correlated with fruit set for *D. rinconis* on *F. cylindraceus* (logistic regression, chi-sq = 4.558, df = 1,  $P = 0.0328$ ,  $n = 97$  visits), but not on *F. wislizeni* (data not shown). Visit duration was not correlated with fruit set for either of the other two cactus bees on *F. wislizeni* (data not shown), nor was visit duration correlated with mean seeds per fruit for any of the three cactus bees (data not shown).

## Discussion

This study provides a counter-example to the prevailing idea that plant-pollinator systems are mostly generalized. The visitor assemblages of both species of *Ferocactus* were dominated by three species of cactus-specialist bees (a functional group of relatively unrelated bees), and these bees were virtually the only pollinators of these plants. Pollen-collecting visits by these bees were equally effective in pollinating the flowers as nectar-collecting visits, thus illustrating that, despite the predictions of some bee biologists (Harder and Barclay 1994, Westerkamp 1996) the pollen-collecting visits of pollen-specialist bees are not necessarily antagonistic to the plants they visit.

*Taxonomic composition of the visitor assemblages*

These plants produce brightly-colored, open flowers with accessible pollen and nectar. The large size of the flowers permit visitation by visitors of a wide range of body sizes, and the actinomorphic shape means that floral visitors do not need to assume a particular orientation or position to access the floral rewards. Because of this generalized floral morphology, and because diverse and variable visitor assemblages are thought to be the rule rather than the exception (Herrera 1987, Thompson 1994, Ollerton 1996), I predicted that the visitor assemblage to these plants would be diverse in composition and variable in time. Instead, the surprising result was that the visitor faunas of both plants were dominated by cactus specialists, which were reliably present in all years. Of all visits recorded in 1996-1997, 83% (*F. cylindraceus*) and 68% (*F. wislizeni*) were by cactus bees.

Previous work characterizing floral visitors of cacti suggest that some cacti may have more diverse assemblages than those observed in this study. The Catalog of Hymenoptera (Krombein et al. 1979) lists many more bee taxa as visitors to prickly-pear and cholla cacti (*Opuntia* spp.: 88 bee species) than to barrel cacti (13 bee species), but these numbers undoubtedly reflect various sampling biases. Mandujano et al. (1996) found that 89% of all visits recorded to *Opuntia rastrera* were by cactus-specialist bees; however, Johnson (1992) found that only 30% of all visits to *Echinomastus* were by cactus bees. Other pollination studies of cacti have not reported visit numbers broken down by taxa. Several studies have found *Megachile* spp. to be abundant or important visitors to cacti (Spears 1987, Osborn et al. 1988, Johnson 1992, Mandujano et al. 1996), although other studies did not (Parfitt and Pickett 1980, Breckenridge and Miller 1982, McFarland et al. 1989).

Very few visits were made by the introduced honeybee (*Apis mellifera*; Table 1) and none by the generalist bees *Bombus* and *Xylocopa* both of which are common in the desert. Honeybees are major visitors to *Prosopis*, *Cercidium*, and *Carnegiea* flowering at the same time and place as *F. cylindraceus* (pers. obs.). The apparent avoidance of cactus flowers by honeybees is in agreement with several other studies (Beutelspacher 1971, Breckenridge and Miller 1982, Schmidt and Buchmann 1986, Osborn et al. 1988, McFarland et al. 1989). Other studies have described honeybees, *Bombus* and *Xylocopa* (all generalists) as important pollinators of cacti (Grant and Grant 1979a, Grant et al. 1979, Spears 1987, Lockwood 1995), but data were not presented.

If some generalist bees actually do avoid the flowers of certain cacti, it could be related to pollen chemical or nutritional characteristics. The chemistry of cactus pollens has not been investigated. The presence or absence of starch in cactus pollen varies among species (Franchi et al. 1996, Roulston and Buchmann 2000), and has not been characterized for *Ferocactus* pollen. In a recent survey of pollen protein content of 377 plant species (Roulston et al. 2000), *Opuntia* (Cactaceae) pollen was found to be relatively low in protein content (22%), although Cactaceae was one of only two families surveyed that showed substantial variability in protein concentration among species. Protein content of *Ferocactus* pollen was not investigated.

Schmidt and Buchmann (1986) found that only very small amounts of *Opuntia* (prickly-pear and cholla) pollen were collected by honeybee colonies, although 24% of their pollen intake during the same period was *Carnegiea* (saguaro) pollen, suggesting that saguaro pollen is much more acceptable to honeybees than *Opuntia* pollen. If pollen odor screens out certain visitors, cactus flowers that are morphologically generalized could be functionally specialized for some visitors. Although studies have shown that specialist bees use pollen odor as a factor in recognizing their pollen host plants

(reviewed in Dobson and Bergström 2000), the role of pollen odor as a possible selective sieve (*sensu* Thompson 1994) for floral visitors has yet to be experimentally addressed. Pollen nutrition has come under recent scrutiny as a possible correlate of visitor taxa, but thus far no significant correlations have been found (Roulston and Buchmann 2000, Roulston and Cane 2000).

That these flowers were almost unvisited by generalist species of *Apis*, *Bombus* and *Xylocopa* highlights the fact that just because floral rewards are accessible does not necessarily mean that they are desirable to generalists. If the flowers or pollen of these plants do in fact possess qualities that repel many potential visitors, it would demonstrate that there is more to restricting (specializing on) floral visitors than hiding the rewards from view or making them difficult to collect.

#### *Pollinator effectiveness of floral visitors*

I predicted that pollen-specialist bees would act as pollinators, but that they would comprise only a part of the pollinator assemblage. The second part of this prediction was not supported: the cactus-specialist bees were even more dominant among the pollinating visitors than they were among the visitors in general. Of visits that resulted in a mature fruit, 99% (*F. cylindraceus*) and 94% (*F. wislizeni*) were by cactus bees. Other visitors were either effective pollinators but rare (*Megachile* and *Halictus* spp.), or were relatively common but never pollinated the flowers (small halictids such as *Augochlorella* spp.) (Table 1). Whether the non-pollinators were actually antagonistic in their interaction with *Ferocactus* depends on the degree to which their removal of nectar and pollen negatively affected visitation by the mutualists. In light of the very small body size of the small halictids relative to the flower size and

the large volume of pollen produced in *Ferocactus* flowers, it is most likely that they act as commensals.

Previous work on the pollination biology of *Ferocactus* species is limited to an observational paper by Grant & Grant (Grant and Grant 1979a) that found that *Lithurge*, *Diadasia* and *Megachile* species visit *F. wislizeni* near Superior, Arizona, and an observational study by Simpson and Neff (Simpson and Neff 1987) that found that *Lithurge echinocacti* and *Perdita echinocacti* were abundant visitors to *F. wislizeni* near the Silverbell Mountains in Arizona. Other work on the pollination of cacti with large, open-bowl flowers, has similarly been limited to observing visitors, and identifying the putative pollinators based on body size, the presence and purity of pollen on the body, or behavior such as touching the stigma (Grant et al. 1979, Grant and Grant 1979a, b, Grant and Hurd 1979, Parfitt and Pickett 1980, Breckenridge and Miller 1982, Leuck and Miller 1982, Simpson and Neff 1987, Spears 1987, Osborn et al. 1988, McFarland et al. 1989, Johnson 1992, Schlindwein 1992, Lockwood 1995, Schlindwein and Wittmann 1995, Mandujano et al. 1996, Schlindwein and Wittmann 1997).

Previous studies comparing the pollination effectiveness of specialist versus generalist bees on a single plant species have found that there was no difference (Motten et al. 1981, Neff and Simpson 1990, Keys et al. 1995). It should be emphasized that in this study, the most important component of the pollinator effectiveness of the cactus bees was their abundance, not their per-visit effectiveness. Herrera (1991) has pointed out that relative measures of plant reproductive success (such as fruit set and mean seeds per fruit) may not be as good predictors of variation in fecundity as absolute measures -- in this case, number of visits. Herrera (1991) and Pellmyr and Thompson (1996) use total realized fecundity as the best summary of which pollinators are the most important for the overall female reproductive success of the plant.

To what extent is the importance of the cactus bees as pollinators of *Ferocactus* related to their specialization on cactus pollen? Specialists might be better quality pollinators than generalists if they possess morphological or behavioral traits that suit them to the flower. This is not the case in this system, because the generalized morphology of *Ferocactus* flowers require no particular traits to access the floral rewards. It is likely that the body size and amount of vestiture of, e.g., *D. rinconis* – traits that are not related to their status as cactus specialists – contribute significantly to their per-visit effectiveness. If the small, relatively hairless halictids observed in this study were specialists on cacti, for example, it is likely that they would still be ineffective pollinators.

In terms of quantity, the abundance of a particular visitor on the flowers of a particular plant is the product of the visitor's absolute abundance and its degree of floral constancy (Cane and Payne 1993). For both specialists and generalists, absolute abundance varies, but whereas the floral constancy of a generalist to a particular plant is also variable, the floral constancy of a specialist is fixed (Cane and Payne 1993, Weislo and Cane 1996). Therefore, the relative abundance of cactus-specialist bees on the flowers of *Ferocactus* is likely linked to their status as pollen specialists.

The relative abundances of the cactus bees observed in this study did not vary greatly among years (Table 1). This, in conjunction with the fact that so few (pollinating) generalists visited *Ferocactus* flowers suggests that, if the specialists were to disappear, these plants might experience reduced fruit set and seed production, unless a lack of other floral resources occurred at the same time and forced some of the generalists to switch to *Ferocactus*. Studies of floral visitors to *Ferocactus* in other localities are needed to determine if the dominance of cactus-specialist bees observed here in these two populations is prevalent elsewhere.

All three cactus bees were found to be capable of pollinating the flowers of both species of *Ferocactus*. For *F. cylindraceus*, virtually the only pollinator was *D. rinconis*, primarily because of its abundance (Fig. 3a). For *F. wislizeni*, visits were equally divided among the three cactus bees in quantity, but the higher quality of *D. rinconis* visits made this bee the main pollinator for this plant also (Fig. 3b). It is likely that *I. duplocincta* is actually a better pollinator than indicated in this study, because of the way my methodology interacted with their behavior. Unlike *D. rinconis* and *A. opuntiae* bees, which collect pollen while alighted on the flower, *I. duplocincta* females usually transfer pollen from the body to the scopae in flight. This made it difficult to accurately identify pollen-collecting visits. Further, both sexes of *I. duplocincta* usually make numerous brief landings on a flower. To standardize my results across different taxa, I allowed only one landing per bee. If I had allowed a "complete encounter," including all landings made by a single bee to a single flower from the time the bee first arrived at the plant until it left, it is likely that the estimate of the pollinator quality of *I. duplocincta* would be higher than reported here.

#### *Pollen-collecting visits versus nectar-collecting visits*

I predicted that pollen-collecting visits would be significantly different in per-visit quality than nectar-collecting visits. This was not the case: there were no significant differences between pollen-collecting visits and nectar-collecting visits. Because these cacti were primarily pollinated by pollen-specialist bees, one might ask, why should cacti make nectar? It would seem wasteful in a water-limited environment to produce nectar if pollen-collecting visits provide sufficient pollination service. The fact that nectar visits were more abundant than any other visit type, for all three cactus

bees, however, suggests that the costs of producing nectar might be offset by the gain in quantity of pollinator visits.

Previous studies examining the benefits to the plant of nectar visits versus pollen visits have reached contrasting conclusions. Free (1993 p. 25) asserts that pollen-collecting bees (in particular, honeybees) are more likely to pollinate flowers than nectar-gatherers. However, others have claimed that pollen-foraging honey bees are likely to be antagonists because they meticulously groom pollen from their bodies, either discarding it or placing it in their corbiculae (pollen baskets) after mixing it with nectar to form it into a solid lump (Westerkamp 1991). Pollen thus treated is unlikely to leave the corbicula (pollen basket, a smooth concave surface) during floral visits. Many solitary bees, in contrast, including the cactus bees observed in this study, carry pollen “dry” (Thorp 2000) in their scopae (pollen brushes composed of branched hairs, structures quite different from pollen baskets), and this pollen may be more likely to leave the bee and pollinate receptive stigmas during subsequent floral visits. However, Jacob-Remacle (1989) found for honeybees that although nectar visits were more abundant, pollen visits were more likely to effect pollination. He found that for a species of *Andrena*, a solitary bee, pollen visits were more abundant and more likely to pollinate flowers than nectar visits. Wilson and Thomson (1991) found that nectar-collecting *Bombus* were better pollinators than pollen-collecting *Apis* on jewelweed, and Gómez and Zamora (1999) found nectar-gatherers of many different species were better pollinators than many pollen-collectors. Conner et al. (1995) found that nectar-feeding butterflies had a higher pollination efficiency on *Raphanus* than nectar- and pollen-feeding honeybees.

It is likely that the differential effects of nectar- versus pollen-gathering on a plant will be determined more by that plant's floral morphology, and how that interacts

with the morphology and behavior of the visitor, than by the reward sought *per se*. In many cases flower morphology forces nectar-gatherers to assume a particular position on the flower, and this position is optimal for (passively) picking up and transferring pollen (Harder and Wilson 1998). In such cases available pollen may be produced in minute quantities, because precise placement on the visitor body may make pollen use more efficient. In these situations, one would expect to find that nectar-gatherers are better pollinators, because if (as is usual) pollen is located in a different part of the flower than nectar, then pollen-collecting visitors may assume the "wrong" position on the flower. For example, the pollen-collecting honeybees studied by Wilson and Thomson (1991) hung upside down in the flower while collecting pollen, whereas the nectar-collecting bumblebees entered the flower the "right" (right side up versus upside down) way.

Males of both species visit the flowers for nectar; males of *D. rinconis* (and possibly *I. duplocincta*, although this is unknown) also patrol the flowers for unmated females. Several authors have postulated that male *D. rinconis* are or should be better pollinators than females, because they use the stigma as a landing platform when they alight, and because they may travel more often between plants in search of females than females do when foraging (Grant and Hurd 1979, Simpson and Neff 1987, Johnson 1992). Johnson (1992) found that male *D. rinconis* visits were much more abundant than female visits to an *Echinomastus* at Organ Pipe Cactus National Monument. The results reported here differ: female *D. rinconis* visits were more abundant than male visits, on both species of plant, although proportions varied among years. I found no significant differences in pollinator quality between male and female bees of *D. rinconis*. Once again, quantity is the more important of the two components of pollinator effectiveness.

The limitations of this study are as follows. The single-pollinator visit method used here characterizes the effect of a visitor on only female reproductive success of plants, and is not informative about how different visitors contribute to male reproductive success of plants. Also, this method does not take into account the fact that pollinator effectiveness of a particular visitor can be conditional, depending on the behavior, effectiveness, and abundance of other visitors (Thompson and Pellmyr 1992, Thomson and Thomson 1992, Arizmendi et al. 1996). This method does, however, provide a common basis for comparing diverse visitors, and because it is commonly used, it facilitates comparisons among different studies. Using actual seeds produced is preferable to counting pollen grains on the stigma because only conspecific, viable, and (possibly) outcross pollen will actually result in seeds. Thus, this measure is relevant to the actual fecundity of the plant (Pellmyr and Thompson 1996). Furthermore, because these plants have a very large number of ovules per ovary (up to 3,000; Appendix A), the number of seeds per fruit resulting from a single visit is a relatively good measure of the pollen load delivered (Herrera 1987, Gómez 2000).

I did not directly measure the abundance or visitation rates of different taxa, and I usually recorded visits only between 9 a.m. and 1 p.m. This limited observation period probably sampled the vast majority of floral visitors, however. For example, in a study of *Opuntia rastrera* in México, Mandujano et al. (1996) found that 81% of all insect visits to flowers occurred between 9 a.m. and 2 p.m. In a study of *Opuntia imbricata* in Colorado, McFarland et al. (1989) did not observe bees visiting the plants either before 9 a.m. or after 3 p.m. Finally, in a study of visitors to *Larrea*, Minckley et al. (1999), species accumulation curves revealed that a morning to midday census yielded more than 85 to 95% of all taxa represented in an all day census. For *F. cylindraceus*, I conducted experiments only during the first peak of bloom (April through June), and did

not record visitors during the remainder of the blooming period (July through September). Observations of these plants at other times and during other experiments suggest that I did not, however, exclude any important pollinators from this study.

Only one population of each plant species was studied, and it is likely that the visitor assemblages and pollinator assemblages of these plants are geographically variable. Because the experimental flowers were covered until visited, they undoubtedly had levels of nectar and pollen that were substantially greater than those normally encountered by flower visitors. This may have had a significant effect on for example visit duration (Neff and Simpson 1990); however, it should have affected all visitors equally. Finally, the effects of different groups or types of visits on the post-dispersal success of plant offspring, effects which can potentially swamp pre-dispersal effects, were not measured (Gómez 2000, Herrera 2000). A holistic approach that evaluates the overall effect of visit type or visitor group on the lifetime reproductive success of the seeds resulting from those visits is obviously preferable to a reductionist examination of slight differences between the effects of visitors or visit types on fruit set or seed set (Zamora 2000).

### *Conclusions*

The most striking result of this study is that despite their generalized morphology, the flowers of both species of barrel cacti are predominantly visited by, and almost exclusively pollinated by, a handful of pollen-specialist bees. The lesson for pollination biologists is that, just as apparently specialized flowers may be visited and pollinated by a more diverse assemblage than one would assume from their appearance (Fishbein and Venable 1996), apparently generalized flowers may be visited and

pollinated by a much more restricted group than would be expected. The almost complete absence from the visitor assemblage of common generalists such as species of *Apis*, *Bombus* and *Xylocopa*, suggests that despite the apparently accessible pollen and nectar of these flowers, they may possess a hidden mechanism for deterring many floral visitors. Perhaps these plants have managed to evolve an effective "selective sieve" for screening out certain visitors as postulated by Thompson (1994). Further study is needed to reveal whether such a sieve actually exists in these flowers, and to determine its nature.

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*Table 1.* Composition of floral visitor taxa, by number of visits (relative abundance), 1996-1998. "Non-pollinating" taxa are those whose visits never resulted in a fruit being set (see Methods). Visits by some non-pollinating taxa were not recorded in 1998 (indicated by "--"). Within category, taxa are arranged in order of decreasing frequency. Differences in total visits recorded among years / plant species reflect differences in sampling effort, not visitation rates. "F.cyl" = *F. cylindraceus*. "F.wis" = *F. wislizeni*.

