Preliminary Report on the Reproductive Biology of the Threatened Chisos Mountain Hedgehog Cactus

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Abstract: The Chisos Mountain hedgehog cactus (*Echinocereus chisoensis*, Cactaceae) is a narrow endemic restricted to an approximately 100 square mile area in Big Bend National Park, Texas. It was listed as threatened in 1987 as *Echinocereus chisoensis* var. *chisoensis*. An investigation of the reproductive biology and pollination ecology conducted in 1999 and 2000 revealed the taxon to be homogamous, self-incompatible, xenogamous, and heavily dependent upon the cactus oligolectic bee, *Diadasia rinconis* (Anthophoridae) for pollination. Despite infrequent bee visitation, fruit set from open pollination is high and fruits produce large numbers of seeds. Predation in 2002, probably from rodents as a result of severe drought conditions, was severe on plants, flower buds, and fruits.

The Chisos Mountain hedgehog cactus, or Chisos pitaya (*Echinocereus chisoensis* W. Marshall), is 1 of 20 threatened or endangered cacti listed by the U.S. Fish and Wildlife Service for Region 2 (http: // ecos. fws.gov/ webpage/ webpage_lead.html? lead_region=2&type=L&listings=1). In 1987 it was added to the federal lists (53 FR 38453) of endangered and threatened wildlife and plants as threatened because of its restricted distribution, low numbers, loss of viability in existing populations, vulnerability to private and commercial cactus collectors, and habitat disruption (U.S. Fish and Wildlife Service 1993). As with other protected cacti, the lack of basic scientific information about the species is limiting the ability of conservation agencies to evaluate the limiting factors and to prescribe management activities. This study, which is part of a long-term investigation of the taxon's life history, presents a description of the pollination ecology and breeding system of *E. chisoensis* and a discussion as to how these features relate to the species' fitness.

Echinocereus chisoensis, a small cylindrical cactus, is restricted to flat alluvial fans and terrace deposits in Big Bend National Park in Brewster County, Texas at elevations of 650–750 m in well-developed desert hardpan of the Chamberinos, Pantera, and Upton-Nickel soil associations (USDA Soil Conservation Service 1985). Plants are typically found growing either under or within dominants of the lowland desert shrub community. Common associates are dog cholla (*Opuntia schottii* G. Engelmann), creosote (*Larrea tridentata* (DC) Cov.), ratany (*Krameria grayi* Rose & Painter and *K. erecta* C. von Willdenow ex J. A. Schultes), whitethorn acacia (*Acacia constricta* A. Gray), tasajillo (*Opuntia leptocaulis* DC.), ocotillo (*Fouquieria splendens* K. Kunth), leatherstem (*Jatropha dioica* V. de Cervantes), lechuguilla (*Agave lechuguilla* J. Torrey), and ceniza (*Leucophyllum frutescens* (J. Berlandier) I. M. Johnston). An earlier study (Hendershott et al. 1992) did not show specific *E. chisoensis*–nurse plant associations, but rather showed associations as a consequence of soil conditions that provide a hospitable environment for a diversity of species or the exploitation by *E. chisoensis* of conditions that are provided by the presence of any nurse plant. The *E. chisoensis* habitat is shared with several other cacti, including blind prickly pear (*Opuntia rufida* Engelm.), purple prickly pear (*O. macrocentra* Engelm.), brown-spine prickly pear (*O. phaeacantha* Engelm.), strawberry cactus (*Echinocereus stramineus* (G. Engelmann) F. Seitz), rainbow cactus (*E. dasyacanthus* G. Engelmann), devil's-head cactus (*Echinocactus horizonthalonius* C. Lemaire, *Echinomastus warnockii* (l. Benson) C. Glass & R. Foster), and coryphantha (*Coryphantha macromeris* R. O. Baird, *C. echinus* (G. Engelmann) N. Britton & Rose)).

Material and Methods

Data were collected over a 2-week period in mid-March 1999 and 2000 at two sites, designated as S1 and ET, that are approximately 4 miles apart. In 2000 additional observations were made approximately every 3 days until April 22. In 1999 *E. chisoensis* plants at the two sites were mapped and individual plant locations were marked with numbered metal tags for subsequent identification of individual plants. In 2000 additional plants were marked at each site to increase sample sizes. Observations and crossing experiments were made with these plants (1999 S1, n = 50; 2000 S1, n = 84; 1999 ET, n = 34; 2000 ET, n = 70), and additional observations of insect visitors were made on unmarked plants.

Phenology

Floral phenology was determined by daily observation of marked plants. Events noted consisted of the time of day the flower opened and closed, flower longevity, pre-pollination and post-pollination floral behavior, the time of anther dehiscence, and the relative position of floral parts. The number of open flowers by individual plant was recorded each day. The status of buds, flowers, and fruits was monitored for each plant throughout the study.

