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BAT AND HUMMINGBIRD POLLINATION OF AN AUTOTETRAPLOID COLUMNAR CACTUS, *WEBERBAUEROCEREUS WEBERBAUERI* (CACTACEAE)[1]

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I present data on flower morphology, pollination, breeding system, and genetic diversity of the columnar cactus *Weberbauerocereus weberbaueri* at 2500 m elevation in southwestern Peru. *Weberbauerocereus weberbaueri* is a self-compatible columnar cactus that is visited and pollinated by one species of rare endemic bat, *Platalina genovensium,* and two species of hummingbirds, *Patagona gigas* and *Rhodopis vesper. W. weberbaueri* exhibits pronounced interplant variation in floral color and size, and flowers exhibit traits corresponding to both bat and hummingbird pollination syndromes. Starch-gel electrophoresis of flower bud tissue indicated that *W. weberbaueri* is an autotetraploid and that genetic diversity (H_{ep} = 0.257) of the study population was high relative to diploid plants but similar to other tetraploid species. Initial fruit set from pollinator exclusion experiments conducted in 1991 and 1993, at the onset of and after a drought associated with the El Niño event of 1991–1992, revealed that bats were the most important pollinators in 1991, but that hummingbirds and diurnal insects were most important in 1993. In both years, however, autogamy and lepidopteran larval infestation of fruits reduced differences in mature fruit production among pollinator exclusion treatments so that differences in mature fruit set were not statistically significant. Reduced bat pollination in 1993 is attributed to the reduced abundance of bats at the study site during a drought caused by El Niño. I hypothesize that interaction among several factors, including tetraploidy, autogamy, larval infestation of developing fruits, and variation in pollinator abundance, may not result in strong selection for a bat vs. hummingbird floral morph, thus allowing the persistence of floral variation in this cactus.

Key words: Andean habitats; bats; Cactaceae; hummingbirds; Peru; pollination; polyploidy; *Weberbauerocereus.*

In arid habitats, a large proportion of plants belonging to the Cactaceae and Agavaceae exhibit floral adaptations for bat and/or hummingbird pollination (Porsche, 1939; Vogel, 1969). Several recent field studies in Mexico, Curaçao, and Venezuela have experimentally demonstrated the importance of bats (Nassar, 1991; Petit, 1995; Fleming, Tuttle, and Horner, in press) and/or hummingbirds (Fleming, Tuttle, and Horner, in press) as pollinators of columnar cacti. Traits associated with bat pollination of cacti include large, funnelform, nocturnal, white, and pungent-smelling flowers that produce large amounts of pollen and nectar. "Hummingbird" flowers, on the other hand, tend to be tubular, red, diurnal, and odorless, but may also produce copious nectar and large amounts of pollen (Faegri and van der Pijl, 1979; Helverson, 1993). Perhaps because bat and hummingbird floral syndromes share several characteristics, evolutionary transitions between these syndromes within a plant lineage may occur relatively frequently (Helverson, 1993). In fact, several plant families (including the Cactaceae), genera, and even species, exhibit floral traits corresponding to both bat and hummingbird pollination syndromes (e.g., Barthlott and Hunt, 1993; Helverson, 1993; Kress and Stone, 1993; Sazima, Sazima, and Buzato, 1994) yet few studies have compared the relative contribution of these pollinators to fruit and seed set.

The purpose of this study was to investigate the importance of bat and hummingbird pollination in *Weberbauerocereus weberbaueri* (tribe Trichocereeae), a columnar cactus occurring in southwestern Peru that exhibits floral traits associated with both bat and hummingbird pollination. Several factors motivated this study: first, the arid regions of Peru contain the second richest cactus flora after Mexico, with ≈44 genera and 249 species distributed primarily along the western Andean slopes (Weberbauer, 1945; Hunt, 1992). Yet, despite the diversity, abundance, and prominence of these plants, virtually nothing is known regarding their ecology and reproductive biology. Second, habitats occurring along the western slopes of the Andes are underrepresented within Peru's national reserve system, and have been ranked as deserving of high priority for research and conservation (Centro de Datos para la Conservacion, 1986). Third, the only nectarivorous bat occurring at mid-to-high elevation sites in southwestern Peru, *Platalina genovensium,* is an extremely rare and endemic bat for which no ecological information existed prior to this study. Finally, *W. weberbaueri* exhibits considerable within-population variation in several floral morphological characters including size, shape, and color, although this variation had never