Floral Visitors	F.cyl 1996	F.cyl 1997	F.cyl 1998	F.wis 1996	F.wis 1997	F.wis 1998
Total visits recorded:	<i>n</i> = 36	<i>n</i> = 91	<i>n</i> = 127	<i>n</i> = 35	<i>n</i> = 144	<i>n</i> = 192
Cactus-specialist bees						
<i>D. rinconis</i>	25	70	113	2	36	61
<i>I. duplocincta</i>	1	4	14	18	29	58
<i>A. opuntiae</i>	0	6	0	8	28	62
Total cactus bees	72% (26)	88% (80)	100% (127)	80% (28)	65% (93)	94% (181)
Other pollinators						
<i>Megachile</i> sp.	1	0	0	0	3	6
<i>Halictus</i> sp.	0	0	0	0	2	0
unidentified large bees	0	1	0	0	0	0
Total other pollinators	3% (1)	1% (1)	0	0	3% (5)	3% (6)
Non-pollinating bees						
<i>Augochlorella</i> spp.	1	0	--	4	36	--
<i>Lastiglossum</i> spp.	0	2	--	3	9	--
Anthidiinae	2	0	0	0	0	0
<i>Apis mellifera</i>	0	1	0	0	0	4
unidentified large bees	0	2	0	0	0	1
unidentified small bees	0	1	--	0	0	--
Total non-pollinating bees	8% (3)	7% (6)	0	20% (7)	31% (45)	3% (5)
Other non-pollinating visitors						
Bombyliidae (Diptera)	6	0	0	0	0	--
Syrphidae (Diptera)	0	4	0	0	0	--
Pieridae (Lepidoptera)	0	0	0	0	1	--
Total other non-pollinators	17% (6)	4% (4)	0	0	<1% (1)	--

Table 2. Variation in the relative abundances of the cactus bees across years, 1996-1998. "F.cyl" = *F. cylindraceus*. "F.wis" = *F. wislizeni*.

	F.cyl '96 <i>n</i> = 26	F.cyl '97 <i>n</i> = 80	F.cyl '98 <i>n</i> = 127	F.wis '96 <i>n</i> = 28	F.wis '97 <i>n</i> = 93	F.wis '98 <i>n</i> = 181
<i>D. rinconis</i>	96%	88%	89%	7%	39%	34%
<i>I. duplocincta</i>	4%	5%	11%	64%	31%	32%
<i>A. opuntiae</i>	0	7%	0	29%	30%	34%

Table 3. Summary statistics for cactus bee taxa. (a) *F. cylindraceus*; (b) *F. wislizeni*.

For fruit set and seeds per fruit, mean  $\pm$  SE are shown; data were summarized for each individual plant before means were calculated. For fruit set and seeds per fruit,

numbers in parentheses are numbers of plants. Number of fruits (NF) = number of visits (NV) \* mean fruit set per visit (FS). Total seeds produced (TS, or realized fecundity) = NF \* S/F (seeds per fruit).

(a) *F. cylindraceus*

	NV # visits (%)	FS fruit set per visit	NF # fruits (%)	S/F seeds/fruit	TS total seeds (% of all)
<i>D. rinconis</i>	155 (88%)	0.50 $\pm$ 0.39 (17)	60 (90%)	157 $\pm$ 125 (13)	8394 (92%)
<i>I. duplo</i>	16 (9%)	0.11 $\pm$ 0.33 (9)	3 (4%)	66 (1)	197 (2%)
<i>A. opuntiae</i>	6 (3%)	0.72 $\pm$ 0.26 (3)	4 (6%)	140 $\pm$ 94 (3)	510 (6%)

(b) *F. wislizeni*

	# visits (%)	fruit set per visit	# fruits (%)	seeds/fruit	total seeds (% of all)
<i>D. rinconis</i>	78 (33%)	0.47 $\pm$ 0.27 (9)	31 (51%)	243 $\pm$ 167 (9)	6909 (79%)
<i>I. duplo</i>	86 (36%)	0.27 $\pm$ 0.36 (11)	17 (28%)	46 $\pm$ 36 (6)	743 (9%)
<i>A. opuntiae</i>	75 (31%)	0.12 $\pm$ 0.18 (12)	13 (21%)	119 $\pm$ 136 (6)	1065 (12%)

*Table 4.* Relative abundances of the genders of cactus bees (1996-1998 pooled).

Percentages are percent of visits for that taxon. Numbers in parentheses are numbers of visits.

	<i>F. cylindraceus</i>	<i>F. wislizeni</i>
Female <i>D. rinconis</i>	78% (153)	71% (70)
Male <i>D. rinconis</i>	22% (44)	29% (29)
Female <i>I. duplocincta</i>	74% (14)	69% (70)
Male <i>I. duplocincta</i>	26% (5)	31% (32)

## Figures

*Figure 1.* Relative abundances and pollinator effectiveness of taxa visiting *Ferocactus* flowers. A: visits to *F. cylindraceus*, 1997. B: visits to *F. wislizeni*, 1997. D.rinc = *D. rinconis*. I.duplo = *I. duplocincta*. A.opun = *A. opuntiae*. unid.lg.bee = unidentified large bees. Lasio. = *Lasioglossum* sp. (Halictidae). Apis = *Apis mellifera* (Apidae). unid.sm.bee = unidentified small bee. Mega. = *Megachile* sp. (Megachilidae). Halict. = *Halictus* sp. (Halictidae). Augo. = *Augochlorella* sp. (Halictidae). Pier. = Pieridae (Lepidoptera).

*Figure 2.* Per-visit pollinator effectiveness of the three cactus bees. A: Mean fruit set per visit; B: Mean seeds per fruit per visit. Shown are means  $\pm$  SE. Statistics are from Kruskal-Wallis test. Bars with different letters were significantly different in a post-hoc Tukey-Kramer HSD test of multiple comparisons. A. opun. = *A. opuntiae*. D. rinc. = *D. rinconis*. I. duplo. = *I. duplocincta*.

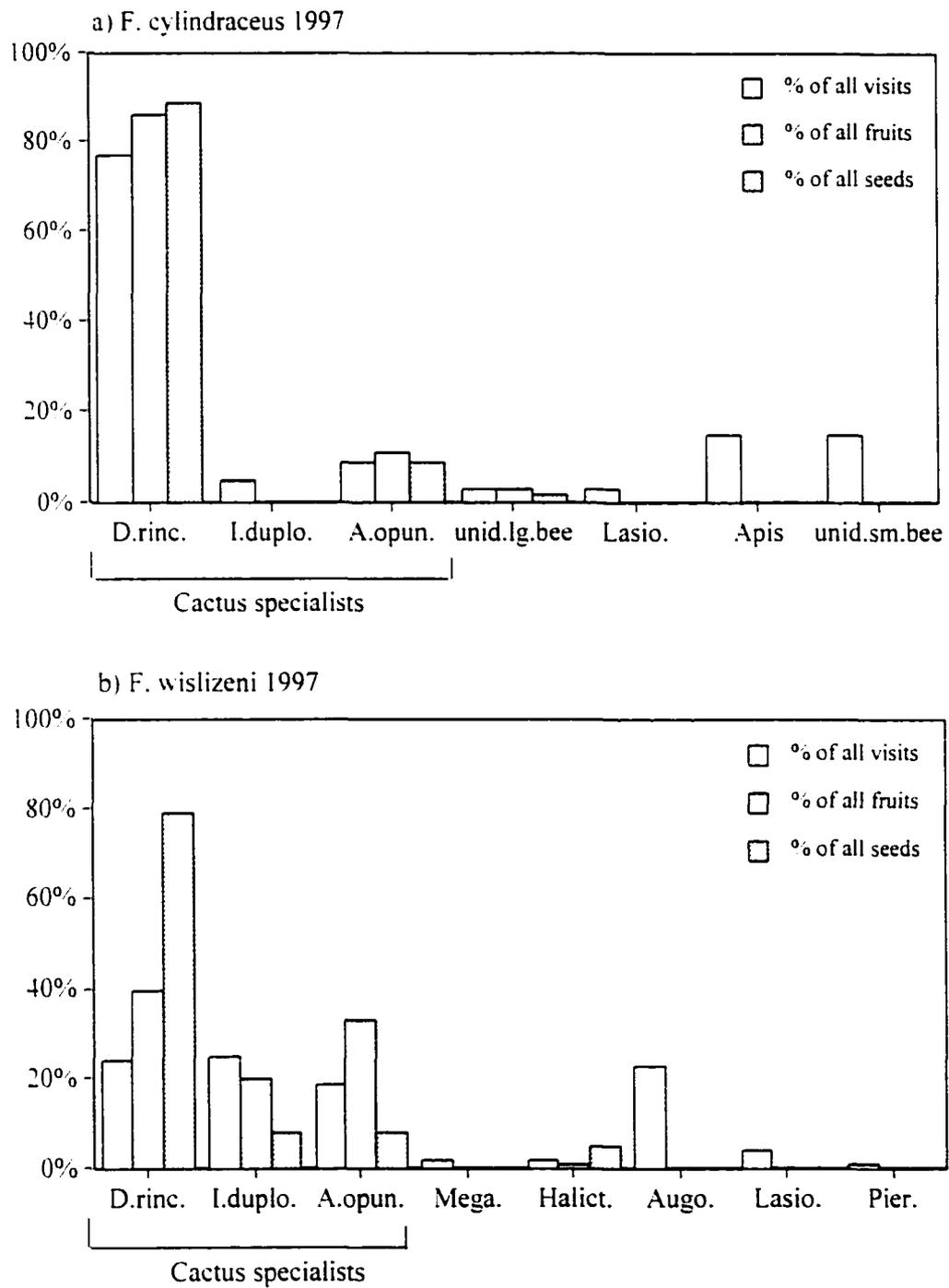
*Figure 3.* Relative abundances and pollinator effectiveness of three cactus bees. A: *F. cylindraceus*; B: *F. wislizeni*. D.rinc. = *D. rinconis*. I.duplo. = *I. duplocincta*. A.opun. = *A. opuntiae*.

*Figure 4.* Relative abundances and pollination effectiveness of different visit types. A: *D. rinconis* visits to *F. cylindraceus* and *F. wislizeni*; B: *I. duplocincta* visits to *F. wislizeni*; C: *A. opuntiae* visits to *F. wislizeni*. Brief = visits during which no floral reward was collected; Nectar = nectar-collecting visits; Pollen = pollen-collecting visits; N&P = visits during which both nectar and pollen were collected.

*Figure 5.* Relative abundances and pollinator effectiveness of different genders of bees.

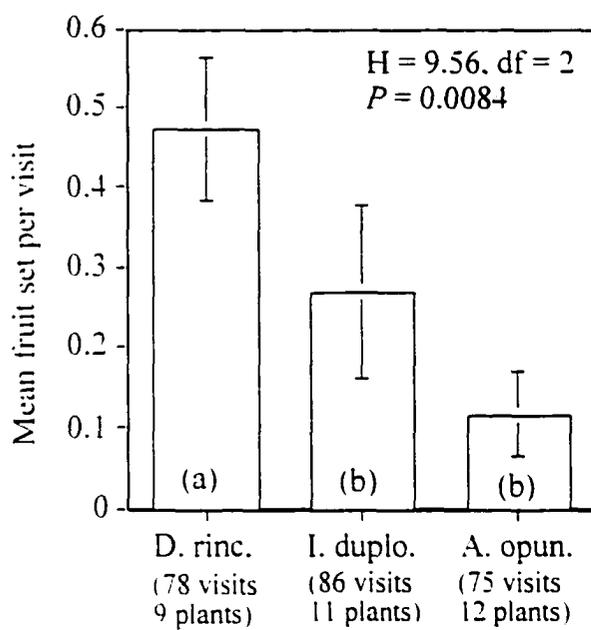
A: *D. rinconis*; B: *I. duplocincta*.

Figure 1.



**Figure 2.**

## a) Fruit Set



## b) Seeds per fruit

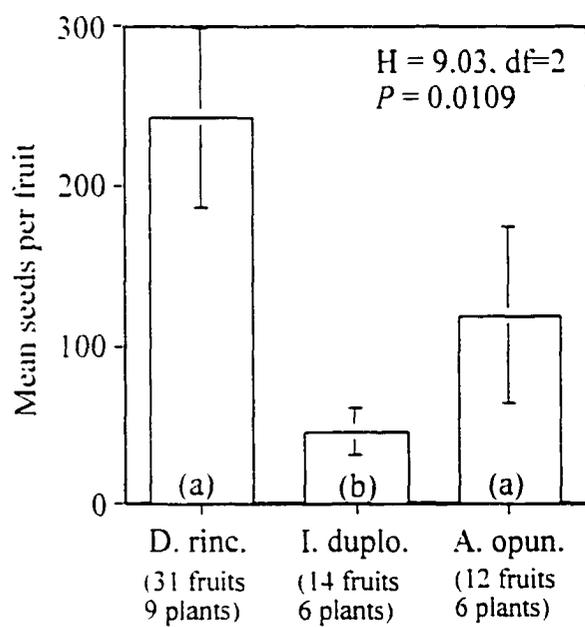


Figure 3.

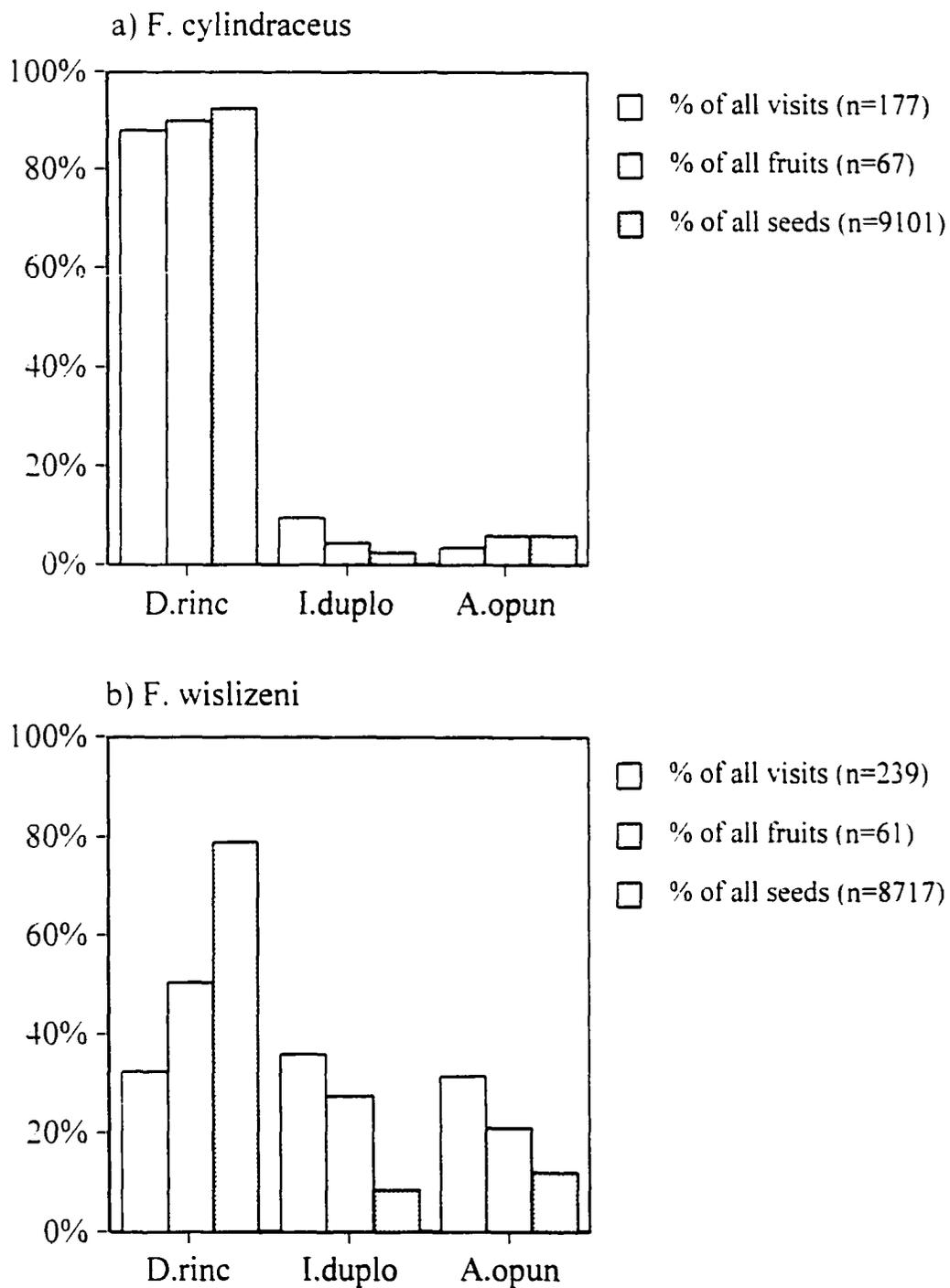


Figure 4.