To determine stigma receptivity, bagged flowers (n = 18) were cross pollinated and self-pollinated by hand at 1 hour intervals beginning at 1000 hrs CDT and continuing to 1500 hrs. Between 0800 and 0915 hrs the following morning, styles were excised and preserved. They were then stained and the stigmas were examined for pollen tube growth with a fluorescence microscope (Martin 1959). Four of the flowers with excised stigmas were marked for later evaluation of seed set. In another test, pollen germination was compared between cross and self-pollinated flowers (n = 16). For this investigation, either plants having two open flowers were used with one flower receiving cross pollen and the other flower receiving self-pollen, or for plants with only a single open flower, half of the stigma received cross pollen and the other half was self-pollinated. Stigmas were collected 2 hours after treatment; they were preserved, stained, and examined for pollen tube growth using a fluorescence microscope (Martin 1959).

Breeding Systems

The breeding system of *Echinocereus chisoensis* was defined through a series of crossing experiments. Pollination was controlled throughout the life of the flower by covering flowers, beginning with mature buds, with insect exclusion bags made of bridal veil fabric. Individual flowers were color coded by treatment with either plastic cable ties or yarn to track fruit development. To determine the role of self-pollination or apomixis, flowers (n = 28 in 1999, n = 16 in 2000) remained covered. Self-compatibility was tested by hand pollinating flowers (n = 37 in 1999, n = 37 in 2000) with their own pollen. In cross-compatibility tests in 1999, flowers (n = 55 in 1999) were hand pollinated using pollen harvested from at least three distant plants. In 2000, cross pollinations (n = 60) were made with pollen from only a single plant, and for 38 of the crosses the distance between the parents was measured. For comparison, fruit set from open pollinated flowers (n = 143 in 1999, n = 71 in 2000) was obtained by marking flowers that were not caged. In 1999 a sample of fruits (n = 63) was harvested for each treatment from each site, and they were sent to the Desert Botanical Garden in Phoenix where the numbers of seeds per fruit were counted. Results from the remaining treatments were recorded as either aborted fruit or set fruit. Chi-square tests (Sokal and Rohlf 1981) were used to determine if significant differences exist among treatments or between populations for fruit set. An analysis of variance was used to determine if the number of seeds produced per fruit was significantly different by treatment or by site. A pollen/ovule ratio and an outcrossing index (Cruden 1977) were prepared as another means of predicting the breeding system.

Attractants and Rewards

The presence of nectar was determined by observing the behavior of insect visitors and by using microcapillary tubes to probe flowers that were protected from insect visitors. Pollen production was estimated by placing all of the anthers of a single flower in a known volume of 3:1 lactic acid/glycerine solution, macerating the anthers to liberate the pollen, and then shaking the suspension with a Vortex Junior Mixer for 30 seconds. A small sample of the pollen suspension was removed with a pipette and placed on a hemocytometer. Pollen grains in the four corners and center square of the hemocytometer were counted, with repetitive counts made for each flower (n = 6). The number of pollen grains for the flower was estimated through the known volume of suspension (Dafni 1992). The number of ovules was determined by dissecting a mature ovary, and with the aid of magnification, counting the ovules.

Flower color was described using the *Naturalist's Color Guide* (Smithe 1975). Floral insect colors and patterns were determined by photographing the flowers, along with a gray scale for comparison, with Tri-X black-and-white film using a series of monochromatic filters (Kodak 35, 61, 48, 25, 65, 18). The resulting photographs were analyzed using Kevan's (1978, 1983) trichromatic technique for insect vision.

Pollen Vectors

Pollinators were determined through observation of flowers and visitors' intrafloral behavior. Voucher specimens of pollinators (i.e. those exhibiting appropriate floral behavior) were collected and sent to appropriate experts for identification.

Results

Phenology

Echinocereus chisoensis flowers have on average a 61.5 mm (SD ± 11.3) outside diameter, ranging from 34 to 85 mm (n = 28). The inside diameter is on average 35.7 mm (SD ± 9.6), ranging from 26 to 46 mm (n = 23). The degree of tepal reflex is determined by environmental conditions, but flowers usually remain cup shaped at full anthesis. The tricolored tepals are showy. The outer tips of the tepals are magenta-rose with a mid-circle of white followed by a band of deep magenta at the tepal base. The time of flower opening and closing varies with ambient temperature, sunlight, and wind, but typically during the peak flowering period flowers open between 0900 and 0930 hrs CDT (ranging from as early as 0800 hrs to as late as 1100 hrs) and close at approximately 1630 hrs (ranging from 1330 hrs to 1730 hrs). Most flowers last a single day, with rare individuals lasting two days. Anther dehiscence closely coincides with time of flower opening. Stamens are numerous and are attached to the bottom and along the curved sides of the floral cup. As a result anthers achieve different heights, with those nearest the style the shortest and those attached on the outer circle, the tallest. At first the stamens are bunched around the style. Shortly after the flower opens or with the first visitation, the stamens become vertical and no longer crowd the style. The anther mass is from 5 to 12 mm ($\mu = 9.1 \pm 1.3$ mm, n = 28) below the stigma. The green stigma is multi-lobed (ca. 10–17) with surface diameter ranging from 4 to 17 mm ($\mu = 8.9$ mm ± 3.4, n = 18). The lobes of the stigma may be vertical, spread horizontally, or spread with the tips erect. Regardless of initial position, the lobes do not change position appreciably during the life of the flower.