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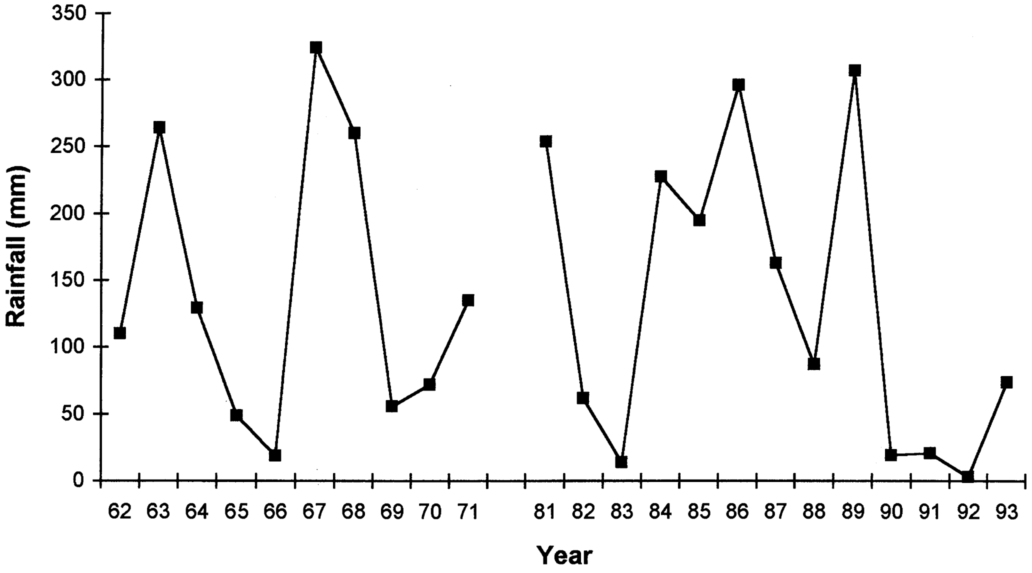

Fig. 1. Rainfall data for 1962–1971 and 1981–1993 for the Characato weather station (2 400 m above sea level). This station is ≈15 km from the main study site and occurs within the same life zone. El Niño events occurred in 1963–1965, 1969, 1972–1973, 1976, 1982–1983, and 1991–1992 (no rainfall data was available for 1969 and 1972–1973 events). Rainfall data from 1961 to 1976 from ONERN, 1974.

previously been quantified. Among-plant variation in floral color ranges from bright pink-red to white. This variation in floral color, along with the presence of long, stout, funnelform-tubular corollas and both diurnal and nocturnal nectar production, suggests adaptation to both bat and hummingbird pollination. Prior to this study, however, the relative importance of bat and hummingbird pollination for fruit production in *W. weberbaueri* was unknown.

To elucidate possible ecological and genetic mechanisms contributing to the presence of floral morphological variation in this cactus, I addressed the following five questions: (1) What is the morphological and genetic diversity of *W. weberbaueri* at the study site? (2) What is the extent of outcrossing vs. selfing occurring within the study population? (3) What is the relative importance of bats, hummingbirds, and insects as pollinators of *W. weberbaueri* and is the importance of pollinators constant between years? (4) What factors, in addition to effective pollination of flowers, influence successful development of maturing fruits? Finally, because this study coincided with the occurrence of the 1991–1992 El Niño southern oscillation, (5) How did climatic fluctuations associated with El Niño influence the pollination biology of this cactus?

MATERIALS AND METHODS

Study site and study organisms—The main study site is located in the department of Arequipa, Peru, 25 km south of the city of Arequipa (16.27° S, 71.30° W) on the western slopes of the Andes, at ≈2 500 m elevation. The site corresponds to the subtropical low-montane desert scrub life zone according to the Holdridge system (ONERN, 1976). Average annual rainfall for this life zone is 222 mm/yr, but varies substantially among years. Between 1990 and 1993, for instance, a drought associated with an El Niño event resulted in a drastic decrease in rainfall at the site (Fig. 1).

Weberbauerocereus weberbaueri is the most abundant columnar cactus in the region around the city of Arequipa (Aragon, 1982; C. T. Sahley, personal observation). Although the population around Arequipa has been classified as three species by Ritter (1981), the continuous variation among plants in flower morphology and color and the lack of any diagnostic alleles among morphs, has led me to consider them as one highly variable species, *W. weberbaueri,* in accordance with Hunt (1992).