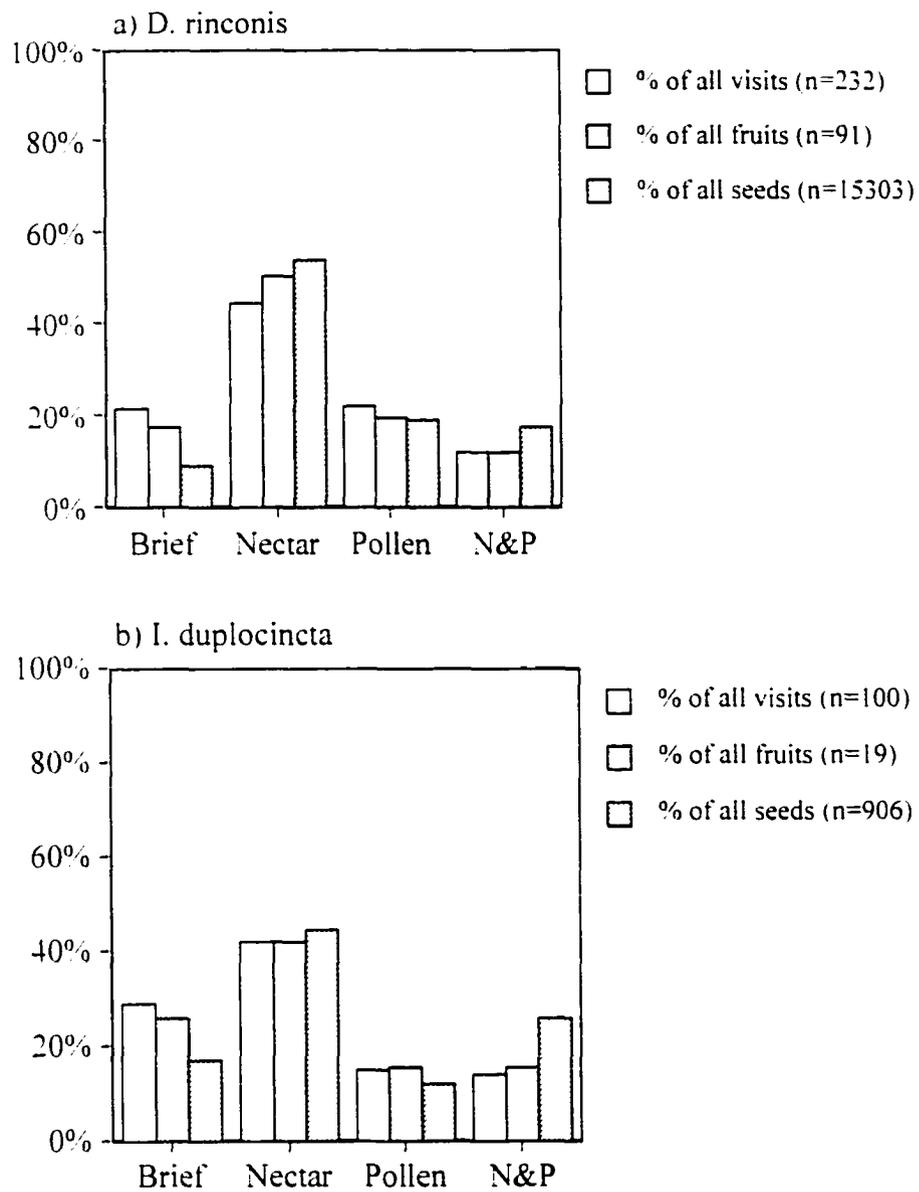


Figure 4. continued

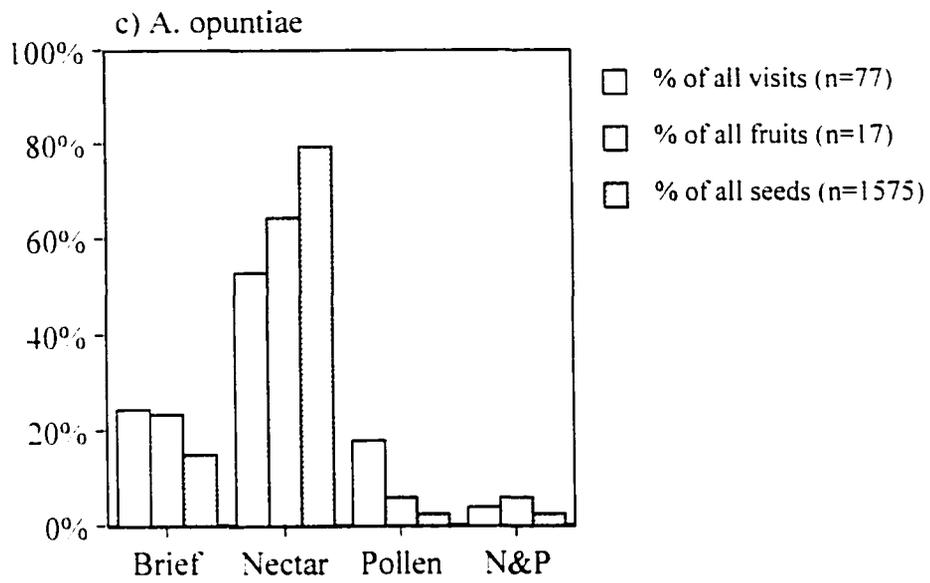
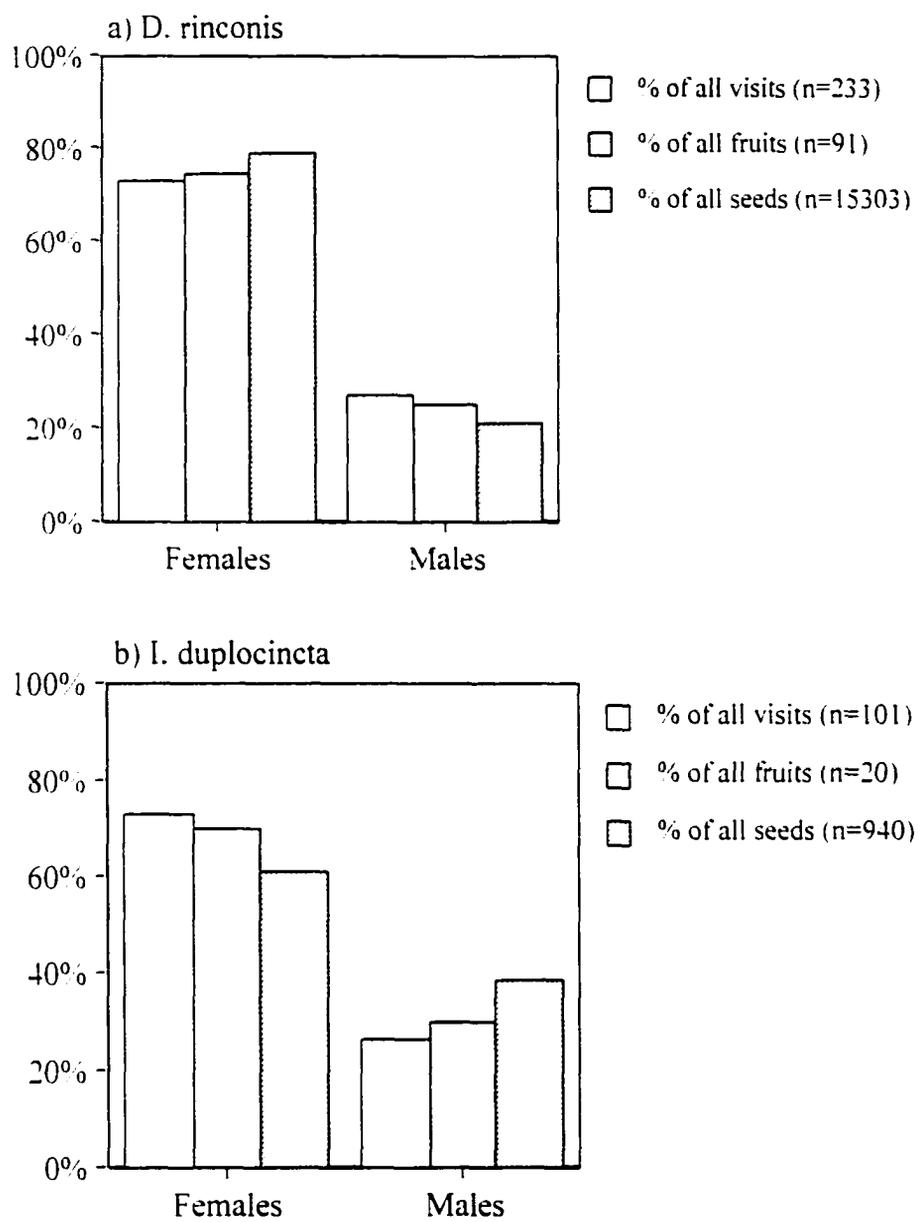


Figure 5.



**APPENDIX D**  
**POLLEN PREFERENCES OF FOUR SOLITARY,**  
**CACTUS-SPECIALIST BEES**  
**FORAGING FOR LARVAL PROVISIONS**

Pollen preferences of four solitary, cactus-specialist bees  
foraging for larval provisions

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## Abstract

Empirical studies of the host ranges of herbivorous insects provide important insights into the forces that shape species interactions. In particular, investigating the host range of different insect herbivores that specialize on the same plants can reveal much about the products of convergent evolution, and can also suggest the future trajectories of the evolutionary process. Like many other herbivorous insects, bees tend to specialize on their host plants, particularly those used as a source of pollen for larval provisions. Several relatively unrelated bee species have converged on using cactus pollen for larval provisions. I tested the pollen preferences of four species of solitary, cactus-specialist bees in and near Tucson, Arizona, in 1999 and 2000. Bees were offered cactus flowers in which the stamens had been removed and replaced with novel pollens, in a choice assay that always included unmanipulated cactus flowers. *Diadasia rinconis*, *Lithurge apicalis*, and *Idiomelissodes duplocincta* were tested in a free-flying setting, and marked individuals of *D. rinconis* and *D. opuntiae* were tested in a flight cage. Naive, newly emerged *D. rinconis* females were also given a no-choice test in a flight cage. Non-host cactus pollens were accepted to some degree by all four species, although the two species of *Diadasia* accepted more non-cactus pollens than did either *L. apicalis* or *I. duplocincta*. Both species of *Diadasia* showed significant acceptance of *Sphaeralcea* (Malvaceae) pollen, a host plant used by many other species of *Diadasia*. Caged, naive *D. rinconis* bees did not initiate nesting when presented with *Sphaeralcea* flowers alone; nesting began immediately when *Opuntia* (Cactaceae) flowers were offered. However, once nesting was underway, six of seven bees switched readily to *Sphaeralcea* when cactus flowers were removed. In both *D. rinconis* and *D. opuntiae*, there were striking differences in degree of pollen specificity and in pollen preferences among caged individuals. These results highlight differences among bees that have

converged on specializing on the same resource, and suggest that host shifts to non-cactus hosts might be especially likely in cactus-specialist *Diadasia*.

## **Introduction**

Delineating the host range of herbivorous insects is a challenging but essential first step in unraveling the evolution of plant-insect interactions. Although host specialization is thought to be widespread in many groups of herbivorous insects (Futuyma 1983, Futuyma and Moreno 1988, Jaenike 1990, Bernays and Wcislo 1994), the nature and extent of such specialization must be documented before we can begin to address the baffling question of why specialization is so prevalent. Further, examining the host range of different insects that have converged on the same host plants can provide clues to the mechanisms of host recognition and acceptance (Futuyma 1983). Finally, variation in host specificity among individuals within a population of herbivorous insects can indicate the scope for further evolutionary change (e.g., host shifts: Via 1990, Futuyma et al. 1995).

Bees are numerically abundant and play a dominant ecological role worldwide in the pollination of flowering plants. Although bees are well-studied as pollinators, however, they are seldom studied as herbivores. The pollen host ranges of bees are a potentially important but little-known research subject in the study of plant-insect interactions.

Female bees actively collect pollen and nectar for larval provisions. In solitary bees, the pollen and nectar are mixed into a provision mass which is placed in an individual cell and on which the female lays an egg. The cell is then sealed, and the larvae have no opportunity to select an alternate host. Thus, pollen- and nectar-

collecting in bees is the functional analogue of oviposition in other herbivorous insects: it is during this behavior that the adult female chooses the host plant(s) for her larvae.

Paralleling patterns found in other groups of herbivorous insects, specialization is common in bees: most bees collect pollen from only a few closely related plant species (Müller 1996, Wcislo and Cane 1996). Exceptions to this pattern are social bees (roughly 10% of the 16,325 species of bees worldwide: Michener 2000), which are pollen generalists. (Both social and solitary bees are nectar generalists.)

A common and intriguing pattern seen in bees (and in other herbivorous insects: Futuyma and Moreno 1988) is that closely related species may specialize on plants from two or more families that do not appear to share many similarities and that are not closely related. This pattern may signal the existence of hidden similarities between the plant families: for instance, in other herbivorous insects it has been found in some cases that such plants may have convergently evolved chemical similarities (Bernays and Chapman 1994).

One such group of plant families used by closely related bees is Cactaceae, Malvaceae, and Convolvulaceae. Use of pollen from plants in both Cactaceae and Malvaceae is found in a lineage of twenty species of *Perdita* (Andrenidae: Danforth 1991). Use of pollen from plants in both Cactaceae and Convolvulaceae is found in two closely related species of *Ancyloscelis* (Apidae: Alves-Dos-Santos and Wittmann 1999). Use of pollen from plants in all three families is found both in the genus *Lithurge* (Mitchell 1938, Houston 1971, Parker and Potter 1973, Brach 1978, Roberts 1978, Michener 1983, 1988, O'Toole and Raw 1991, Camillo et al. 1994, Schlindwein 1998), and in the genus *Diadasia* (Linsley and MacSwain, 1957, 1958; Sipes and Wolf 2001).

These three plant families are from different orders (Cactaceae: Caryophyllales, Malvaceae: Malvales, Convolvulaceae: Solanales; Judd et al. 1999), and so the

“hidden” similarities are not derived from a shared ancestor. Host shifts might be facilitated by factors intrinsic to the plants, such as floral morphology, pollen odor, pollen nutritional content, and pollen grain size. Ecological factors may also come into play: for example, representatives from these three families may tend to occur in the same localities or habitats and to bloom at the same time. Many have noted that plants specialized on by bees tend to have reliable abundant flowers that offer large quantities of accessible pollen and nectar (the “predictable plethora” syndrome, as defined by Weislo and Cane 1996). Such plants attract richly diverse guilds of bees, both generalist and specialist.

By testing the preferences of bees for different pollens, while holding extrinsic factors constant, we may gain clues as to the relative importance of intrinsic versus extrinsic factors in the pollen host choice of bees. The exclusive use of cactus pollen has arisen independently in several bee lineages (Table 1). I used a combination of open-air and flight-cage choice tests to explore the pollen specificity of four species of cactus-specialist bees that occur near Tucson, Arizona: *Diadasia rinconis* and *D. opuntiae* (Apidae), *Idiomelissodes duplocincta* (Apidae), and *Lithurge apicalis* (Megachilidae). I placed novel pollen into emasculated cactus flowers, and recorded pollen-collecting visits (and other visits) to treated and control flowers. I also conducted a no-choice test on naive bees. I focused on short-range host acceptance, rather than on long-range host finding.

It can be difficult to persuade foraging bees to sample flowers they might normally ignore, especially if the bees are not enclosed. By placing novel pollen into cactus flowers, I promoted contact between cactus bees and non-cactus pollens, albeit in a situation they would never encounter in nature. This technique is also useful for decoupling pollen-specific cues from other floral cues, making it likely that different

responses to different pollens are related to the pollen itself, not other floral characteristics.

The goals of this study were to determine whether non-cactus pollens are acceptable to cactus bees, whether all cactus pollens are equally acceptable to cactus bees, and to search for behavioral variation in pollen specificity within populations of each species of bee.

If the appearance or odor of the flower (apart from the pollen itself) is what triggers host recognition, we would expect that cactus bees would collect any pollen found in a cactus flower. If acceptance takes place at the level of the pollen itself, we would expect to see non-host pollens rejected, even if they are found in a cactus flower. If pollen characteristics only affect pollen-foraging bees, we would expect similar responses to control and treated flowers by bees that are not collecting pollen. Finally, if adult experience leads to induction of preference (via learning, imprinting, etc.), we would expect naive bees to be less selective than experienced bees.

## **Methods**

### *Study Sites and Organisms*

Experiments took place at three sites in or near Tucson, Arizona: 1) in central Tucson at a 470 m<sup>2</sup> vacant lot in May 1999 and May 2000 (lat 32°14'N, long 110°56'W, 720 m elevation); 2) at the Santa Rita Experimental Range (a semidesert grassland research preserve, lat 31°52'N, long 110°50'W, 1110 m elevation, 40km south of Tucson) in June 1999; and 3) at an urban park on the southeastern outskirts of Tucson in

August 1999 and 2000 (Lincoln Park near the Rincon unit of the Saguaro National Park: lat 32°11'N, long 110°49'W).

I tested the pollen specificity of four species of cactus bees (Table 1). *Diadasia rinconis* and *D. opuntiae* (Hymenoptera: Apidae: Emphorini) are solitary ground-nesting bees. *D. opuntiae* is active in spring, *D. rinconis* in spring and summer. *Diadasia* is a New World genus with ca. 30-40 species, most of which are pollen specialists on plants in the Malvaceae (Sipes and Wolf 2001). *Lithurge apicalis* (Hymenoptera: Megachilidae: Lithurgini), active in the spring, is a solitary wood-nesting bee. *Lithurge* is a world-wide genus with ~50 species. In the New World, all species of *Lithurge* are cactus specialists. *Idiomelissodes duplocincta* (Hymenoptera: Apidae: Eucerini) is a ground-nesting solitary bee active in the summer and early fall. It has been described as a specialist on the pollen of barrel cacti (*Ferocactus*) (Zavortink 1975, Bouseman 1977). The genera *Diadasia*, *Lithurge* and *Idiomelissodes* are not closely related (Roig-Alsina and Michener 1993), indicating that the use of cacti has arisen independently in each lineage.

### *Experiments*

I tested the pollen specificity of cactus bees by offering them cactus flowers in which the cactus pollen had been removed and replaced with other kinds of pollen. Trials were performed on free-flying bees in the field (*D. rinconis*, *L. apicalis*, and *I. duplocincta*), and in a field-based flight cage with marked individuals (*D. opuntiae* and *D. rinconis*). Bees were offered a multiple choice array that always included fresh pollen of their preferred host species of Cactaceae as one of the choices. For the bees that are active in spring, *D. opuntiae*, *D. rinconis* and *L. apicalis*, the host cactus was

the common prickly pear *Opuntia engelmannii*. For the late summer bee *I. duplocincta*, the host cactus was the barrel cactus *Ferocactus wislizeni*. In both the flight cage and the free-flying settings, experiments took place in the morning and early afternoon, for periods ranging from 1/2 to 3 h, usually between 0900 to 1300.

In all experiments (Table 2) I presented bees with 3 types of flowers: 1) control: a fresh flower of the preferred host cactus; 2) "damaged" control: a fresh host flower in which the stamens had been cut out with scissors and then replaced in the flower (to control for the effect of cutting the stamens); 3) novel pollen: a host flower in which the stamens had been cut out and removed, and replaced with novel pollen. It was not usually possible to find three flowers of similar age equally distant from each other and oriented such that all three could be observed simultaneously on a single plant. Hence, all three experimental flowers were removed from their original plant and (in the free-flying experiments) positioned on another nearby host plant, or (caged experiments) randomly placed in an array on the top of a small table. In order to present flowers that had not been previously visited by bees, experimental flowers were either removed from the original plant before they had opened (*Ferocactus*), or visitors were excluded by wrapping unopened buds with tissue paper (*Opuntia*). In the free-flying experiments, the three flowers were placed on a host plant in a triangular configuration. For the flight-cage experiments on marked individual bees (Table 2), several flowers of each treatment were presented, and more than one kind of novel pollen was offered at a time. I also provided caged bees with emasculated *O. engelmannii* flowers for supplemental nectar.