Pollen germination commences approximately 2 hours after cross pollination regardless of time of pollen deposition (1000 hrs CDT, 1100 hrs, 1200 hrs, 1330 hrs), suggesting similar stigma receptivity throughout the life of the open flower. Stigmas harvested approximately 17 hours after pollination did not interfere with seed production, indicating rapid pollen tube growth through the style. Results were inconclusive concerning pollen germination on self-pollinated stigmas.

Flower production begins around March 12 followed by a 2-week peak flower production. At S1 in 2000 the 84 plants observed produced a total of 273 flowers (including 17 flowers that had opened prior to the observation period) from shortly before March 12 to April 8, with 95 percent of the flower crop expended by March 24 (Figure 1). Likewise, in this period, 85 percent of the plants had completed flowering. In comparison, the ET population showed a slight delay in flowering with only 2 of the 169 flowers appearing before March 12. By April 10, 95 percent of the flowers had been produced and 85 percent of the 70 plants under observation had completed flowering (Figure 2). The percentage of flower completion at ET is skewed slightly because of the April 1 production of 21 flowers, 15 of which were produced on two plants. Flower production follows mast cycling with flowers appearing for 2 or 3 days followed by a day or more of no or few flowers (Figures 1 and 2). For the 2 study years, early March to mid-April defines the flowering period. On a few occasions, new flower buds were formed late in the flowering period. In years with adequate rainfall, the flowering period may be prolonged beyond what was observed during the droughty study period.

Of the 154 plants observed for flower production, 25 failed to produce flowers (Figure 3). At ET the cause was predation of flower buds (14) or, in one case, the death of the plant. At S1 plants either did not produce buds, buds stopped development, or they were eaten. Most plants (125) produced four or fewer flowers; 29 of the plants produced five or more flowers (Figure 3) with the record number of 17 flowers produced by an ET plant having seven stems. Generally, plants producing more than four flowers were multi-stemmed or multi-headed plants (3.16 average number of stems per head for plants with five or more flowers versus 1.46 for plants with fewer flowers).

Unsuccessful crosses result in abortion of the ovary approximately 1 week after flowering. When the ovary falls, there is a distinct depression surrounded by white cottonly trichomes on the stem where the ovary was attached. Fruits take approximately a month to mature, with little change in coloration. The dry fruits dehise longitudinally.