Flowers of *W. weberbaueri* are hermaphroditic, begin opening between 1500 and 1700, remain open throughout the night, and generally close by 1100 the following morning. Nectar production is continuous, but variable, while the flower is open (Sahley, 1995). Although flower and fruit production peaks in September and October, some flower and fruit production occurs year-round (Sahley, 1995). *Weberbauerocereus weberbaueri* is visited by the rare and endemic nectar-feeding bat, *Platalina genovensium* (Phyllostomidae), two species of hummingbirds, *Patagona gigas* and *Rhodopis vesper* (Trochilidae), small, unidentified nocturnal moths, diurnal flies, bees, and rarely, butterflies (C. T. Sahley, personal observation).

Flower and fruit morphology—My observations indicated that variation in floral morphs was among, rather than within plants, so I collected one flower from each of 97 cacti in 1991 and one flower from 22 additional cacti in 1993. Unfortunately, low flower production per plant coupled with the necessity of using flowers for exclusion experiments hindered efforts to collect additional flowers per plant during the course of the study. However, long-term, repeated observations of plants confirmed that basic floral attributes such as color, shape, and presence or absence of stigma exsertion were at least qualitatively consistent within plants. I recorded outer corolla tube length, outer corolla tube width at both widest and narrowest points, petal length, stigma exsertion, and style length. The number of stigma lobes, corolla tube color, and petal color were also recorded. As the study progressed, additional floral characters were measured. Thus, in 1993 I also recorded ovary length, width, and inner corolla tube length and width. Petal color was scored in one of four categories: white, white tinged with varying degrees of reddish brown, maroon, and bright pink-red.

I collected one mature fruit from each of 115 plants. Fruits when immature were green and took ≈2 mo to mature; when mature they usually changed color and soon after opened to expose the fruit pulp and seeds. I recorded width and length of all fruits and external fruit color of 69 fruits. Seeds were counted from 14 open-pollinated fruits, and individual seed masses were recorded for five seeds per fruit for 26 fruits.

Genetic diversity and mating system—To sample genetic diversity in the study population, flower bud tissue was collected from 25 adults in 1993. Bud tissue was crushed with liquid nitrogen prior to addition of phosphate-polyvinylpyrrolidone (PVP) extraction buffer. For the mating system analysis, one mature fruit was collected from each of 31 plants. Seeds were germinated on moist filter paper in petri dishes inside growth chambers. Germination usually occurred within 10–14 d. Six to 24 seedlings at the cotyledon stage ($\approx$3 wk old; mean number seedlings per fruit = 14) per fruit were prepared for electrophoresis by crushing with a PVP extraction buffer (see Mitton et al., 1979).

Starch gel electrophoresis was performed on prepared tissues using buffer systems and staining recipes of Soltis et al. (1983). Eight enzyme systems with 15 loci were assayed for the bud tissues. Of these, one probable monomorphic (*Pgm*-1) and two polymorphic loci (*Fe*-2, *Aat*-1) were not consistently scoreable and were not used in analyses. The enzyme systems and loci used were: phosphoglucoisomerase (*Pgi*-1, *Pgi*-2), alcohol dehydrogenase (*Adh*), phosphoglucomutase (*Pgm*-2), mannose reductase (*Mnr*-1), fluorescent esterase (*Fe*-1), triose-phosphate isomerase (*Tpi*-1, *Tpi*-2, *Tpi*-3), leucine-amino peptidase (*Lap*), 6-phosphogluconate dehydrogenase (*6PGD*), and aspartate amino transferase (*Aat*-2). Buffer system 8- was used for *Fe, Mnr,* and *Aat,* system 11 for *Pgm* and *6PGD,* and system 34 for *Tpi, Adh,* and *Lap.* Adult genotypes were scored for all of the above loci. Only *Adh*-1, *Lap, Tpi*-3, *Aat*-1, *Mnr,* and *6PGD* were used in mating system analyses. Maternal genotypes were inferred from the genotypes of the seedlings.

Electrophoretic data from flower bud tissue collected from adult plants were used to calculate the following genetic parameters as in Hamrick and Godt (1990): percentage polymorphic loci, mean number of alleles per locus, and genetic diversity. Genetic diversity (H_{ep}) was calculated for each locus as $H_{ep} = 1 - \Sigma p_i^2$, where p_i is the frequency of the i^{th} allele. An overall mean H_{ep} was obtained by averaging over all loci.