In all of the experiments, I tallied pollen-collecting visits to control and treated flowers, to test whether non-cactus pollens would be acceptable, and to test whether there were pollen preferences among different cacti. I also recorded all other landings

on the flowers, i.e., nectar-collecting visits and visits in which neither pollen nor nectar was collected.

In the free-flying experiments, the number and identities of individual foraging bees could not be known. For visits by free-flying *Diadasia* bees, I calculated the minimum number of individuals observed during a daily experiment, based on the number of pollen-collecting visits observed per unit time during the experiment. The calculation employed three sources of information: my own flight cage observations, observations of free-flying marked bees in the Tucson plot, and published records of *D. rinconis* provisioning behavior (Neff and Simpson 1992). In using the data from caged *D. rinconis* bees, for each 5 min period of observation, I noted how many bees were pollen-foraging, and how many total pollen visits were made during that period (Table 3a). From these data I created a rough extrapolation table (Table 3b). In some cases I observed several bees foraging on the experimental flowers simultaneously, which allowed me to increase the number of individuals observed above that suggested by the extrapolation table. The estimated number of individuals (Table 3b) is conservative, because it assumes that each individual bee is visiting only the three experimental flowers, returning to them repeatedly, and not visiting any other flowers. Although individual bees did tend to return repeatedly to the same flower (pers. obs.), in the 470 m<sup>2</sup> lot there were over 200 *D. rinconis* nests, of which as many as 170 were active (with a nesting female digging and/or provisioning cells, as opposed to closed or abandoned nests) on any one day (unpub. data).

Experiment 1 (testing the pollen preferences of free-flying *D. rinconis* bees) took place from 3 to 21 May 1999 and from 21 to 25 April 2000 in the urban vacant lot. In 1999, experimental flowers were observed for 60 to 120 min (usually 90 min) and all visits were recorded. The minimum number of individual bees observed per day ranged

from one to eight. In 2000, experimental flowers were observed until 21 pollen-collecting visits were recorded: there was usually more than one trial per day. The protocol was changed from a set time period to a set number of visits to facilitate comparison among treatments. Twenty-one visits was selected as a rough minimum for detecting preferences. The minimum number of individuals observed during a trial in 2000 ranged from two to five.

Experiment 2 (testing the pollen preferences of free-flying *L. apicalis* bees) took place from 7 to 21 May 1999 in the urban Tucson vacant lot. Experimental flowers were observed for 60 to 120 min (usually 90min) and all visits were recorded.

Experiment 3 (testing the pollen choices of free-flying *L. duplocincta* bees) took place from 17 to 26 Aug 1999 and on 15 Aug and 19 Aug 2000 in Lincoln Park. Trials took place in four different locations (sub-sites) within the park, separated by at least 200 m, to increase the likelihood of testing different individuals. I also marked 48 individual *L. duplocincta* bees at the sub-site that was in the center of the park, and observed flowers over the next five days at the other three sub-sites. Only one marked individual was observed once at a sub-site other than the central marking sub-site, indicating that the sub-sites were sufficiently distant that observations were nearly always on different individuals. Hence, sub-sites were used as replicates. Experimental flowers were observed until 21 pollen-collecting visits were recorded: there was usually more than one trial per sub-site per day.

Experiment 4 (testing the pollen preferences of caged *D. opuntiae* bees) took place at the Santa Rita Experimental Range from 3 to 10 June 1999. While nesting females were quiescent in their nests in early morning, I placed a 1.83 m x 1.83 m x 1.83 m flight cage over a group of existing *D. opuntiae* nests. Five experienced *D. opuntiae* nesting females were thus caged, and after three days they resumed

provisioning their nests with pollen. In all caged experiments, bees were marked for identification with enamel paint. Caged experiments were used because the behavior of different individuals of the same species, even from the same population, can be significantly divergent, and hence data from free-flying insects can mask this behavioral diversity.

Experiment 5 (testing the pollen preferences of caged *D. rinconis* bees) took place at the vacant lot in urban Tucson from 30 April to 5 May 2000. Seven naive newly-emerged female bees were captured in emergence cages and placed in a flight cage, along with males for mating. After mating (which takes place immediately after emergence; *D. rinconis* females mate only once; Ordway 1984, 1987), the males were removed. Following the no-choice experiment described below, choice trials were performed. Three sets of treatments were presented, and each set was presented on two days. For each individual bee, if the bee foraged on both days on which a particular set of treatments were presented, day was the replicate. Otherwise, there was no replicate. Experiment 6 (no-choice tests on caged *D. rinconis* bees) took place before and after experiment 5 (above) using the same bees. In this experiment, only one kind of flower (either the normal host cactus, or *Sphaeralcea*: Malvaceae) was available for pollen.

#### *Pollens used*

I used novel pollens from a variety of sources (Table 4). Because the goal of these experiments was to test pollen preferences of cactus bees, I attempted to include pollens with a range of grain sizes (because it has been suggested that cactus bees prefer large pollen grains: Houston 1971, Schlindwein 1998), pollens from plants blooming in the same locality and at the same time as the cacti (biologically realistic choices for host

shifts), and pollens from plant families used as host plants by bees that are closely related to cactus bees (e.g., Malvaceae and Convolvulaceae). I also had access to and used a variety of previously frozen pure pollen samples. I estimated the size of the pollen grains used in the study by measuring the diameter of ten grains and averaging them. Pollen samples were prepared in glycerin with a fuschin stain, and examined using a compound microscope.

Nearly all pollens used in these experiments are documented to be collected by some bees at some time; even the wind-pollinated *Zea* and *Ephedra* are occasionally collected by honey bees (Buchmann et al. 1989). *Typha* is not known to be collected by bees, but is used as a supplemental food source for commercial honey bee colonies (Schmidt et al. 1989). To test whether cactus bees prefer pollen from some cacti to that of other cacti, I also used several different cactus pollens. Flowers of *Opuntia* and *Ferocactus* are visited primarily by cactus-specialist bees (pers. obs.: Appendix C), although they appear to be avoided by honeybees (Appendix C). *Carnegiea* (saguaro) flowers, on the other hand, are visited by bats, insects including honeybees, and birds. The difference in pollinator assemblages suggests that cactus bees might find the pollen of *Opuntia* and *Ferocactus* flower more acceptable than that of *Carnegiea* flowers (although *Diadasia rinconis* has been recorded as visiting *Carnegiea* flowers; McGregor et al. 1959).

Cactus flowers generally have numerous stamens bearing large quantities of pollen (*Opuntia engelmannii*, mean of 577 stamens per flower,  $n = 5$ ; *Ferocactus wislizeni* stamens are more numerous: pers. obs.). Because the amount of pollen in a flower may influence foraging preferences, I aimed to replace the cactus pollen with an equal amount of novel pollen. However, purifying pollen by separating it from plant parts is a time-consuming task. For this reason I often used hand-collected anthers,

stamens, staminal columns, or sometimes whole flowers or inflorescences, instead of pure pollen, for the novel pollen treatments (Table 4). These were also sometimes refrigerated (for up to six weeks) or frozen (up to two yr) in glass vials. To determine the effects of freezing pollen on bee foraging behavior, previously frozen host cactus pollen was also offered in some trials. Pollens are referred to as "host cactus pollen," "novel cactus pollen," and "novel non-cactus pollen."

### *Data analysis*

For each experiment, I first tested whether pollen-collecting visits to the control flower(s) were significantly different in frequency than pollen-collecting visits to the "damaged control" flower(s), using a G-test, with the null expectation that pollen-collecting visits would be equal to control and damaged control. If the pollen-collecting visits were not different from the null expectation, I pooled "control" and "damaged control" treatments, and tested the novel pollen treatments against the pooled control. For example, if one control, one damaged control, and one novel pollen flowers were offered, the expected proportion of pollen-collecting visits was 66% to (pooled) control, 33% to novel.

To test the null hypothesis that all pollens would be equally acceptable, I compared actual numbers of pollen-collecting visits made to the different treatments against the expected numbers of pollen-collecting visits, using a univariate categorical chi-square test. Although this approach can demonstrate clear differences among treatments, it is recognized that the data include variable numbers of visits from variable numbers of individuals. This null hypothesis does not allow one to statistically distinguish between two situations that are biologically quite different: cases where the

novel pollen was accepted, but at lower levels than the control pollen, and cases in which no pollen-collecting visits at all were made to the novel pollen. In some cases, because the number of flowers offered varied across treatments, and because bee foraging behavior can be influenced by the relative abundances of different flowers, the expected numbers were weighted by numbers of flowers.

In experiment 4, a different set of treatments was offered on nearly every day. For this reason, results could not be pooled across days. In addition, in almost all cases the numbers of pollen-collecting visits per bee per day did not meet the sample-size requirements of the chi-square test (with two categories, the smallest expected value should be five or more; with more than two categories, smallest expected value should be 1 or more, and no more than 20% of the expected values should be less than five; SAS Institute Inc. 1989-99b). Because the pollen preferences of individual bees were so different, pooling data across bees would have created misleading results. Therefore no statistical tests were performed for experiment 4.

All statistical tests were performed with JMP IN® software (SAS Institute Inc., 1989-99a).

## Results

**Experiment 1:** Do free-flying experienced *D. rinconis* bees prefer some pollens to others?

In both years, numbers of pollen-collecting visits to control and damaged control flowers were not significantly different, so these were pooled in further tests (1999: 411 visits, 54% control, 46% damaged control;  $P = 0.1524$ , G-test; 2000: 146 visits, 46% control, 54% damaged control;  $P = 0.3204$ , G-test). In both years, because I was

essentially testing the same group of bees across days, trials in which the same treatments were offered were pooled across days.

In 1999 no pollen-collecting visits were made to *Zea* pollen (Figure 1c). Significantly fewer visits than expected were made to flowers with the pollen of previously frozen host cactus pollen (*Opuntia engelmannii* stamens, Fig. 1a), previously frozen *Ferocactus* pollen (novel cactus pollen, Fig. 1b), fresh *Prosopis* anthers (Fig. 1e) and fresh *Argemone* anthers (Fig. 1d). Pollen-collecting visits were not significantly fewer than expected to *Sphaeralcea* (Fig. 1f), indicating that this pollen was as acceptable as the control pollen. In 2000 no pollen-collecting visits were made to *Cucurbita* (Fig. 1k), *Argemone* (Fig. 1l), or *Ipomoea* (Fig. 1n). Significantly fewer visits than expected were made to the pollen of *Helianthus* and *Kallstroemia* (Fig. 1i and 1j). Pollen-collecting visits were not significantly fewer than expected to the novel cactus pollens of *Carnegiea* and *Ferocactus* (Fig. 1g and 1h), and to the novel non-cactus pollen of *Sphaeralcea* (Fig. 1m).

**Experiment 2:** Do free-flying experienced *L. apicalis* bees prefer some pollens to others?

Numbers of pollen-collecting visits to control and damaged control flowers were not significantly different, so these were pooled in further tests (87 visits, 54% control, 46% damaged control:  $P = 0.4527$ , G-test). Previously frozen host cactus pollen (*Opuntia engelmannii* stamens) was accepted, but not as frequently as fresh (control) host pollen (Fig. 2a). Previously frozen *Ferocactus* pollen (novel cactus pollen) was also accepted at about the same level as host pollen (12% *Opuntia*, 11% *Ferocactus*; Fig. 2b). All of the novel pollens (*Sphaeralcea*, *Argemone*, and *Prosopis* Fig. 2c-e) were rejected (pollen-collecting visits were significantly different than expected for

*Sphaeralcea* and *Argemone*, the only two treatments with enough pollen-collecting visits to be tested statistically).

**Experiment 3:** Do free-flying experienced *I. duplocincta* bees prefer some pollens to others?

In both years, numbers of pollen-collecting visits to control and damaged control flowers at each site within the park were not significantly different, so these were pooled in further tests (1999: site 1, 192 visits,  $P = 0.0602$ ; site 2, 176 visits,  $P = 0.4509$ ; site 3, 177 visits,  $P = 0.3283$ ; site 4, 169 visits,  $P = 0.1434$ ; 2000: site 1, 21 visits,  $P = 0.2729$ ; site 2, 21 visits,  $P = 0.2729$ ; G-tests).

Among the cactus pollen treatments, pollen-collecting visits were made to previously frozen pure *Opuntia* pollen (Fig. 3a) and to previously frozen *Carnegiea* anthers (Fig. 3c), although the number of pollen-collecting visits was significantly lower than expected. No pollen-collecting visits at all were made to previously frozen *Opuntia* stamens (Fig. 3b), indicating that the inclusion of previously frozen plant parts could have a deterrent effect, or that this pollen might have lost its odor. Among the novel pollens, including four previously frozen and five fresh pollens, only *Kallstroemia* was accepted (Fig. 3j), albeit at very low levels (2.5% of pollen-collecting visits). In every case the number of pollen-collecting visits was significantly lower than expected; i.e., no treatment pollens, whether cactus or non-cactus, were as acceptable as control (fresh host cactus) pollen.

Four pollens rejected by *I. duplocincta* were accepted to some degree by generalist halictids (*Augochlorella*) foraging at the same time: *Sphaeralcea*, *Argemone*, *Prosopis* and *Helianthus* (Table 5). The occurrence of pollen-collecting visits to these

treatments by this generalist bee clearly indicates that these pollens were not intrinsically unacceptable to bees.

**Experiment 4:** Do caged, experienced *D. opuntiae* bees prefer some pollens to others?

All five bees visited control and damaged control flowers in proportions not significantly different from 50% to each (Table 6). Small sample sizes (see Methods) precluded further statistical tests. Individual bees showed striking differences in pollen preference (Figure 4). "Red" bee accepted only fresh host cactus (control) pollen. "Copper" bee accepted previously frozen host cactus pollen, in addition to control pollen, but no novel pollens. "Yellow" bee accepted novel cactus pollen (*Ferocactus*) and previously frozen host cactus pollen. "White" bee accepted *Sphaeralcea* pollen and previously frozen host cactus pollen. "Blue" bee preferred *Carnegiea* (novel cactus) flowers to fresh host cactus flowers, and also accepted previously frozen host cactus pollen, *Sphaeralcea*, and *Helianthus*. The *Carnegiea* flowers were rejected by the other 4 bees.

**Experiment 5:** Do caged, experienced *D. rinconis* bees prefer some pollens to others?

Four of the seven bees showed a statistically significant preference for either control or damaged control flowers (Table 7). Hence, pollen-collecting visits to control and damaged control flowers were not pooled. As with *D. opuntiae*, individuals behaved quite differently (Figure 5). "RW," "White," and "WY" bees accepted only fresh host cactus pollen. "GW" made 1 visit to *Sphaeralcea* and "Red" made 1 visit to *Prosopis*. "Blue" accepted *Ferocactus*, *Carnegiea*, *Sphaeralcea*, and *Kallstroemia*, but to lesser degrees than host cactus pollen. "Yellow" accepted *Ferocactus*, *Carnegiea*, and *Sphaeralcea* at levels significantly greater than the host cactus pollen. The only

case in which pollen-collecting visits to treatment pollens were not significantly different than expected, i.e., in which the novel pollen was as acceptable as the control pollen, were *Carnegiea*, *Ferocactus* and *Sphaeralcea* for "Blue" bee.

**Experiment 6:** Do naive, caged *D. rinconis* bees exhibit innate preferences among different pollen-host flowers?

Two newly-emerged females were caged on 20 April, and five more were added on 25 April (Table 8). During this period they were offered large bunches of fresh *Sphaeralcea* flowers as a pollen source. Flowering potted *Scabiosa* plants were also available as a supplemental nectar source (these flowers bear minute amounts of pollen), and the bees readily accepted them for nectar. They also nectared on the *Sphaeralcea* flowers, but no nests were initiated. On 28 April, after the first two bees had been in the cage for 8 days, and the other five for 3 days, the *Sphaeralcea* flowers were removed and replaced with fresh *Opuntia engelmannii* flowers (host cactus). Four bees began digging nests immediately, and by the second day (29 April), all seven had dug nests. Pollen-foraging (to provision cells) began on 30 April. During 30 April through 5 May, the choice experiments (Experiment 5) took place.

On 6 May, after the choice experiments were complete, I removed the *Opuntia* flowers and presented them with *Sphaeralcea* again. Three of the seven immediately began collecting pollen from *Sphaeralcea*, and three others accepted *Sphaeralcea* the next day. One bee, RW, never switched to *Sphaeralcea*. This bee had been one of the most conservative in the choice test (experiment 5; Figure 5). On 9 May, I continued to offer *Sphaeralcea*, but added one *Opuntia* flower to the cage. All 7 bees immediately switched to collecting pollen from the *Opuntia* flower.

## Discussion

Although insect herbivores are generally antagonists of their host plants, most bees are thought to be mutualists of the plants they visit (see Appendix C for the importance of cactus bees as pollinators of cacti). Comparing host selection in pollen-foraging bees to host selection in other herbivorous insects thus provides a useful contrast, one that may suggest which aspects of plant-insect interactions are specific to whether the interaction is mutualistic or antagonistic.

This study documents the pollen preferences of several species of cactus bees, and the results suggest that all four are indeed strongly specialized to the pollen of cactus flowers. The genera *Diadasia*, *Lithurge* and *Idiomelissodes* are only distantly related (Roig-Alsina and Michener 1993), hence the use of cactus pollen has evolved independently in these three lineages. The fact that both species of *Diadasia* tested showed significant acceptance of *Sphaeralcea* pollen, which was however rejected by *L. apicalis* and *I. duplocincta*, suggests that although all four species specialize on cactus pollen, their independent evolutionary histories may have led them to use different cues to recognize cactus pollen. *Sphaeralcea* is believed to be the ancestral host plant for the genus *Diadasia* (Sipes and Wolf 2001), and it may be that the cactus-specialist *Diadasia* use a chemical cue (for example) that is shared by cacti and mallows. The level of behavioral variability in pollen preferences demonstrated for *D. rinconis* and *D. opuntiae* in the caged experiments suggests that these bees may possess the potential for future host shifts.