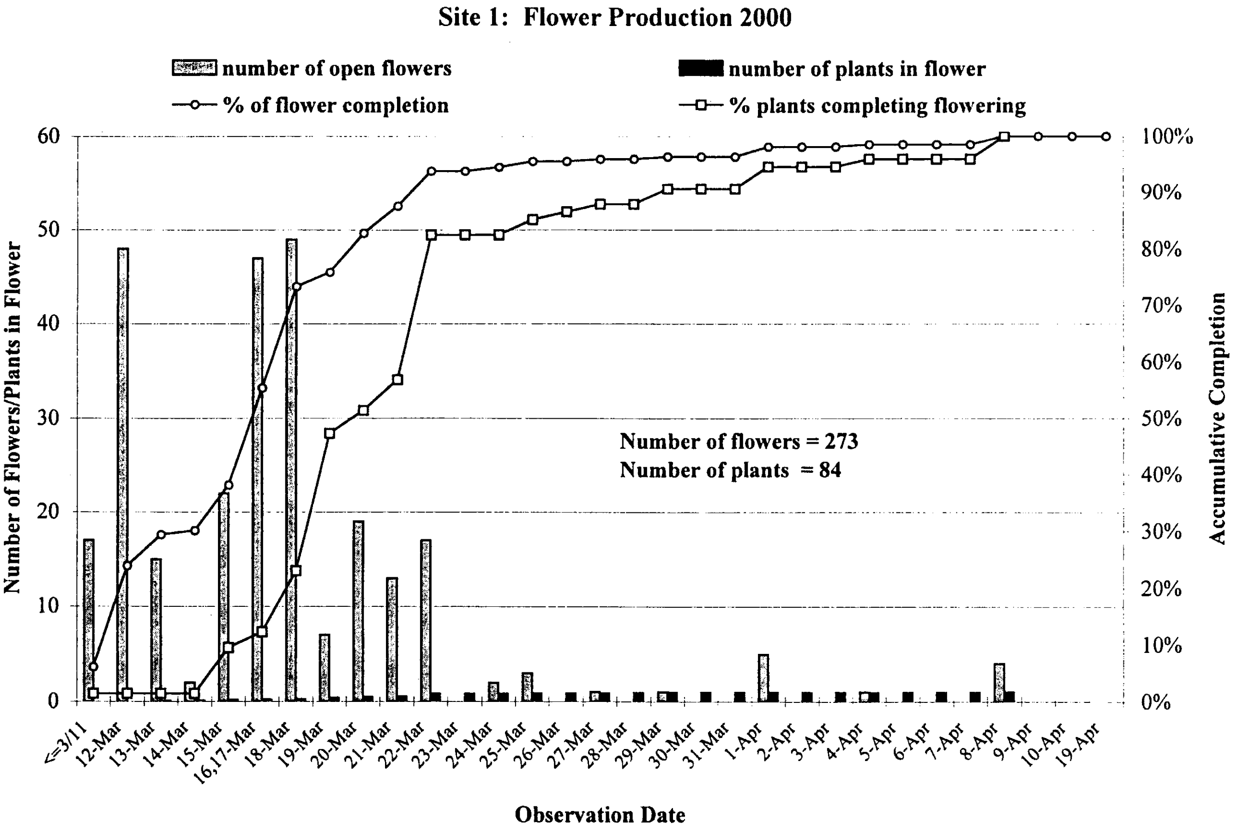

Figure 1. Flower production for S1 in 2000 with the bars showing the number of open flowers each day and the number of plants that produced flowers each day. The lines show accumulative completion of flower production as a percentage of flowers produced and by the percentage of plants completing flower production.

Breeding System

Results from non-manipulated and self-pollination tests were similar with few of the 44 non-manipulatechd flowers (1999, 11%; 2000, 0) or the 74 self-pollinated flowers (1999, 8%; 2000, 5%) forming fruits (Table 1). Chi-square tests showed no significant difference between sites or years (all $p > 0.05$). In 1999 fruit production was high for both the cross-pollinated (93%) and open-pollinated flowers (90%). There was no significant difference between the treatments.

In 2000 many of the ovaries from the cross-pollination (67%) and open-pollination (63%) tests were eaten. Fruit set data for non-manipulated and self-pollinated treatments were less affected because failure to set fruit is typically marked by abortion of the ovary about a week after flowering (Table 2). To use data from the 2000 crossing experiments, a Kruskal-Wallis non-parametric one-way analysis of variance was used to compare the number of days from treatment to result, with the result being defined as abortion, eaten fruit, or mature fruit. Results for self-pollinations were compared with cross and open pollinations. Because this analysis showed a significant difference (x^2 approximation = 54.6, df = 1, $p < 0.01$) between the two treatments, it is likely that ovaries that remained on the plant longer than the average for self-pollinations were forming seeds and being recorded as set fruit. Based on this assumption, comparisons between years showed no marked difference in regard to treatment or site (Table 1).

Based on breeding study results, *E. chisosensis* is self-incompatible, requires a pollinator, and is xenogamous. This is supported by a pollen/ovule ratio of 1416.7 which is within the range for facultative xenogamy (Cruden 1977). An outcrossing index of four also defines *E. chisosensis* as an outcrossing species (Dafni 1992).

Seeds per fruit ranged from 0 to 758, with an average of 399 (n = 73). For fruits with one or more seeds, there was no significant difference (F = 2.03,