To determine the degree of outcrossing vs. selfing in the population, genotypic data from 416 seedlings of 31 open-pollinated fruits were analyzed using the multiallelic version of the TETRAT program written by Ritland (1990), which is fully described in Murawski et al. (1993). The program inferred maternal genotypes from their progeny arrays by calculating probabilities of progeny derived from selfing and outcrossing and then calculated population-level multilocus and single-locus estimates of outcrossing rates. The program also calculated standard errors of the estimates using the bootstrap method. Fifty among-family (i.e., fruit) bootstraps were used for population estimates.

Pollinator exclusion experiments—Pollinator exclusion experiments were conducted from September to November in 1991 and 1993. Replication of experiments in 1992 was not feasible due to the extremely low flowering frequency of *W. weberbaueri* that year. Flowers from 48 plants in 1991 and 51 plants in 1993 were subjected to six pollination treatments in a randomized block design, so that when possible, each plant underwent all six treatments. Because daily flowering frequency is low in *W. weberbaueri,* generally only one treatment per night per plant was applied; exclusion experiments thus took several weeks to complete. In a few instances, more than one treatment per night was applied to a plant. This occurred when it appeared that not enough flowers would be available in the future to complete the experiment. The pollinator exclusion treatments applied in 1991 and 1993 were: (1) open control, which allowed access to all flower visitors; (2) total exclusion, which prohibited access to all visitors; (3) diurnal exclusion, which permitted bats and nocturnal insects access to flowers, but excluded diurnal visitors such as hummingbirds and insects; (4) nocturnal exclusion, which permitted hummingbirds and diurnal insects access to flowers but excluded nocturnal visitors; (5) bat and diurnal exclusion, which permitted access to nocturnal insects only; and (6) hummingbird and nocturnal exclusion, which permitted access to diurnal insects only (Table 1). Materials used to exclude potential pollinators consisted of either 2.54 cm (1 inch) hexagonal wire mesh (used to exclude vertebrates, but not insects) or bridal veil netting (used to exclude all visitors). For treatment (1), no excluders were used, while for treatment (2) (total exclusion) bridal veil netting was placed over the flower prior to opening and removed after the flower closed the following day. The diurnal exclusion treatment consisted of putting bridal veil netting over the flower in the late afternoon prior to opening, removing the netting at sunset (between 1740 and 1800), and replacing it again just before sunrise (between 0445 and 0515). The nocturnal exclusion treatment consisted of placing bridal veil netting over flowers after opening at sunset and removing the netting the following morning just prior to sunrise. Treatment (5), the bat and diurnal exclusion, involved placing bridal veil over flowers prior to opening and replacing the bridal veil netting with wire mesh at sunset. Before sunrise, the wire mesh was again replaced with bridal veil netting, which was removed the following day after flowers had closed. Treatment (6), the hummingbird and nocturnal exclusion, involved placing wire mesh excluders over flowers prior to opening and replacing the wire mesh excluders with bridal veil netting at sunset. Before sunrise, the bridal veil netting was replaced with wire mesh, which was removed later that day after flowers had closed.

TABLE 1. Pollinator exclusion treatments performed in 1991 and 1993 for *Weberbauerocereus weberbaueri.*

Treatment number	Pollination treatment	Description of flower treatment	Flower visitors allowed
1	Open	No treatment	Insects, bats, hummingbirds
2	Total exclusion	Bridal veil during entire time flower open	None
3	Diurnal exclusion	Bridal veil netting during day	Bats, nocturnal insects
4	Nocturnal exclusion	Bridal veil netting during night	Hummingbirds, diurnal insects
5	Diurnal and bat exclusion	Bridal veil netting during day and wire mesh during night	Nocturnal insects
6	Nocturnal and hummingbird exclusion	Bridal veil netting during night and wire mesh during day	Diurnal insects

All cacti used in the pollinator exclusion experiments were tagged and numbered. Each experimental flower was also individually tagged and numbered using soft aluminum tags inserted into the cactus 2 cm below the flower. Each flower was censused once a week until the fruits matured. I recorded whether flowers and fruits were on or off the plant, whether fruit development had initiated (indicated by swelling of ovaries 1 wk after treatment; this was used as an indication of effective pollination), and presence or absence of lepidopteran larval infestation. After fruits began to develop, 0.635 × 0.635 cm (¼ inch) wire mesh was placed over them to prevent fruit predation by vertebrates. In 1991 all undamaged, mature experimental fruits were collected and their seeds were stored in labeled envelopes. Seeds from 14 open-pollinated fruits were counted and weighed, and all seeds from the other treatments were weighed. In 1993 fruit set was recorded, but fruits were not collected.