Individuals of both species of *Diadasia* accepted more non-cactus pollens than did the individuals of either *L. apicalis* or *I. duplocincta*. Of the three cactus bee species, *Lithurge apicalis* was the only one to accept only cactus pollens; however, this

bee was tested on only three novel pollens (Experiment 2). The only novel non-cactus pollen accepted by *Idiomelissodes duplocincta* was *Kallstroemia grandiflora*, which blooms at the same time and place as their host cactus *Ferocactus* (but pollen-collecting visits to *Kallstroemia* represented only 2.5% of all pollen-collecting visits; Experiment 3). Fresh pollens from other non-cactus plants blooming at the same time and place as the experiments (*Acacia*, *Caesalpinia*) were rejected by *I. duplocincta*. In addition, many of the novel non-cactus pollens tested were in the form of previously frozen anthers or stamens (*Sphaeralcea*, *Argemone*, *Prosopis*). *I. duplocincta* bees rejected previously frozen *Opuntia* stamens with pollen while accepting previously frozen pure *Opuntia* pollen, suggesting that pollens that include previously frozen plant parts may be less acceptable to this bee than to the other species tested.

There were very low levels of acceptance by *Diadasia* of *Prosopis*, *Argemone*, and *Kallstroemia*, but many other novel non-cactus pollens were rejected entirely (Experiments 1, 4 and 5). For both species of *Diadasia*, the novel non-cactus pollen chosen most frequently was *Sphaeralcea*. Although individuals varied widely in their acceptance of *Sphaeralcea*, at least some individuals of both species found *Sphaeralcea* pollen equally acceptable as host cactus pollen. In contrast, *Sphaeralcea* was rejected by both *L. apicalis* (fresh stamens) and *I. duplocincta* (previously frozen stamens).

Pollen of the host cactus was preferred to novel cactus pollens by almost all individuals of all four cactus bee species (Table 9). However, in all four species there was some level of acceptance for all novel cactus pollens tested.

With regard to the third question, whether or not there is behavioral variation within populations of each species that might allow host shifts onto non-cactus hosts, firm conclusions are not warranted due to small sample sizes. However, for both species of *Diadasia*, even with a small number of individuals, major differences were found

among them, and there was a large degree of acceptance of *Sphaeralcea* pollen. If this variation in preference is genetically based, it could be acted upon by natural selection, leading to host shifts. However, the fact that naive *D. rinconis* females would not initiate nesting with *Sphaeralcea* may mean that these bees would not nest if no cactus flowers were available during their adult lifetime, which would preclude future host shifts from cactus to mallows. Further tests would be needed to show if this is truly so.

The mixed histories and formats of the pollens offered in these experiments (pure pollen versus anthers or stamens, previously frozen versus fresh) require that the results must be interpreted with caution. In general, I have chosen to emphasize the results obtained from offering fresh pollen or stamens (Table 9). With regard to experiments in which previously frozen pollen or plant parts were offered, I suggest that if such materials were rejected, we can conclude little about the factors contributing to the rejection. However, in cases where such materials were accepted (e.g., frozen *Carnegiea* and *Ferocactus* anthers), I find it unlikely that the acceptance was related primarily to the freezing process. In some cases pure pollen was offered, in others I used complete anthers, stamens, or inflorescences. The presence of plant tissues other than the pollen itself could be attractive or repellent, or the response could depend on the particular plant. Comparing control versus damaged control indicates that in most cases the damaged stamens of the host plant had little effect on bee behavior; when there was a statistically significant difference, some bees preferred the control and some the damaged control. Again, however, damaged cactus stamens cannot be equated with damaged stamens from other plants.

*Possible factors linking Cactaceae, Malvaceae and Convolvulaceae*

Several lineages of bees contain species that use two or three of these plant families, suggesting that plants of these families possess hidden similarities. One factor mentioned by several authors that may tie these three plant families together is pollen grain size (Houston 1971, Schlindwein 1998): many plants in these families have pollen grains that are large (50 to 100  $\mu\text{m}$ ) or very large ( $> 100 \mu\text{m}$ ). Although morphological specialization to particular pollens is not ubiquitous in bees (Thorp 1979), it has been noted that in general, bees that collect small-grain pollens have fine multibranched scopal hairs (scopa = pollen-collecting brush on hind legs or abdomen), whereas bees specializing on large-grained pollens have stout unbranched scopal hairs (Thorp 1979, Houston 1989). This does not hold true for the bees in this study: all have branched scopal hairs that are not particularly stout (pers. obs.). However, a range of grain sizes is found in Cactaceae, Malvaceae and Convolvulaceae (Table 4). In these experiments, *Diadusia rinconis* bees accepted the pollens of *Sphaeralcea* (41  $\mu\text{m}$ ), *Ferocactus* (50  $\mu\text{m}$ ), *Carnegiea* (60  $\mu\text{m}$ ) and *Opuntia* (110  $\mu\text{m}$ ), while rejecting those of *Ipomoea* (94  $\mu\text{m}$ ) and *Cucurbita* (135  $\mu\text{m}$ ). This does not rule out large grain size as a factor in host choice, but it suggests that it is not an overriding factor.

Other pollen characteristics likely to affect host choice in bees are chemistry (including odor) and nutritional composition. In plants whose pollen odor and chemistry have been studied, it has been found that pollen has an odor that is distinct from that of other plant parts, and that solitary specialist bees may have an innate recognition of the pollen odor of their host plants (reviewed in Dobson and Bergström 2000). Many specialist herbivorous insects have extreme sensitivity to one or very few chemical signals that they use to recognize their host plants (Bernays and Funk 1999), a phenomenon consistent with the neural-constraint hypothesis for the prevalence of specialization in herbivorous insects (Bernays and Wcislo 1994, Bernays 1996). We

lack detailed information on the chemistry (including volatiles) of pollens in the Cactaceae, Malvaceae, and Convolvulaceae, but it is plausible that they share convergently evolved chemicals or groups of chemicals and that these are used as cues by pollen-specialist bees. A chemical cue used by cactus-specialist *Diadasia* to recognize cactus pollen may also appear in *Sphaeralcea* pollen, for example. It could also be that *Lithurge apicalis* bees, which rejected *Sphaeralcea* in this study, use a different chemical cue to recognize cactus pollen, one that does not also appear in *Sphaeralcea*.

Increased efficiency in flower-handling of complex floral morphologies, another mechanism related to information-processing constraints, has been cited as a factor promoting specialization and floral constancy in bees (Strickler 1979). Although the flowers of Cactaceae, Malvaceae and Convolvulaceae are similar in some ways (all are actinomorphic, and cactus and mallow flowers are both bowl-shaped), they do not possess complex morphological traits requiring special handling skills. In the flowers of all three families, the pollen is apparent and is readily available to any insect landing on the flower. Floral constancy might not always be related to efficiency in information processing, however: hover-flies showed floral constancy while foraging on flowers with apparent and available pollen (Goulson and Wright 1998).

Additional data from this study suggest that contact chemoreception may not be needed to reject unacceptable host pollen. For most experiments I recorded all landings on the experimental flowers, whether or not they were pollen-collecting visits. In comparing pollen-collecting visits to "other visits" (nectar-collecting visits, plus those in which the bee did not collect any reward), I found in general that fewer "other visits" were made to the novel pollen flowers than to the control flowers (a pattern similar to that found for pollen-collecting visits; unpub. data). This suggests that the bees may

evaluate the suitability of the pollen before landing on the flower, which in turn suggests that a contact chemosensory evaluation is not always necessary. Instead, bees may rely on visual and olfactory cues at close range to determine if the pollen is suitable (Dobson and Bergström 2000, Lunau 2000). It may also suggest that non-host pollens make host flowers repellent to bees even if they are not foraging for pollen.

Pollen nutrition may also be a factor linking the pollens of Cactaceae, Malvaceae and Convolvulaceae. Pollens differ widely in their nutritional content (Schmidt et al. 1989, Franchi et al. 1996, Roulston and Buchmann 2000, Roulston and Cane 2000). Two components that have been suggested as important to pollen-consuming floral visitors are starch and protein. In Cactaceae, the presence or absence of starch varies by species: plants of Malvaceae have been found to have starchy pollen, and Convolvulaceae pollens seem to all be starchless (Franchi et al. 1996, Roulston and Buchmann 2000). Thus, it is unlikely that the presence or absence of starch is one of the hidden similarities that link these plant families.

In contrast to starch content in pollen, protein concentration is probably the most important nutritional component of pollen for larval performance (Roulston and Cane 2000). There is no evidence that foraging bees can evaluate the protein content of the pollens they are collecting (Wcislo and Cane 1996, Roulston and Cane 2000), although bumble bees were found to be sensitive to the protein availability at a foraging site as a whole, and foraged to maximize the site-specific efficiency of pollen collection (Rasheed and Harder 1997). If adults are unable to assess the protein content of different pollens, then pollen specialization may function to ensure uniformity of nutrition for the larvae (Velthuis 1992). In a recent survey of pollen protein of 377 plant species (Roulston et al. 2000), *Opuntia* (Cactaceae) pollen was found to be relatively low in protein content (22%), although Cactaceae was one of only two families

surveyed that showed substantial variability in protein concentration among species. Protein content was found to be near the middle of the range for *Ipomoea* and *Sphaeralcea* (both about 29%). Genera that host pollen-specialist bees did not produce pollen higher in protein than those genera not hosting pollen specialists (Roulston et al. 2000). It thus seems unlikely that protein content facilitates host shifts among Cactaceae, Malvaceae, and Convolvulaceae.

Even if the selection of a particular host plant does not automatically increase larval performance on that host, an increased ability to digest a particular pollen may evolve over time in a specialist species. In tests of larval performance of native solitary specialist and generalist bees on different pollens, the evidence is equivocal: in some cases, the performance of larval bees was worse on the novel pollen compared with the normal host (Guirguis and Brindley 1974, Bohart and Youssef 1976), and in some cases it was the same (Rozen 1963, Horne 1995).

Pollen from *Ipomoea* (Convolvulaceae) was rejected by both *D. rinconis* and *I. duplocincta* (the only two cactus bees tested on this pollen). This was surprising because pollen from plants in the Convolvulaceae is used by one species of *Diadasia* and by two of the four species in the sister clade to the genus *Diadasia* for which host plants are known (Sipes and Wolf 2001). Convolvulaceae is also used by species of *Lithurge* that are not cactus-specialists, and individuals of *Ancyloscelis fiebrigi* collect pollen from both Cactaceae and Convolvulaceae (Alves-Dos-Santos and Wittmann 1999).

*Sphaeralcea* pollen was accepted by both species of *Diadasia* at levels much higher than any other novel pollen. This is particularly interesting in that Malvaceae are thought to be the ancestral host plant family for the genus (Sipes and Wolf 2001); pollen of this plant family is used by 18 of 25 species of *Diadasia* for which host plants

are known (Sipes and Wolf 2001). It may be that the cactus-specialist species of *Diadasia* (a monophyletic clade nested within the other Malvaceae-using species of the genus) still retain the ability to recognize Malvaceae pollen as a suitable food for larvae, perhaps because of a chemical cue shared with Cactaceae.

#### *Adult pollen preferences and host shifts*

In many herbivorous insects, larvae have been shown to perform well on hosts rejected by the adult (Janz and Nylin 1997, Carriere 1998, Ballabeni and Rahier 2000), indicating that adult choice may be more important in explaining the host range of an insect than larval performance. In the case of bees, because the larvae have no choice, host shifts must begin with changes in adult foraging behavior. Such shifts require one of the following: larval conditioning that influences adult behavior, adult conditioning, or genetic variation in innate preference (Rojas and Wyatt 1999). Larval conditioning, or the "Hopkins host selection principle," postulates that cues experienced during larval feeding become relatively more acceptable to individuals in the adult stage. This principle has not been supported by most studies that tested for it (e.g., vanEmden et al. 1996, Rojas and Wyatt 1999), including a recent study involving a specialist bee (Dobson and Ayasse 2000). Adult conditioning (learning, imprinting, induction of preference) is well-established as an important factor in host choice in herbivorous insects (Papaj and Prokopy 1989, Jaenike and Papaj 1992, Cunningham et al. 1998), including honeybees (Guirfa and Nunez 1989, Chittka et al. 1995). It is not omnipresent in herbivorous insects (Parmesan et al. 1995), however, and even if present, may not completely override innate preferences (Lunau et al. 1996).

It does not seem to be the case that foraging experience can override innate preferences in *D. rinconis*. In the case of the naive caged *D. rinconis*, foraging on *Sphaeralcea* and *Scabiosa* for up to eight days before beginning to forage on cactus flowers did not seem to interfere with their ability to recognize or prefer cactus flowers. Furthermore, after foraging for pollen exclusively on *Sphaeralcea* for three days, and after being exposed to *Sphaeralcea* for the first 3-8 days of their adult life, all 7 bees immediately switched back to *Opuntia* when a single *Opuntia* flower was introduced, in the continued presence of abundant *Sphaeralcea* flowers. This demonstrates that the preference for cactus pollen is not easily overridden by foraging experience, even successful foraging for pollen on other plants, and also demonstrates that the preference for cactus pollen is robust in the face of marked differences in the abundances of the two flowers. In addition, cactus-experienced *Diadasia* (both caged and free-flying) often accepted *Sphaeralcea* pollen from cactus flowers, indicating that induced preference is not an overriding factor in host choice. In general, pollen preferences of naive caged *D. rinconis* bees were indistinguishable from those of experienced free-flying bees from the same population, again indicating the strength of innate preference.

Free-flying *D. rinconis* bees in the vacant lot in urban Tucson sometimes accepted *Sphaeralcea* pollen from *Opuntia* flowers, even in the presence of abundant *Opuntia* pollen nearby. However, when *Sphaeralcea* flowers were placed among the *Opuntia* plants in the vacant lot, they were never visited by *Diadasia* bees (unpub. data). Because there were very few *Sphaeralcea* plants blooming in the neighborhood at the same time, especially in comparison to flowering *Opuntia* plants, it seems likely that individual bees that accepted *Sphaeralcea* pollen in cactus flowers had no prior foraging experience with *Sphaeralcea*. This is also true of the Santa Rita Experimental Range site, where flowering *Sphaeralcea* plants were rarely observed (pers. obs), and where

caged, experienced *D. opuntiae* bees sometimes accepted *Sphaeralcea* pollen from *Opuntia* flowers.

Some specialist bees have been found to switch to alternative hosts if the preferred host is not available (Linsley and MacSwain 1958, Linsley et al. 1963, Thorp 1969, Linsley 1978, Parker 1978, Neff et al. 1982, Cane and Payne 1993, Martins and Borges 1999), whereas others may delay nesting or even enter diapause until the next year in the absence of the normal host (Eickwort 1973, Bohart and Youssef 1976, Parker 1978, Minckley et al. 1994, Wcislo and Cane 1996, Minckley et al. 1999). The majority of host shifts in specialist bees observed in these studies took place at the end of the foraging season, when the host had ceased to bloom; host shifts were relatively rare at the beginning of the season, when the host had not yet begun to bloom. This may suggest the hypothesis that, for many bees, presence or abundance of the host plant may be a prerequisite to nesting, but that host choice becomes flexible once nesting is underway.

One of the most striking findings of this study is that newly emerged *D. rinconis* did not initiate nesting in the absence of cactus flowers. Acclimation to the flight cage undoubtedly disrupted their behavior to some extent: caged experienced *D. opuntiae* females (experiment 5) did not resume nesting activity until the third day of captivity. However, when cactus flowers were brought into the cage, all seven *D. rinconis* bees began nesting, despite the fact that they had been in captivity for different amounts of time: five had been caged for three days, and two for eight days. One bee did show signs of being ready to nest during the period when only *Sphaeralcea* flowers were available: it flew low over the ground repeatedly and alighted to dig briefly on April 24 (four days before cactus flowers were introduced), but never actually dug a nest.

If egg load (or some other factor tied to age; Agnew and Singer 2000) reduces selectivity in specialist bees, as it does in other insects (Papaj 2000), it is possible that if the bees had not been offered *Opuntia* flowers, some or all of the bees would have eventually accepted *Sphaeralcea* and begun nesting using its pollen. However, egg load-related changes in specificity occur in only some insects (Papaj 2000). In many other herbivorous insects, including pollen-feeding beetles, oogenesis (Hopkins and Ekbohm 1996) or egg maturation (Rana and Charlet 1997) is delayed in the absence of the host plant. Indirect evidence for delayed egg maturation in pollen specialist bees has been found: in a population of bees that specialize on *Oenothera*, in a year in which *Oenothera* failed to bloom, adult females retreated into nests to diapause until the next year, and one such hibernating female was found to have degenerated ova (Bohart and Youssef 1976). Other specialist bees in captivity have been observed to delay nesting up to two weeks in the absence of their preferred host, and only begin nesting when the host is introduced (N. Williams, pers. comm.).

In some cases, oogenesis may be stimulated by a host-related activity, instead of directly by the host itself: in burying beetles, the behavior of carcass preparation stimulates oogenesis (summarized in Papaj 2000). It could be that, in pollen-specialist bees, the presence of the preferred host plant stimulates nest-building, which in turn promotes oogenesis.

In terms of the potential for future evolutionary host shifts, in both of the populations of *Diadasia* tested, I found significant variation among individuals in the degree of pollen specificity. In spite of the fact that only five individuals of *D. opuntiae* and seven individuals of *D. rinconis* were tested, a wide range of host-specificity was revealed. In both species, there were individuals that would only accept control flowers, individuals that actually preferred novel, non-cactus pollens to control host plant pollen,

and individuals with intermediate levels of specificity (Figures 4 and 5). This would suggest that there is sufficient behavioral variability in these populations to potentially allow a host shift to *Sphaeralcea*, especially in the absence of cactus flowers.