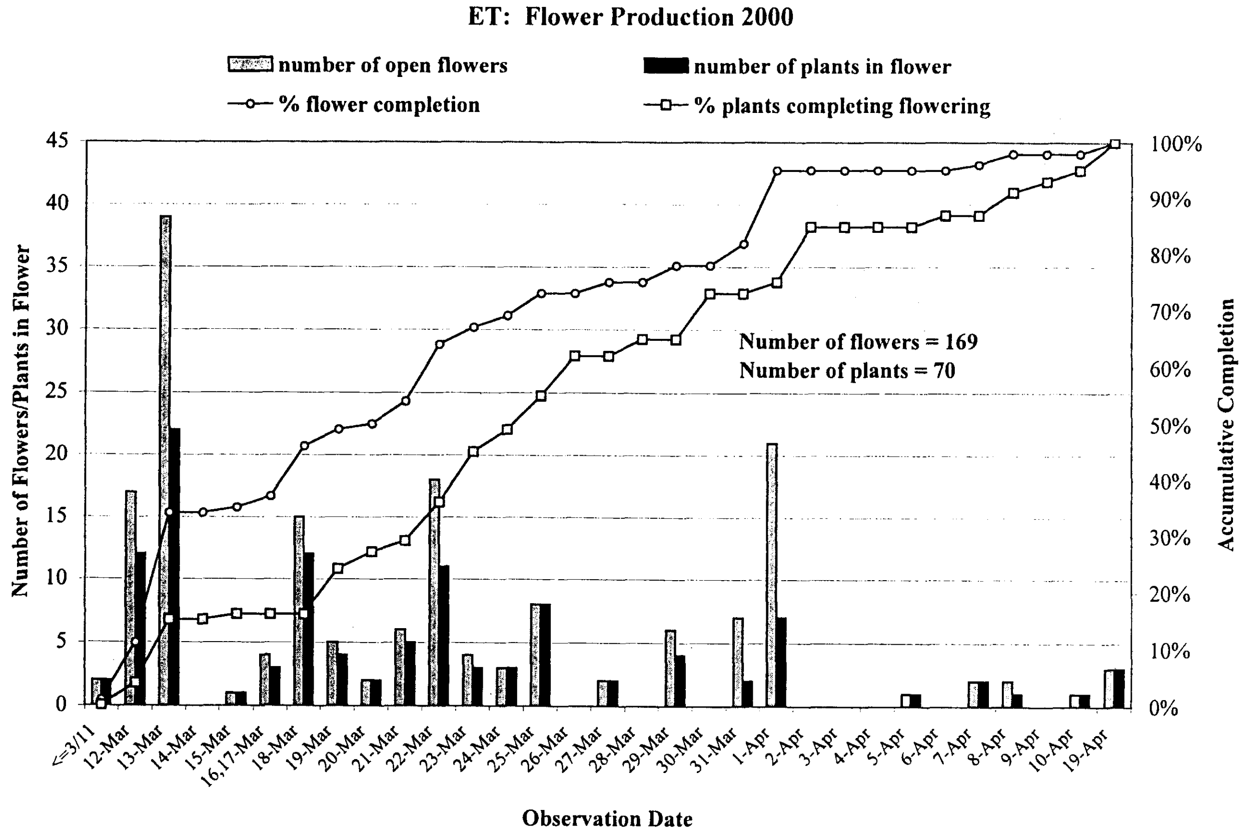

Figure 2. Flower production for ET in 2000 with the bars showing the number of open flowers each day and the number of plants that produced flowers each day. The lines show accumulative completion of flower production as a percentage of flowers produced and by the percentage of plants completing flower production.

df = 3, 63, p = 0.11) in the number of seeds formed at each site or between treatments (Table 3). Because of the rarity of fruit formation for the non-manipulated and self-pollinated treatments, only three fruits were harvested for each of these treatments and these six fruits are probably a result of experimental contamination. The bridal veil exclusion cages must be loosely attached to the cactus to prevent damaging the plant and are occasionally partially dislodged by the wind, allowing insect access. Self-pollinations conducted on three of the six plants in 2000 did not result in fruit formation.

In 2000, cross pollinations were made between parents separated by distances ranging from less than a meter (3 dm) to 157 meters. Only one of the flowers (2.7 m) failed to initiate fruit development (seed counts could not be evaluated because of high loss of fruits to predation). Based on these limited data, the distance between pollen donor and seed plant is not a limiting factor in successful reproduction. This study also involved only a single pollen donor for each cross, as compared to the use of three or more in 1999 cross pollinations. The high success with one pollen donor suggests

Table 1. Results from breeding experiments. Fruit set for cross- and open-pollinated treatments for 2000 are estimates. Due to predation, fruit set was based on how long fruit remained on the plant following treatment rather than mature fruits, as in 1999.

Treatment	N / Number of fruits formed	Percentage fruit set
1999		
Non-manipulated	28/3	11%
Self-pollinated	37/3	8%
Cross-pollinated	55/51	93%
Open-pollinated	143/129	90%
2000		
Non-manipulated	16/0	0%
Self-pollinated	37/2	5%
Cross-pollinated	60/54*	90%
Open-pollinated	71/70**	99%

*Six fruits aborted, 15 partially eaten, and 25 completely eaten.
**One fruit aborted, 5 partially eaten, and 40 completely eaten.