Statistical analyses—Data from the pollinator exclusion experiments were analyzed using SYSTAT version 5.3 (Wilkinson, 1991) and SAS

TABLE 2. Morphological measurements for flowers of *Weberbauerocereus weberbaueri*. Coefficients of variation are in parentheses.

	Outer corolla tube length (cm)	Outer corolla tube width (widest) (cm)	Outer corolla tube width (narrowest) (cm)	Petal length (cm)	Style length (cm)	Stigma exsertion (mm)	Number of stigma lobes	Ovary length (mm)	Ovary width (mm)
Mean	7.57	2.20	1.45	2.08	7.49	7.2	11.49	7.02	7.4
	(0.11)	(0.12)	(0.11)	(0.15)	(0.15)	(0.79)	(0.12)	(0.32)	(0.13)
	$N = 96$	$N = 71$	$N = 69$	$N = 95$	$N = 92$	$N = 92$	$N = 51$	$N = 21$	$N = 22$
Minimum	5.2	1.5	1.0	1.2	3.5	0	8	3.7	5.8
Maximum	10.3	2.9	1.9	2.9	10.5	2.5	15	14.9	9.1

(SAS, 1989). Differences among treatments in the frequency of fruit initiation and fruit maturation were analyzed using an experimentwise likelihood ratio χ^2 statistic (*G* statistic; Sokal and Rohlf, 1981) for each year and for both years combined. Individual treatment *G* statistics were also calculated for each year and both years combined following the procedure in Sokal and Rohlf (1981). Chi-square tests were used to conduct pairwise comparisons between the diurnal and diurnal plus bat exclusion treatments, as well as between the nocturnal and nocturnal plus hummingbird exclusion treatments. The *G* statistic was also used to analyze differences in fruit initiation frequencies and the proportion of mature fruit set between years as well as between-year differences in frequency of larval infestation and fruit mortality due to larval infestation.

Seeds from mature fruits collected in 1991 were weighed to the nearest 0.0001 g. A Pearson product-moment correlation coefficient was calculated to determine the relationship between total seed mass and seed number per fruit for the 14 open-pollinated fruits. A one-way analysis of variance was performed to analyze differences in total seed mass per fruit among treatments. Because treatments (5) and (6) did not differ significantly from the total exclusion treatment for both fruit initiation and mature fruit set in either year or in both years combined, seeds from these treatments were pooled with the total exclusion treatment in order to increase sample size.

RESULTS

Flower and fruit morphology—*W. weberbaueri* flowers were tubular to slightly funnelform in shape; flowers were long, ranging from 5.2 cm to 10.3 cm, and corolla tube width was narrow, especially just above the nectary (Table 2). The most variable floral morphological characters were petal length, ovary length, style length, and length of stigma exsertion (Table 2). Despite variation in style and stigma exsertion length, most flowers (82%) exhibited some degree of exsertion. The most common petal color ($N = 117$) was white tinged with varying degrees of reddish-brown (42%) followed by white (38%), maroon (14%), and pink-red (6%) Within-plant flower color was constant and did not exhibit seasonal or annual variation. All flowers regardless of shape or color, opened between 1500 and 1700 (prior to sunset) and remained open until late morning (between 0900 and 1100).

Mature fruits ($N = 69$) ranged in color from bright red (23%), to orange-red (29%), orange (23%), orange-yellow (4%), yellow (4%), and green (15%). Seed number and seed mass in *W. weberbaueri* were more variable than fruit length and width (Table 3). The number of seeds per fruit ranged from 181 to 1 533; individual seeds on average weighed slightly less than 0.001 g.

Genetic diversity and mating system—Banding patterns obtained from assays of all polymorphic enzyme systems in all individuals indicated that *W. weberbaueri* is an autotetraploid. Both balanced and unbalanced heterozygote banding patterns, corresponding to equal and unequal combinations of alleles, were present in adult and offspring tissues. Fixed heterozygotes, which would be expected to occur in allotetraploids, were never observed for any enzyme system.

Of the 12 resolvable loci assayed, eight (66.6%) were polymorphic. The number of alleles in adult heterozygotes ranged from two to four per locus per individual plant (Table 4); the mean number of alleles per locus was

TABLE 3. Fruit morphology of *Weberbauerocereus weberbaueri*. Coefficients of variation are in parentheses.