The level of individual variability that I observed also has implications for the interpretation of data from the experiments with free-flying bees. For example, looking at the results from the free-flying *D. rinconis* bees in 1999 and 2000 (Experiment 1, Figure 1), one might conclude that all individuals showed a significant level of acceptance for *Sphaeralcea*. In fact, the caged bee experiment reveals that some individuals rejected *Sphaeralcea* pollen, some accepted it to some degree, and some preferred it to the control flowers. The host preferences of a population are therefore a composite of sometimes divergent preferences of individuals. Individual variation also has implications for interpreting the result that *L. apicalis* bees rejected all non-cactus pollens. There may actually be individual variability in degree of specificity to cactus pollen in the tested population, but at a lower proportion of individuals, such that it would only be observed if a large number of individuals were tested. Variation in preference might also be revealed if multiple populations were tested. It is also possible that all four species of bees have the same degree of pollen specificity to cactus pollen when they are naive, but that for *L. apicalis* and *I. duplocincta*, forms of adult conditioning such as learning play a stronger role in later foraging behavior than they do in *Diadasia*. Geographic variation in host preferences in all of these bees is also likely.

Host shifts in these bees within the plant family Cactaceae may be relatively easy. *D. rinconis*, *D. opuntiae* and *L. apicalis* have all been described as specializing on *Opuntia* pollen (Krombein et al. 1979, Ordway 1984, 1987, Neff and Simpson 1992), and *I. duplocincta* is identified as a specialist on species of *Ferocactus* (Zavortink 1975). However, the results of these experiments show that although the host cactus

may be preferred to other cacti, preferences of these bees within Cactaceae may be broader than had been previously thought. It is likely that the pollens from different species of cacti have enough shared visual, olfactory or gustatory cues to make them all recognizable as suitable hosts. However, very few non-host cactus pollens were tested, and it would perhaps be more revealing to test pollens from those cacti that are hummingbird, moth, or bat-pollinated. Although *Carnegiea* is pollinated by bats, recent work suggests that the bees and birds often pollinate the flowers (which remain open well into the day, unlike those of other bat-pollinated columnar cacti), and may be influencing the floral characteristics of this species (Fleming 2000).

The results of this study might be taken to suggest that it would be relatively easy for at least some individuals of both species of *Diadasia* to switch to Malvaceae in the absence of cactus flowers. However, if they are unable to initiate nests in the absence of cactus flowers, such a host shift would be impossible. The experimental methods I used may also have created a level of acceptance for non-cactus pollens that would never arise in nature. I offered novel pollens within cactus flowers. This method is useful because it forces contact between specialist bees and pollens they might contact in their natural lives, which is potentially revealing about pollen-specific cues. It also controls for factors related to other parts of the flower, and the host plant itself. On the other hand, because this is an artificial situation never encountered in nature, the pollen preferences revealed in this manner may be curiosities that have little relevance to the lives of these bees in their normal ecological context. The mixed nature of the offering may itself be a repellent factor, and may mask actual floral preferences if pollen acceptance requires the stimulation of the original flower. These concerns would be more troubling if all of the novel pollens had been rejected, or if they had all been accepted. The fact that some and not others were accepted, however, suggests that at

least part of the process of pollen acceptance in bees requires cues from the pollen itself, which is an important finding.

### *Conclusions*

The identification of *D. rinconis*, *D. opuntiae*, *L. apicalis*, and *I. duplocincta* as "cactus bees" is well-founded: in the populations tested, bees of all four species showed a strong preference for cactus pollen and, in many cases, cactus pollens were the only ones accepted. Among the non-cactus pollens, the one that both species of *Diadasia* responded most to was *Sphaeralcea*, a member of the ancestral host plant family for the genus *Diadasia*, suggesting that a host shift back to *Sphaeralcea* may be relatively easy. However, *D. rinconis* resisted initiating nests in the absence of cactus flowers. There was striking variation among individuals in pollen preferences and degree of pollen specificity in both *D. rinconis* and *D. opuntiae*.

The actual pollen host ranges of bees are poorly known, with most of our information coming from collection records (e.g., Krombein et al. 1979), examination of pollen on museum specimens (e.g., Müller 1996), field observations of foraging behavior (e.g., Minckley et al. 1994), and in a few cases analysis of pollen provision masses from nests (e.g., Brach 1978). There are very few data from experiments in which adult bees are offered different pollen hosts, and their behavior observed (but see Cripps and Rust 1989, Horne 1995). By testing the preferences of naive and experienced bees for different pollens in controlled experiments, we may gain clues as to the relative importance of different factors in host choice in bees. It has been suggested that specialization in bees differs from specialization in other herbivorous insects in its mechanisms, evolutionary origins, and ecological consequences (Wcislo

and Cane 1996). Continuing studies of host choice in specialist pollen-foraging bees have the potential to provide data necessary to test these theories.

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Table 1. North American cactus-specialist bees.

Bee Family	Genus	Cactus-using species	Pollens used by congeners	Species tested in this study
Apidae	Diadasia (30–40 spp., New World only)	6 species (New World)	Malvaceae Asteraceae Convolvulaceae Onagraceae	D. rinconis D. opuntiae
Apidae	Idiomelissodes (monospecific)	duplocincta (SW U.S. & México)	unknown	I. duplocincta
Megachilidae	Lithurge (~50 spp. worldwide)	10 species (N. & S. Am.)	Malvaceae Convolvulaceae Asteraceae	L. apicalis
Megachilidae	Ashmeadiella (~55 spp. N. Am. only)	1 species (SW U.S. & México)	unknown	
Andrenidae	Perdita (~800 spp., N. Am. only)	7 spp. in the Macrotera group (SW U.S. & México)	Malvaceae	
Halictidae	Dufourea (~80 N. Am. spp.)	1 species	unknown	
Apidae	Melissodes (~120 N. Am. spp.)	1 species	unknown	

Table 2. List of Experiments.

Exp	Bee tested	Dates	Locality	Cactus Host	Expmnt type
1	<i>D. rinconis</i>	May 99 & May 00	urban Tucson	<i>O. engelmannii</i>	free-flying: choice
2	<i>L. apicalis</i>	May 99	urban Tucson	<i>O. engelmannii</i>	free-flying: choice
3	<i>I. duplocincta</i>	Aug 99 & Aug 00	Lincoln Park	<i>F. wislizeni</i>	free-flying: choice
4	<i>D. opuntiae</i>	June 99	SRER	<i>O. engelmannii</i>	flight cage (5 bees): choice
5	<i>D. rinconis</i>	May 00	urban Tucson	<i>O. engelmannii</i>	flight cage (7 bees): choice
6	<i>D. rinconis</i>	May 00	urban Tucson	<i>O. engelmannii</i>	flight cage (7bees): no-choice

Table 3. Estimation of numbers of *D. rinconis* individuals observed in free-flying experiments, based on observations of caged individual bees. a) data from caged *D. rinconis* bees, 2000. b) extrapolation table used for free-flying bees.

a)

No. pollen-foraging bees	Mean $\pm$ SD visits per 5 min interval (n = intervals: range)
1	1.85 $\pm$ 0.80 (n = 13: 1 to 3)
2	3.89 $\pm$ 1.79 (n = 28: 2 to 9)
3	6.10 $\pm$ 4.92 (n = 18: 3 to 11)
4	7.59 $\pm$ 3.09 (n = 11: 5 to 11)
5	9.76 $\pm$ 3.80 (n = 7: 11 to 16)
6	12 (n = 1)

b)

Visits observed per 10 min interval	Estimated number of individual bees
1 to 4	1
5 to 8	2
9 to 12	3
13 to 16	4
17 to 20	5
21 to 24	6

Table 4. Pollens used. Cactus pollens are listed first; non-cactus pollens are listed alphabetically by plant family.

Plant Family (cactus bee congeners using this plant family)	Plant	Pollen grain size	Flowers naturally near Tucson?	Forms of pollen used
Cactaceae	Opuntia engelmannii (host for expmts 1, 2, 4, 5)	110µm	Spring	- pure pollen, prev. froz. - anthers, prev. froz. - anthers, fresh
Cactaceae	Opuntia phaecantha	110µm	Spring	- pure pollen, prev. froz.
Cactaceae	Carnegiea gigantea	60µm	Spring	- flowers, fresh - anthers, prev. froz.
Cactaceae	Ferocactus wislizeni (host for exp. 3)	50µm	Summer	- anthers, prev. froz.
Asteraceae (Diadasia, Lithurge)	Helianthus		Summer	- pure pollen, prev. froz.
Convolvulaceae (Diadasia, Lithurge, Ancyloscelis)	Ipomoea carnea	94µm	No	- anthers, fresh
Cucurbitaceae	Cucurbita pepo (zucchini)	135µm	No	- anthers, fresh
Ephedraceae	Ephedra	32µm	Spring	- pure pollen, prev. froz.
Fabaceae	Acacia constricta		Spring & Summer	- fresh inflorescences
Fabaceae	Acacia greggii		Spring & Summer	- fresh inflorescences
Fabaceae	Caesilpinia		No	- anthers, fresh
Fabaceae	Prosopis velutina	29µm	Spring	- anthers, fresh
Malvaceae (Diadasia, Lithurge, Perdita)	Sphaeralcea	41µm	Spring	- staminal columns, fresh or frozen
Papaveraceae	Argemone	44µm	Spring & Summer	- anthers, fresh or frozen
Poaceae	Zea mays	82µm	No	- pure pollen, prev. froz.
Typhaceae	Typha	26µm	No	- pure pollen, prev. froz.
Zygophyllaceae	Kallstroemia grandiflora	66µm	Summer	- anthers, fresh or frozen

Table 5. Experiment 3: Comparison of a cactus specialist (*I. duplocincta*) with a generalist bee (*Augochlorella* sp.). Both bees were tested at the Lincoln Park site in 1999. "fr" = previously frozen. "NT" = not tested. "Op." = *Opuntia*. "site" = subsite within Lincoln Park. In the *Augochlorella* column, multiple results for one type of pollen are from different subsites within the study plot.

Pollen	<i>I. duplocincta</i> (cactus specialist)	<i>Augochlorella</i> sp. (pollen generalist)
fr. Op. stamens	Rejected	Rejected (1 site, 20 visits)
fr. Op. pollen	Accepted moderately	Accepted (48% of 25 visits). Accepted (25% of 16 visits). Rejected (1 site, 8 visits)
fr. Carnegiea	Accepted moderately	Accepted (55% of 31 visits) Accepted (78% of 9 visits)
fr. Sphaeralcea	Rejected	Accepted minimally (3% of 36 visits)
fr. Argemone	Rejected	Accepted minimally (4% of 25 visits)
fr. Prosopis	Rejected	Accepted (13% of 15 visits)
fr. Helianthus	Rejected	Accepted (22% of 9 visits)
Kallstroemia	Accepted minimally	Accepted (44% of 9 visits). Accepted (22% of 18 visits)
<i>A. constricta</i>	Rejected	NT
<i>A. greggii</i>	Rejected	NT
Caesilpinia	Rejected	Rejected (1 site, 6 visits)
Ipomoea	Rejected	NT

Table 6. Caged *D. opuntiae* 1999: visits to control vs. damaged control flowers. Within bee, day was the replicate. P-values from G<sup>2</sup> likelihood ratio test.

Bee	n=visits	days foraging	% to control	% to damaged control	p-value
Blue	55	8	58%	42%	0.2239
Copper	42	5	36%	64%	0.0622
Red	120	7	48%	52%	0.5838
White	55	5	53%	47%	0.6858
Yellow	52	5	62%	38%	0.0946

Table 7. Caged *D. rinconis* 2000: visits to control vs. damaged control flowers. Within bee, day was the replicate. P-values from G<sup>2</sup> likelihood ratio test.

Bee	n=visits	days foraging	% to control	% to damaged control	p-value
Blue	98	6	50%	50%	1.0
GW	86	6	36%	64%	0.0092
Red	83	5	35%	64%	0.0110
RW	114	6	50%	50%	1.0
White	52	6	63%	37%	0.0508
WY	26	5	73%	27%	0.0165
Yellow	49	6	65%	35%	0.0308

*Table 8.* Chronology of the no-choice test on 7 naive caged *D. rinconis* bees, 2000. If bees are not actively provisioning a nest, they forage for nectar for their own nutrition. If they are provisioning a cell, they forage for both pollen and nectar.

Dates	Flowers offered for pollen	Bee activities
April 20	Sphaeralcea	Two bees caged (Blue & Yellow). Nectar-foraging only.
April 21-24	Sphaeralcea	Nectar-foraging only.
April 25	Sphaeralcea	Five more bees added (R, RW, W, WY, GW). Nectar-foraging only.
April 25-27	Sphaeralcea	Nectar-foraging only.
April 28	Opuntia	Four bees initiate nests. B & Y had been in the cage 8 days. W & R had been caged 3 days.
April 29	Opuntia	The remaining three initiate nests (RW, WY, GW).
April 30- May 5	Opuntia	Pollen-foraging (provisioning the nests) began on April 30. Apr 30 - May 5, choice experiments (Expmt. 5).
May 6	Sphaeralcea	Three bees immediately switch to collecting Sphaeralcea pollen for nest provisions.
May 7-8	Sphaeralcea	Three more switch to Sphaeralcea on May 7. The 7th bee (RW) does not switch and suspends nesting activities.
May 9	Opuntia and Sphaeralcea	Flowers available: large bunches of Sphaeralcea (hundreds of flowers), plus one Opuntia flower. All 7 bees immediately switch to collecting Opuntia pollen from the one flower.

*Table 9.* Summary of results. Plants are listed as follows: Cactaceae first, others alphabetically by plant family. Levels of preference are defined as follows: “Preferred” = pollen-collecting visits greater in proportion than those to control flowers. “Acc.” = Accepted: pollen-collecting visits in the same proportion as those to control flowers. “Acc. moderately” = pollen-collecting visits less in proportion than those to control flowers, but account for more than 5% of all pollen-collecting visits. “Acc. minimally” = pollen-collecting visits to this pollen 5% or less of all pollen-collecting visits. “Rejected” = no pollen-collecting visits to this pollen. “NT” = not tested. Percentages are percent of pollen-collecting visits. The format and previous history of the pollen used is indicated in parentheses. “fr.” = previously frozen.

Plant (Family)	Exp. 1: free-flying <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 5: Seven caged <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 4: Five caged <i>D. opuntiae</i> Host: <i>Opuntia</i>	Exp. 2: Free-flying <i>L. apicalis</i> Host: <i>Opuntia</i>	Exp. 3: free-flying <i>L. duplocincta</i> Host: <i>Ferocactus</i>
<i>Opuntia engelmannii</i> (Cactaceae)	Acc. moderately (fr. anthers 17%)	NT	Acc. or acc. moderately (4 bees; Rejected (1 bee) (fr. pollen or anthers)	Acc. moderately (fr. anthers 12%)	Acc. moderately (fr. pollen); Rejected (fr. anthers)
<i>Opuntia phaeacantha</i> (Cactaceae)	NT	NT	Accepted: 1 bee; Rejected: 4 bees (fr. pollen)	NT	NT
<i>Carnegiea gigantea</i> (Cactaceae)	Accepted (fr. anthers, 38%)	Preferred: 1 bee; Acc. moderately: 1 bee; Rejected: 5 bees (fr. anthers)	Preferred: 1 bee; Rejected: 5 bees (fresh flowers)	NT	Acc. moderately at 3 of 4 subsites (fr. anthers, 14%)
<i>Ferocactus wislizeni</i> (Cactaceae)	Accepted moderately (1999, 8%) or Accepted (2000, 23%) (fr. anthers)	Preferred: 1 bee; Acc. moderately: 1 bee; Rejected: 5 bees (fr. anthers)	Acc. moderately: 1 bee; Rejected: 4 bees (fr. anthers)	Acc. moderately (fr. anthers, 11%)	NT

Table 9, continued

Plant (Family)	Exp. 1: free-flying <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 5: Seven caged <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 4: Five caged <i>D. opuntiae</i> Host: <i>Opuntia</i>	Exp. 2: Free-flying <i>L. apicalis</i> Host: <i>Opuntia</i>	Exp. 3: free-flying <i>L. duplocincta</i> Host: <i>Ferocactus</i>
<i>Helianthus</i> sp. (Asteraceae)	Acc. moderately (fr. pollen, 10%)	NT	Accepted: 1 bee; Rejected: 3 bees (fr. pollen)	NT	Rejected (fr. pollen)
<i>Ipomoea carnea</i> (Convolvulaceae)	Rejected (fresh anthers)	Rejected (fresh anthers)	NT	NT	Rejected (fresh anthers)
<i>Cucurbita pepo</i> (Cucurbitaceae)	Rejected (fresh anthers)	Rejected (fresh anthers)	NT	NT	NT
<i>Ephedra</i> sp. (Ephedraceae)	NT	NT	Rejected: 5 bees (fr. pollen)	NT	NT
<i>Acacia constricta</i> (Fabaceae)	NT	NT	NT	NT	Rejected (fresh inflorescences)
<i>Acacia greggii</i> (Fabaceae)	NT	NT	NT	NT	Rejected (fresh inflorescences)
<i>Caesalpinia</i> sp. (Fabaceae)	NT	NT	NT	NT	Rejected (fresh anthers)
<i>Prosopis velutina</i> (Fabaceae)	Acc. minimally (fresh anthers 3%)	Acc. minimally: 1 bee; Rejected: 6 bees (fresh anthers)	Rejected: 5 bees (fresh anthers)	Rejected (fresh anthers)	Rejected (fr. anthers)
<i>Sphaeralcea</i> sp. (Malvaceae)	Accepted (fresh stamens, 33%)	Preferred: 1 bee; Acc. mod.: 1 bee; Acc. minimally: 1 bee; Rejected: 4 bees (fresh stamens)	Accepted: 1 bee; Acc. moderately: 1 bee; Rejected: 3 bees (fresh stamens)	Rejected (fresh stamens)	Rejected (fr. stamens)

Table 9, continued

Plant (Family)	Exp. 1: free-flying <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 5: Seven caged <i>D. rinconis</i> Host: <i>Opuntia</i>	Exp. 4: Five caged <i>D. opuntiae</i> Host: <i>Opuntia</i>	Exp. 2: Free-flying <i>L. apicalis</i> Host: <i>Opuntia</i>	Exp. 3: free-flying <i>L. duplocincta</i> Host: <i>Ferocactus</i>
<i>Argemone</i> sp. (Papaveraceae)	Acc. minimally (2%, fresh anthers)	Rejected: 7 bees (fresh anthers)	Rejected: 5 bees (fresh anthers)	Rejected (fresh anthers)	Rejected (fr. anthers)
<i>Zea mays</i> (Poaceae)	Rejected (fr. pollen)	NT	NT	NT	NT
<i>Typha</i> sp. (Typhaceae)	NT	NT	Rejected: 5 bees (fr. pollen)	NT	NT
<i>Kallstroemia grandiflora</i> (Zygophyllaceae)	Acc. minimally (5%, fr. anthers)	Acc. minimally: 1 bee; Rejected: 6 bees (fr. anthers)	NT	NT	Acc. minimally (2.5%, fresh anthers)

## Figure Legends

*Figure 1.* Experiment 1: Observed vs. expected numbers of pollen-collecting visits for free-flying *D. rinconis* bees. If no p-value is shown, the sample size was not large enough to allow a statistical test. In this and all figures, the y-axis is the number of visits. Hatched bars are observed visits to cactus pollens, including control flowers. Shaded bars are observed visits to novel non-cactus pollens. White bars are expected numbers of visits. Charts (a) through (f) are from 1999, and (g) through (n) from 2000.