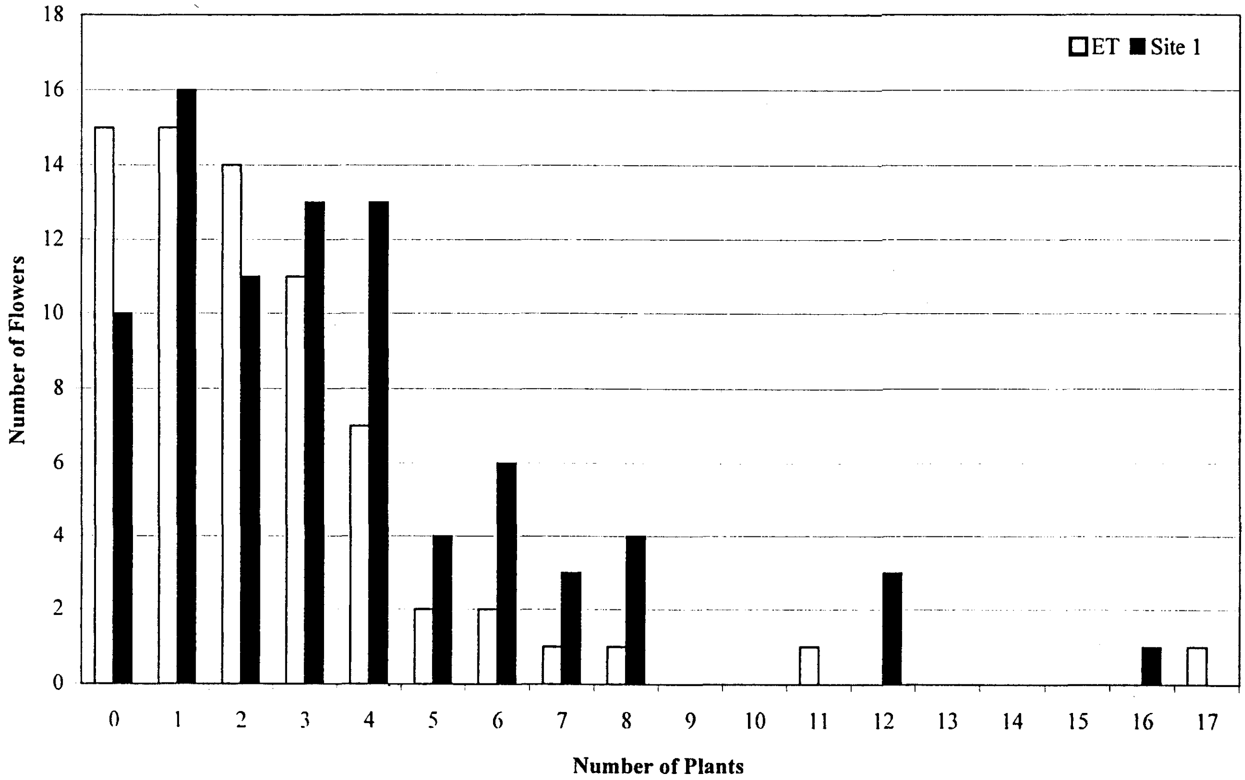

Figure 3. Number of flowers produced by each of the marked plants in 2000.

that pollinators may be equally successful even with short foraging distances or limited diversity in pollen loads.

Floral Attractants and Rewards

Insect visitors were observed harvesting pollen and foraging for nectar. However, nectar production is scanty, at best, because none could be collected using microcapillary tubes on protected flowers at any time during the day. Copious amounts of large, spiny, yellow pollen are produced in each flower (μ = 495,833 ± 185,133 per flower, n = 6). Flowers have a faint sweet aroma. The color pattern for bees is similar to that for humans. The tepals are tri-colored: insect green at the tips followed by a mid-circle of insect yellow, then insect red at the tepal base. The anthers are insect red-orange and the stigma is insect blue-purple. Studies comparing pre- and post-pollination colors and population variation are not complete.

Pollen Vectors

The frequency of all floral visitors was low (i.e. one visitor approximately every 30 min). Diversity of potential pollinators was also low with only three species of bees, *Megachile sidalceae* (Megachilidae), *Dialictus* sp. (Halictidae), and *Diadasia rinconis* (Anthophoridae) collected from the flowers. *Megachile sidalceae* was a rare visitor. The small polylectic *Dialictus* sp. bees were more frequent but ineffective pollinators because they entered and exited the flower without coming in contact with the stigma. The most frequent flower visitor was the mid-sized bee *Diadasia rinconis*. Males foraged for nectar and hunted for females while females visited the flowers for nectar and pollen. Floral arrivals and departures for both males and females occurred on and from the stigma. A single foray within the styles by a *Diadasia* resulted in a liberal dusting of pollen on the bee's body, the inner surface of the tepals, and, upon departure,

Table 2. The average number of days with the standard deviation from the day of flowering in 2000 by treatment to result, with result being defined as aborted fruit, eaten fruit, or harvested fruit. Data for the two sites are combined.

Treatment	Average number of days to result	N
Non-manipulated	8.7 ± 4.5	18
Self-pollinated	11.3 ± 7.6	36
Cross-pollinated	32.1 ± 7.0	59
Open-pollinated	32.4 ± 4.2	72

Table 3. Results from seed counts for 1999 by treatment.

Treatment	No. fruits counted	Range seeds per fruit	Avg seeds per fruit
Non-manipulated	3	457-599	545 ± 77
Self-pollinated	3	14-749	261 ± 423
Cross-pollinated	29	141-758	395 ± 162
Open-pollinated	38	158-735	401 ± 123

the stigma. Subsequent visits to other flowers resulted in pollination.

Diadasia is oligolectic on Cactaceae, especially *Opuntia* (Hurd 1979, Ordway 1987). *Diadasia rinconis* has been listed as an important pollinator or floral visitor for several cacti, including *Echinomastus erectrocentrus* var. *acunensis* (Johnson 1992), *Echinocereus engelmannii* var. *acicularis* (Johnson 1992), *Opuntia phaeacanthha* (Osborn et al. 1988), *Echinocereus dubius* and *E. stramineus* (Breckenridge and Miller 1982), *Opuntia lindheimeri*, *O. discata* (Grant et al. 1979), and *Opuntia basilaris* var. *treleasei* (Grant and Grant 1979a). In central Texas, Neff and Simpson (1992) reported *D. rinconsis* visiting flowers of *Opuntia engelmannii* var. *lindheimeri*, *O. leptocaulis*, *O. macrorhiza*, *Echinocereus reichenbachii*, and *Ferocactus setispinis*. When described, the intrafloral behavior for *D. rinconis* in these cacti is similar to what was observed in *E. chisoensis*.