*Figure 2.* Experiment 2: Observed vs. expected numbers of pollen-collecting visits for free-flying *L. apicalis* bees. If no p-value is shown, the sample size was not large enough to allow a statistical test. Number of treatments tested is less than for *D. rinconis* because *L. apicalis* bees were rarer, and only appeared on the flowers on some days. Hatched bars are observed visits to cactus pollens, including control flowers. Shaded bars are observed visits to novel non-cactus pollens. White bars are expected numbers of visits.

*Figure 3.* Experiment 3: Observed vs. expected numbers of pollen-collecting visits for free-flying *I. duplocincta* bees. For each set of treatments, results were pooled across subsites. All charts are from 1999, except l) 2000. Hatched bars are observed visits to cactus pollens, including control flowers. Shaded bars are observed visits to novel non-cactus pollens. White bars are expected numbers of visits.

*Figure 4.* Experiment 4: Observed numbers of pollen-collecting visits for caged *D. opuntiae* bees (5 bees). Each graph represents foraging by one bee on one day. A unique

set of treatments was offered on nearly each day, so results cannot be pooled across days. Sample sizes were not large enough to allow statistical testing. "Control" and "damaged control" results were pooled. White bars are observed visits to control flowers, hatched bars are observed visits to novel cactus pollens, and shaded bars are observed visits to novel non-cactus pollens.

- Arg. = fresh Argemone anthers
- Carn. = fresh Carnegiea flowers (Cactaceae)
- Con = control plus damaged control
- Eph. = previously-frozen Ephedra pollen
- Fero. = previously-frozen Ferocactus anthers (Cactaceae)
- Heli. = previously-frozen Helianthus pollen (bee-collected)
- Op1 = previously-frozen *O. engelmannii* anthers (Cactaceae)
- Op2 = previously-frozen *O. phaeacantha* pure pollen (Cactaceae)
- Op3 = refrigerated *O. engelmannii* anthers (Cactaceae)
- Op4 = previously-frozen *O. engelmannii* pure pollen (Cactaceae)
- Pros. = fresh Prosopis anthers
- Spha. = fresh Sphaeralcea stamens
- Typh. = previously-frozen Typha pollen

*Figure 5.* Experiment 5: Observed vs. expected numbers of pollen-collecting visits for caged *D. rinconis* bees (7 bees). Each column represents one bee, and each set of treatments (rows) represents data pooled from the two days on which that set of treatments was offered. "Control" and "damaged control" were not pooled. Hatched bars are observed visits to cactus pollens, including controls. Shaded bars are observed visits

to novel non-cactus pollens. White bars are expected number of visits. In these experiments, on each day 9 flowers were offered: 3 control, 3 damaged control, and one each of 3 novel pollens. For this reason, expected visits to control and damaged control are much larger than the expected visits to novel pollens. If no p-value and no expected visits are shown, the sample size was not large enough to allow a statistical test.

"Arg" = *Argemone* anthers (fresh)

"Carn" = *Carnegiea gigantea* anthers (previously frozen)

"Con" = control

"Dam" = damaged control

"Fero" = *Ferocactus wislizeni* anthers (previously frozen)

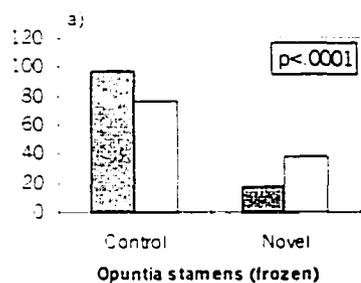
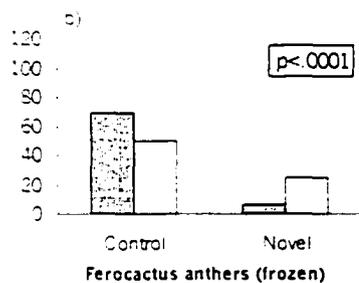
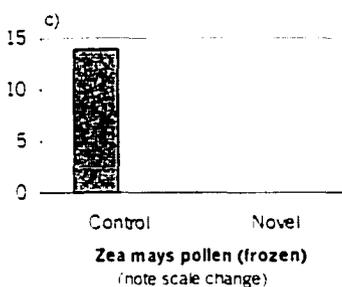
"Ipo" = *Ipomoea carnea* anthers (fresh)

"Kall" = *Kallstroemia grandiflora* anthers (previously-frozen)

"Pros" = *Prosopis velutina* anthers (fresh)

"Spha" = *Sphaeralcea* staminal columns (fresh)

"Zucc" = *Cucurbita pepo* anthers (zucchini: fresh)

Figure 1. Free-flying *Diadasia rinconis* bees, 1999Cactus pollens  
(previously frozen)Non-cactus pollens  
(previously frozen)

Non-cactus pollens (fresh)

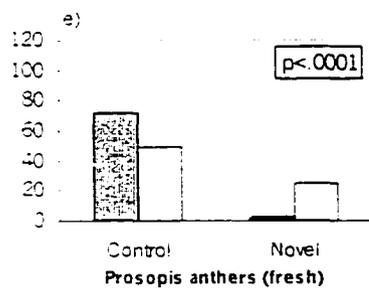
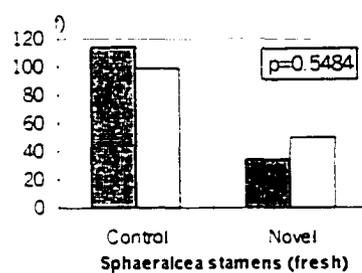
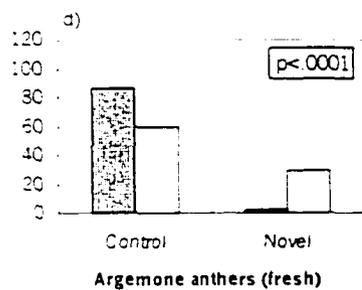
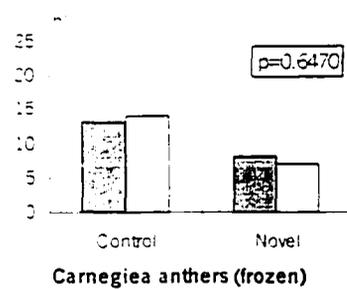
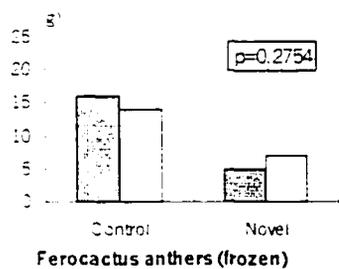
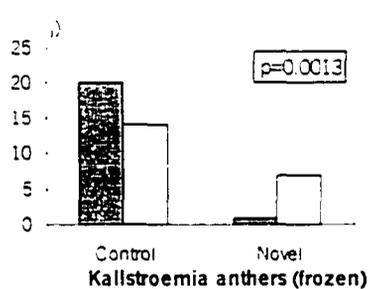
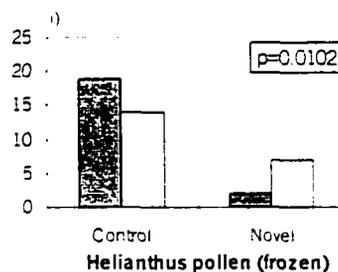


Figure 1. continued. Free-flying *Diadasia rinconis* bees. 2000Cactus pollens  
(previously frozen)Non-cactus pollens  
(previously frozen)

## Non-cactus pollens (fresh)

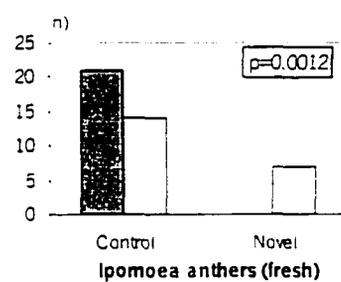
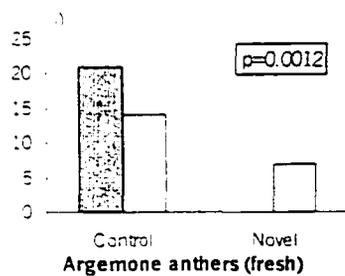
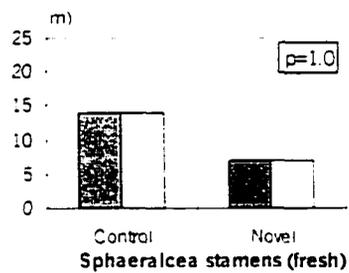
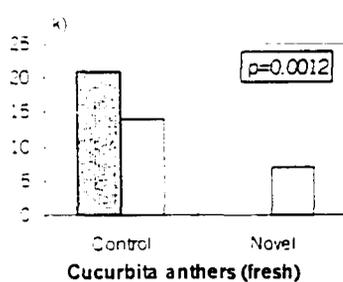
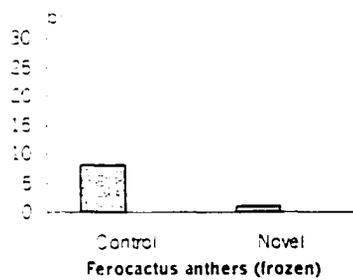
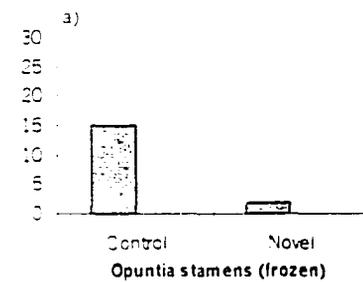


Figure 2. Free-flying *Lithurge apicalis* bees, 1999

Cactus pollens  
(previously frozen)



Non-cactus pollens  
(fresh)

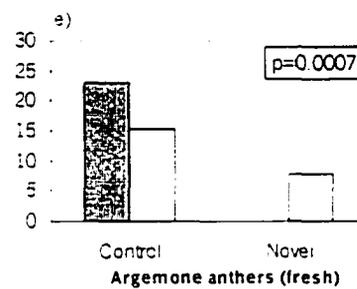
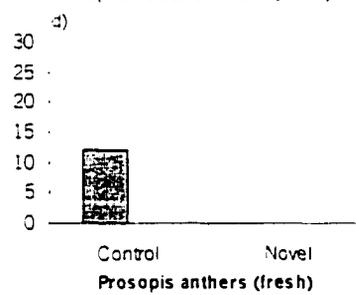
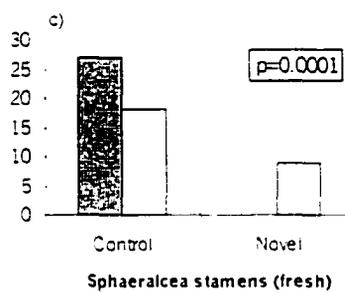


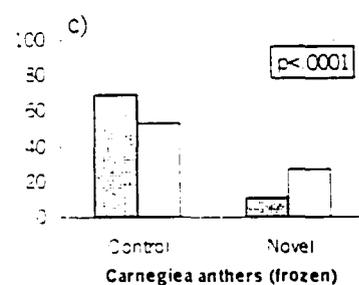
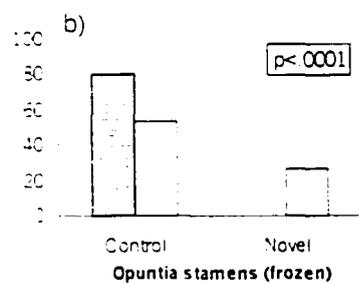
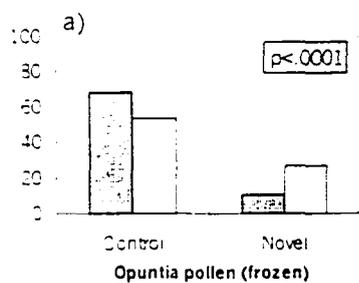
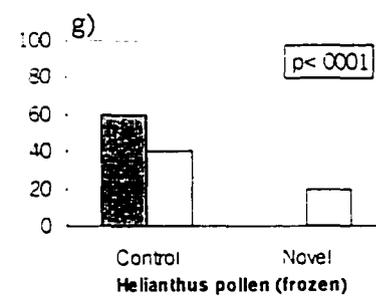
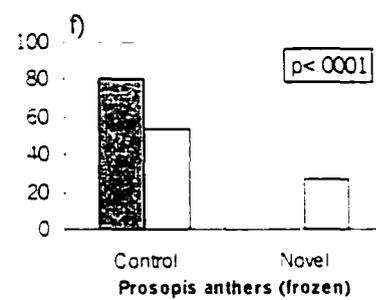
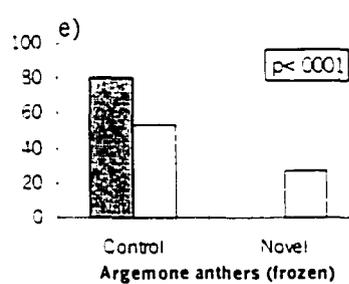
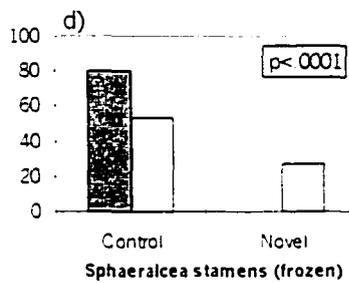
Figure 3. Free-flying *Idiomelissodes duplocincta* bees, 1999Cactus pollens  
(previously frozen)Non-cactus pollens  
(previously frozen)

Figure 3. continued. Free-flying *Idiomelissodes duplocincta* bees. 1999-2000

## Non-cactus pollens (fresh)

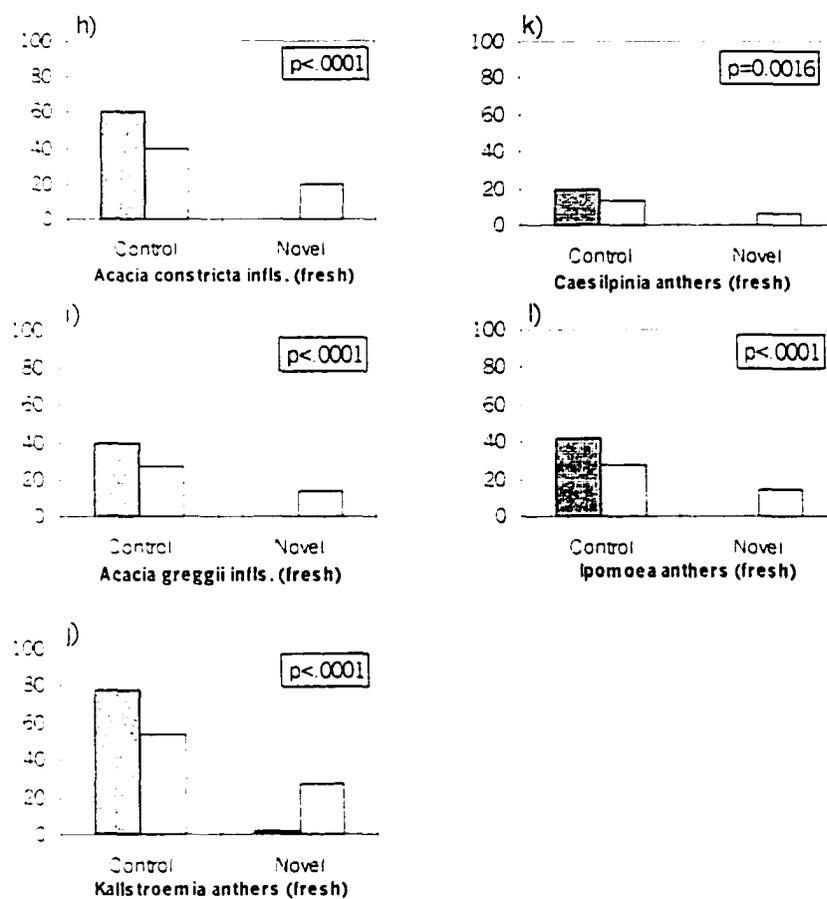


Figure 4: Caged *Diadasia opuntiae* bees (1999): "Blue" bee

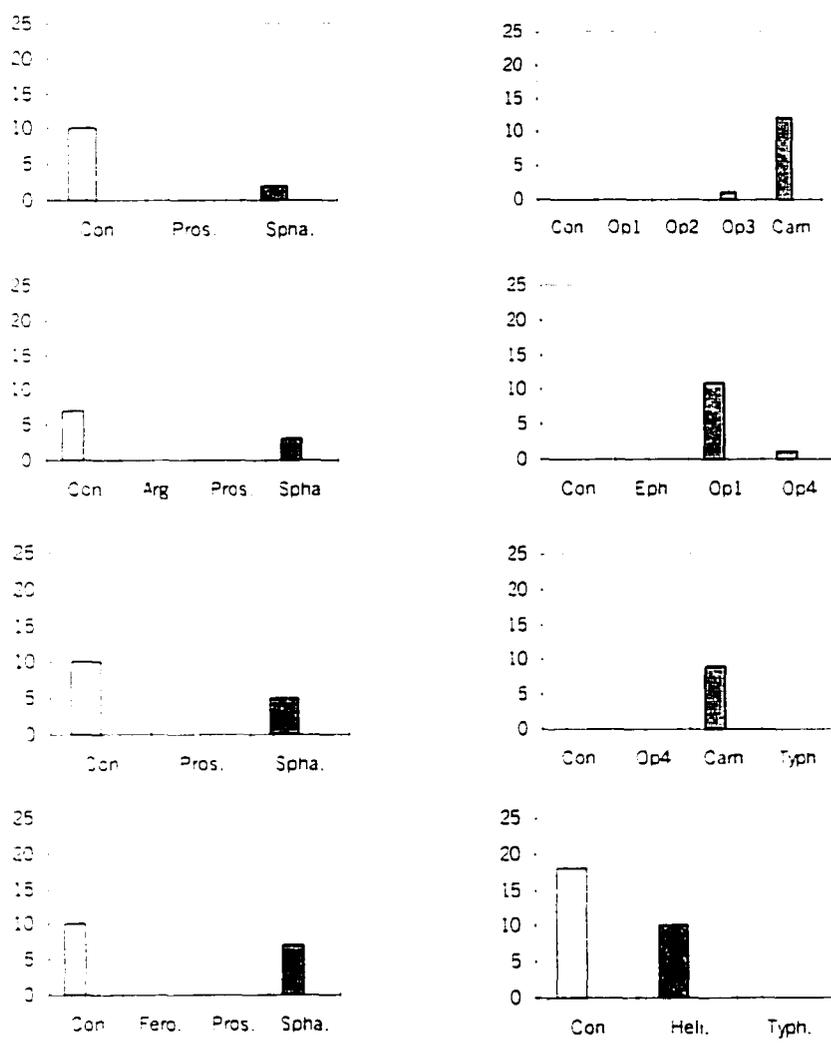


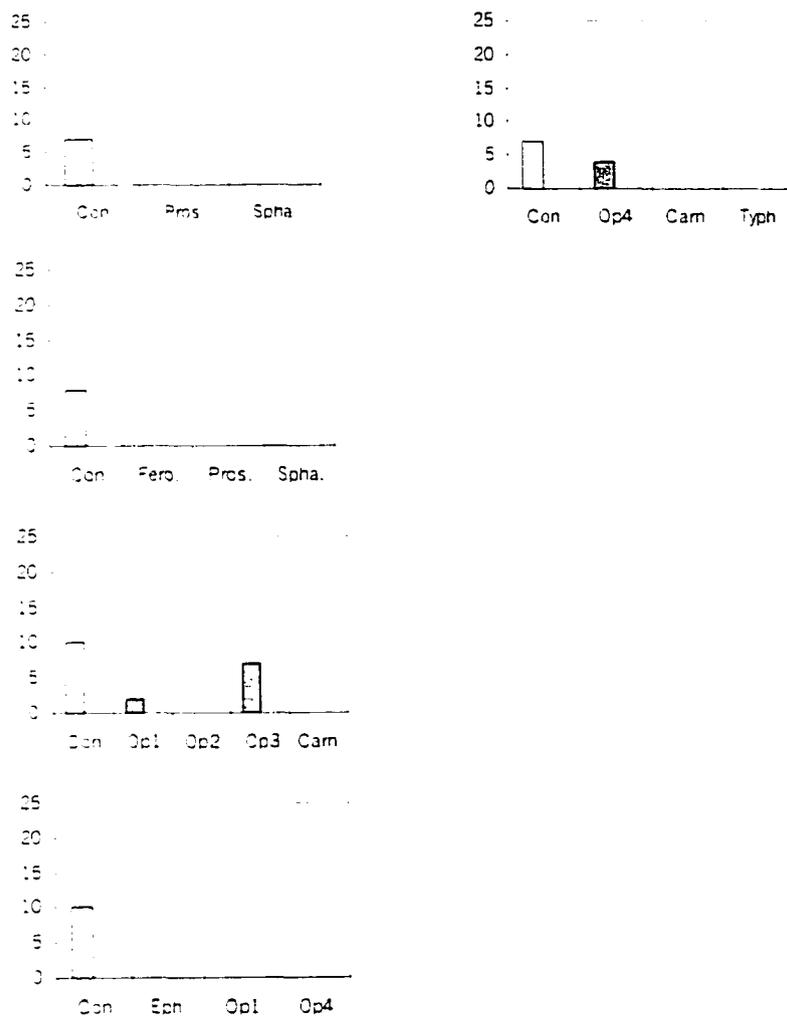
Figure 4. continued. Caged *Diadasia opuntiae* bees (1999): "Copper" bee

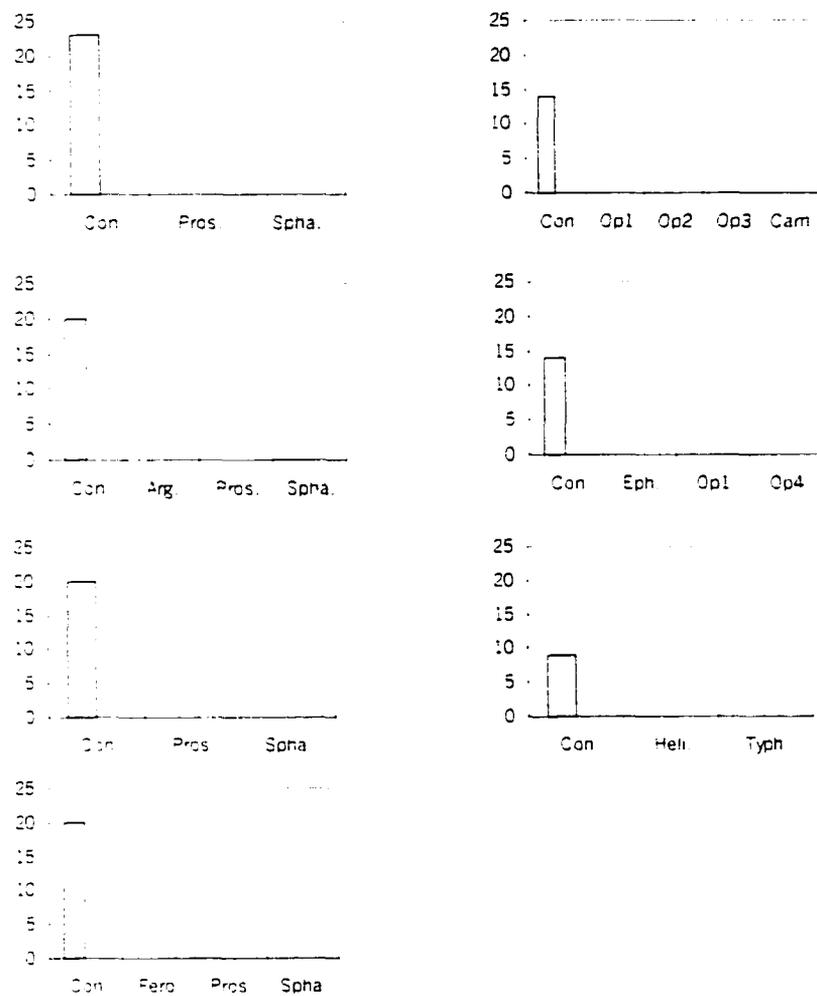
Figure 4. continued. Caged *Diadasia opuntiae* bees (1999): "Red" bee

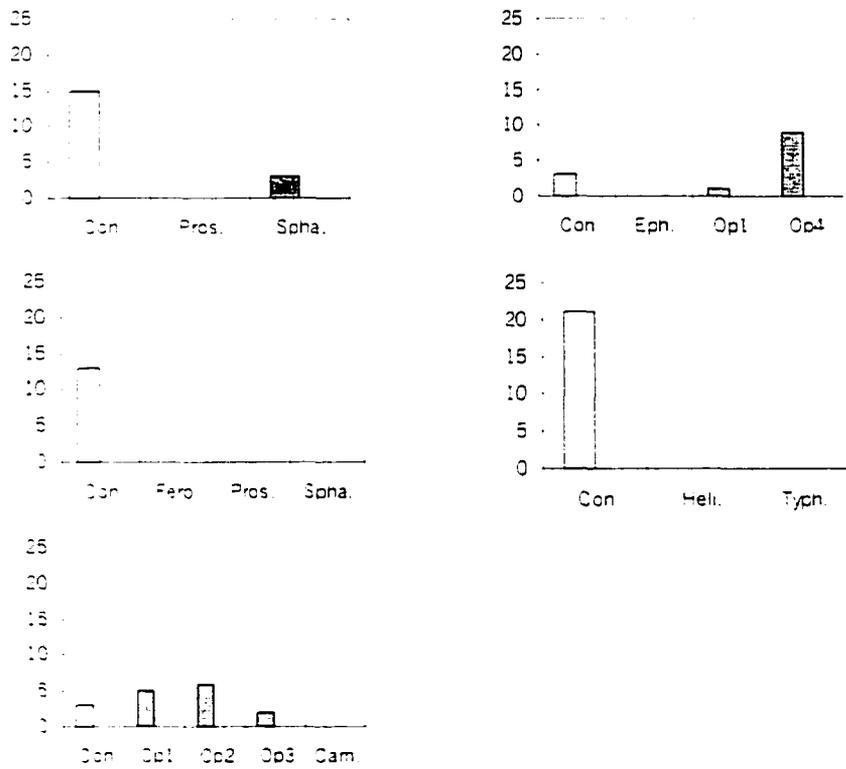
Figure 4, continued. Caged *Diadasia opuntiae* bees (1999): "White" bee

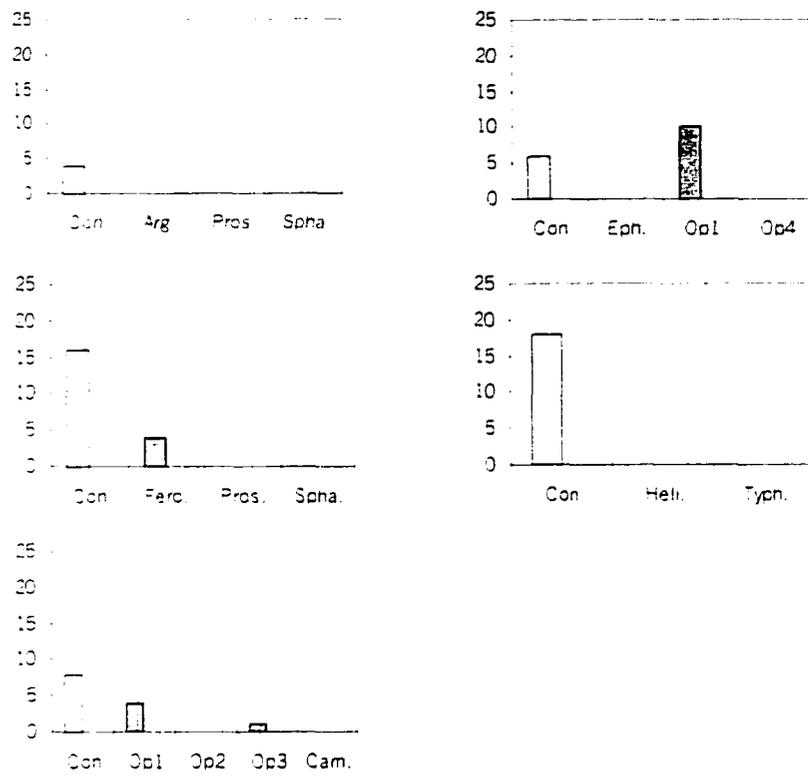
Figure 4. continued. Caged *Diadasia opuntiae* bees (1999): "Yellow" bee

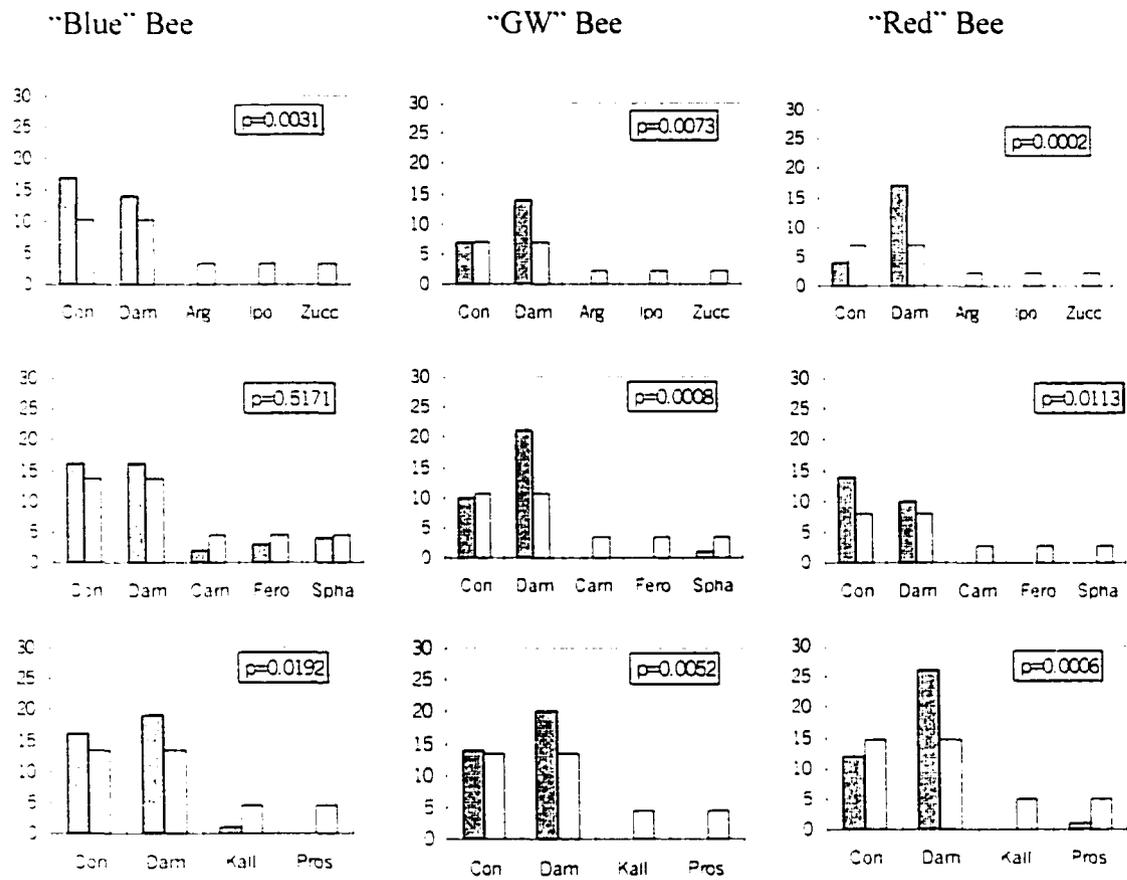
Figure 5. Caged *Diadasia rinconis* bees (2000)

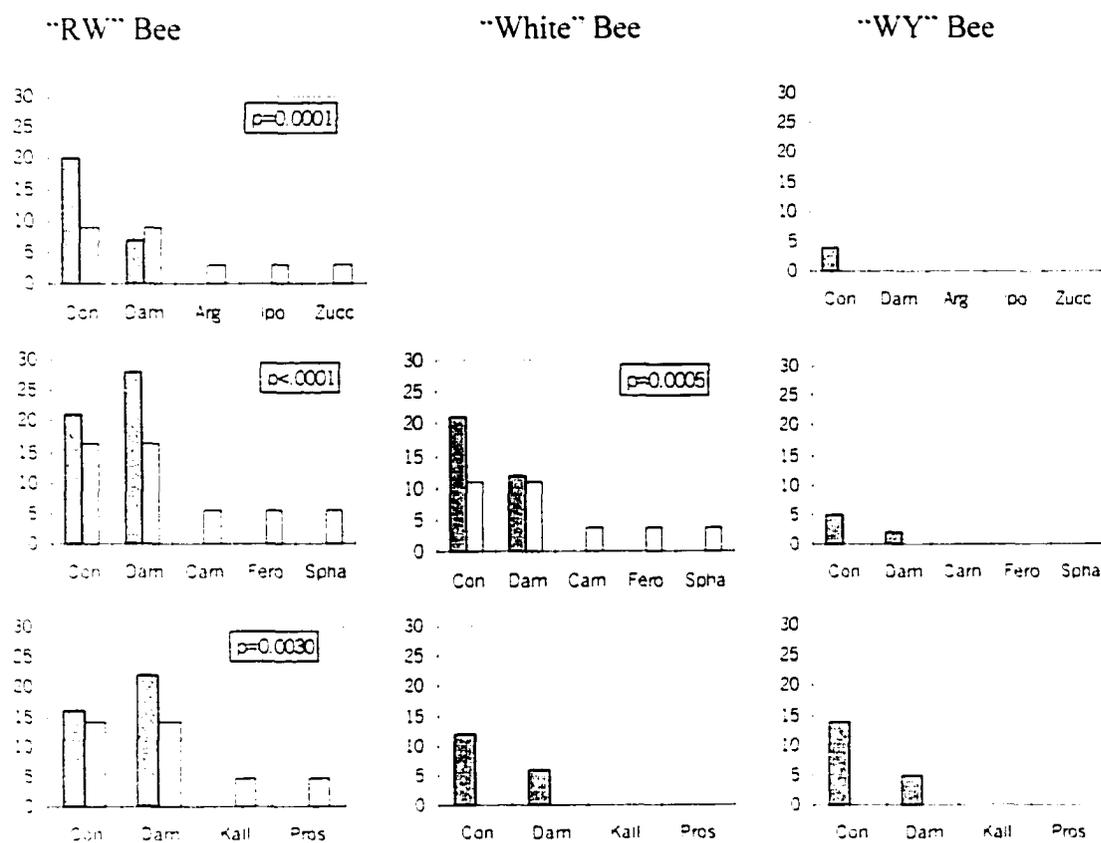
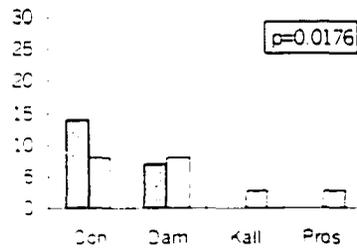
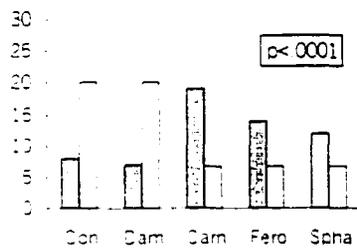
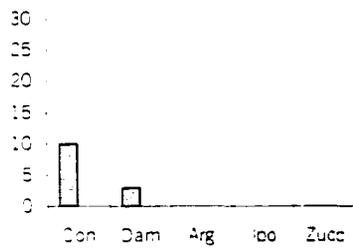
Figure 5. continued. Caged *Diadasia rinconis* bees (2000)

Figure 5. continued. Caged *Diadasia rinconis* bees (2000)

## "Yellow" Bee



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