Discussion

Echinocereus chisoensis displays the features characteristic of Southwestern Cactaceae flowers: medium to large brightly colored bowl-shaped flowers, copious yellow pollen, large stigma, and little to no nectar. It differs from other *Echinocereus* species (Breckenridge and Miller 1982, Johnson 1992, Leuck 1980, Grant and Grant 1979b) in having a flower that lasts a single day rather than 3–4 days. It is homogamous, a feature it shares with members of the *E. viridiflorus* complex, differing from the protandry of other Southwestern *Echinocereus* studied thus far. It is obligately xenogamous and heavily dependent upon the primary pollinator, the oligolectic cactus bee, *Diadasia rinconis*. Despite infrequent insect visitation, at least from the perspective of a human observer, there is no evidence of pollinator limitation as shown by the high percentage of open fruit set and no significant difference between hand cross-pollinated and open-pollinated seed set. Based on these first observations, the taxon's precarious existence does not appear to be directly related to its breeding system or pollination ecology.

Several caveats must be mentioned. The two populations studied thus far are the largest known for the species. These populations are large enough to attract sufficient numbers of pollinators. There is also apparently enough variability present in the population to prevent self-incompatibility from reducing seed set. Many *E. chisosensis* plants occur in small, isolated populations, some containing fewer than a dozen plants. Reduced population size, especially for an obligate xenogamous species, can have dramatic effects on reproductive output (Petanidou et al. 1995). Pollinator behavior and the number of pollen grains transferred are influenced by the geometry (i.e. size, density, shape) of a plant population (Handel 1983). Future studies will compare the reproductive success of the small populations with the larger ones and define the genetic variation for both small and large populations.

Abundant seed was produced in 1999 and would have again in 2000, except for predation caused by dry conditions. Without seed viability tests, we cannot evaluate the value of the seed rain and cannot totally eliminate the breeding system as a possible factor in poor recruitment. Very few seedlings have been observed in the field, albeit they are difficult to find with the nurse plant association. Future studies will address seed viability, germination requirements, and seed and seedling predation.

Another condition to consider is the drought of 1999 and 2000. Rainfall during both of the study years was less than average. In 1999, Rio Grande Village (RGV) and Panther Junction (PJ), the two nearest rainfall collection sites to the populations, showed a respective 27 percent and 31 percent decline in rainfall from 14-year averages of 9.41 (RGV) and 14.30 (PJ) inches (Big Bend National Park Communications Center Weather Data). Other than cacti, few species were in flower at either site. In years with more rainfall, floral resources will be more diverse. Leuck (1980) suggested that reproductive output in the *Echinocereus viridiflorus* complex may be higher during inclement conditions because the cactus flowers are virtually ignored in the presence of taxa with more substantial nectar rewards. However, the primary pollinators reported for *E. virdiflorus* flowers were solitary halictid bees rather than a cactus oligolege. Regardless, both polylectic bees with marked flower preferences and oligolectic bees are known to change floral species in dry years when their preferred hosts are rare or absent (Hurd and Linsley 1975).

If there is a strong dependency upon a single species of pollinator, such as suggested in this 2-year observation, fruit set will be more precarious because undoubtedly pollinator availability will differ from year to year. For example, in their discussion of partial bivoltinism in central Texas, Neff and Simpson (1992) observed 2 years in which *Diadasia rinconis* populations had low densities, despite abundant and appropriate floral resources. They concluded that this may reflect a pattern of skipping years in which a significant proportion of the *D. rinconis* population apparently spends 2 years rather than the usual single year in diapause.

Of particular interest is the continued study of the reproductive relationship between *E. chisoensis* and the other cacti, particularly the *Opuntia*, that share the habitat. *Diadasia rinconis* is a cactus oligolege but perhaps with a preference for *Opuntia* (Neff and Simpson 1992). When there is direct competition for pollinators, one species often competes more successfully than the other (Mosquin 1971). Small populations of rare species, particularly those less effective in attracting pollinators, may be prone to reduced seed set (Petanidou et al. 1995). The flowering period of *E. chisoensis* appears to begin about a week before the sympatric *Opuntias*. Does an earlier flowering time reduce competition between *E. chisoensis* and the *Opuntia*? With the vagarious nature of desert weather, timing of flowering of the cacti in the community is rarely absolute. In years with overlap in flowering times, does *E. chisoensis* produce as many fruits? Does *E. chisoensis* provide resources for the initial *Diadasia* populations which are then available to pollinate the later flowering *Opuntias*, providing pollination facilitation (Petanidou et al. 1995) or sequential mutualism (Mosquin 1971)? These are a few of the questions to be addressed in future years.

Acknowledgments

We thank Kelly McCoy, Angelo State University, for statistical assistance; Kathy Rice, Desert Botanical Garden, for seed counts; Terry Griswold, Utah State University, for bee identifications; Big Bend National Park (BBNP) for allowing the research to be conducted; and the Angelo State "hedgehog crew" of Sam Lightsey, Jason Neil, Mark Pourner, John Shelton, Larisa Watson, and Jody Davila (BBNP) for help in the field. The research was supported in part with a Beta Beta Beta Undergraduate Research Grant and an Angelo State University Carr Undergraduate Research Scholarship awarded to C. Vassiliou. Research was conducted under U.S. Fish and Wildlife Service permit TE006210-0 and Big Bend National Park permits BIBE-98-15 and BIBE-2000-017.

Literature Cited

Breckenridge, F. G. III, and J. M. Miller. 1982. Pollination ecology, distribution, and chemotaxonomy of the *Echinocereus enneacanthus* complex (Cactaceae). Systematic Botany 7:365–378.

Cruden, R. W. 1977. Pollen-ovule ratios: A conservation indicator of breeding systems in flowering plants. Evolution 31:32–46.

Dafni, A. 1992. Pollination ecology, a practical approach. Oxford University Press, New York.

Grant, V., and K. A. Grant. 1979a. Pollination of *Opuntia basilaris* and *O. littoralis*. Plant Systematics and Evolution 132:321–325.

Grant, V., and K. A. Grant. 1979b. Pollination of *Echinocereus fasciculatus* and *Ferocactus wislizenii*. Plant Systematics and Evolution 132:85–90.

Grant, V., K. A. Grant, and P. D. Hurd, Jr. 1979. Pollination of *Opuntia lindheimeri* and related species. Plant Systematics and Evolution 132:313–320.

Handel, S. N. 1983. Pollination ecology, plant population structure, and gene flow. In L. Real, ed. Pollination biology, pp. 163–212. Academic Press, Orlando.

Hendershott, A. J., L. A. Whitehead, A. R. P. Journet, and J. Sadler. 1992. Distribution and plant associations of the threatened chisos pitaya (*Echinocereus chisoensis*) in Big Bend National Park, Texas. Unpublished report to the Resource Management Division of Big Bend National Park, Texas.

Hurd, P. D. 1979. Superfamily Apoidea. In K. V. Krombein and P. D. Hurd, eds. Catalog of Hymenoptera in America north of Mexico, Vol. 2, pp. 2120–2123. Smithsonian Institution Press, Washington, D.C.

Hurd, P. D., and E. G. Linsley. 1975. The principal *Larrea* bees of the southwestern United States (Hymenoptera: Apoidea). Smithsonian Contributions to Zoology 193:1–74.

Johnson, R. A. 1992. Pollination and reproductive ecology of acuna cactus, *Echinomastus erectrocentrus* var. *acunensis* (Cactaceae). International Journal of Plant Science 153:400–408.

Kevan, P. G. 1978. Floral coloration, its colorimetric analysis and significance in anthecology. In A. J. Richards, ed. The pollination of flowers by insects, pp. 51–78. Academic Press, New York.

Kevan, P. G. 1983. Floral colors through the insect eye: What they are and what they mean. In C. E. Jones and R. J. Little, eds. Handbook of experimental pollination biology, pp. 117–141. Van Nostrand Reinhold, New York.

Leuck, E. E. II. 1980. Biosystematic studies in the *Echinocereus viridiflorus* complex. PhD dissertation, University of Oklahoma, Norman.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Techniques 34:125–128.

Mosquin, T. 1971. Competition for pollinators as a stimulus for the evolution of flowering time. Oikos 22:398–402.

Neff, J. L., and B. B. Simpson. 1992. Partial bivoltinism in a ground-nesting bee: The biology of *Diadasia rinconis* in Texas (Hymenoptera, Anthophoridae). Journal of the Kansas Entomological Society 65:377–392.

Ordway, E. 1987. The life history of *Diadasia rinconis* Cockerell (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 60:15–24.

Osborn, M. M., P. G. Kevan, and M. A. Lane. 1988. Pollination biology of *Opuntia polyacantha* and *Opuntia phaeacantha* (Cactaceae) in southern Colorado. Plant Systematics and Evolution 159:85–94.

Petanidou, T., J. C. M. Den Nijs, J. G. B. Oostermeijer, and A. C. Ellis-Adam. 1995. Pollination ecology and patch-dependent reproductive success of the rare perennial *Gentiana pneumonanthe* L. The New Phytologist 129:155–163.

Smithe, F. B. 1975. Naturalist's color guide. American Museum of Natural History, New York.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2nd ed. W. H. Freeman, New York.

U.S. Fish and Wildlife Service. 1993. Chisos Mountain hedgehog cactus (*Echinocereus chisoensis* var. *chisoensis*) recovery plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

U.S. Department of Agriculture, Soil Conservation Service. 1985. Soil survey of Big Bend National Park, part of Brewster County, Texas. United States Department of Agriculture.