	Fruit length (mm)	Fruit width (mm)	Seed number per fruit	Individual seed mass (g)
Mean	27.0	30.6	1 067	0.0009
	(0.15)	(0.14)	(0.39)	(0.31)
	$N = 115$	$N = 113$	$N = 14$	$N = 133$
Minimum	15.7	18.4	181	0.0004
Maximum	37.5	41.5	1 533	0.0020

TABLE 4. Allele frequencies of eight polymorphic loci for adult plants at the main study site. *Tpi*-1, *Tpi*-2, *Pgi*-1, *Pgi*-2 were monomorphic.

Locus	Allele designation	Allele frequency	Genetic diversity (H_{ep})
FE-1	1	0.408	0.704
	3	0.276	
	4	0.197	
	5	0.118	
MNR	2	0.927	0.135
	3	0.073	
PGM-2	2	0.307	0.425
	3	0.693	
6P-2	2	0.546	0.516
	3	0.432	
	4	0.023	
AAT-2	3	0.875	0.219
	4	0.125	
TPI-3	3	0.890	0.196
	4	0.110	
ADH	1	0.060	0.267
	3	0.850	
	4	0.080	
	5	0.010	
LAP	2	0.227	0.625
	3	0.546	
	4	0.113	
	5	0.114	

TABLE 5. Proportion initiated and mature fruits for *Weberbauerocereus weberbaueri* according to pollinator exclusion treatment. *G* statistics are in parentheses.

	1991			1993			1991–1993 Combined		
Treatment	*N*	Initiated fruits	Mature fruits	*N*	Initiated fruits	Mature fruits	*N*	Initiated fruits	Mature fruits
Open	51	0.55	0.35	44	0.64	0.25	95	0.59	0.30
Total exclusion	40	0.40	0.28	40	0.48	0.25	80	0.44 (5.1099)*	0.26
Diurnal exclusion	40	0.70 (5.534)*	0.48	43	0.60	0.16	83	0.65	0.32
Nocturnal exclusion	41	0.56	0.37	46	0.76 (4.927)*	0.35	87	0.67 (3.8610)*	0.35
Bat and diurnal exclusion	32	0.38	0.31	39	0.54	0.26	71	0.46	0.28
Hummingbird and nocturnal exclusion	30	0.47	0.20	42	0.60	0.26	72	0.54	0.23
Experimentwise *G* statistics	234	11.177*	7.003	254	8.671	4.128	488	14.769*	3.127

* $P < 0.05$ (likelihood ratio χ^2 to test hypothesis of equal proportions among treatments).

2.25. Mean genetic diversity (H_{ep}) of adults in the study population was 0.257.

Analysis of the mating system of open-pollinated seedlings from 1991 indicated that population outcrossing levels were high based on both multilocus ($t_m = 0.937$, SE = 0.042) and single locus ($t_s = 0.869$, SE = 0.047) estimates.

Pollinator exclusion experiments*—Fruit initiation frequencies*—Data on fruit initiation frequencies indicated that bats, hummingbirds, and diurnal insects effectively pollinate *W. weberbaueri* flowers but that flowers also produce fruits in the absence of flower visitors (Table 5). In 1991, fruit initiation frequencies among pollinator exclusion treatments differed significantly ($G = 11.177$, df = 5, $P < 0.05$). The diurnal exclusion treatment (bat plus nocturnal insect pollination) accounted for a significantly higher proportion of fruits initiated (70%; $G = 5.534$, df = 1, $P < 0.05$) than the other five treatments. Pairwise comparison between the diurnal exclusion treatment and the bat and diurnal exclusion treatment revealed a significant difference, ($\chi^2 = 6.34$, $P = 0.01$), indicating that the high fruit initiation frequency for the diurnal exclusion treatment was due to bat, not nocturnal insect pollination. Remaining treatments were not significantly different from the hypothesis of equal proportions among treatments. Forty percent of flowers from which all flower visitors were excluded initiated fruit development, indicating the presence of self-compatibility and autogamy in *W. weberbaueri.* Because flowers in this treatment were not emasculated, however, the possibility of apomixis having occurred cannot be ruled out.

In contrast to 1991, differences in fruit initiation frequencies among pollinator exclusion treatments conducted in 1993 were not significant ($G = 8.671$, df = 5, $P > 0.05$). Examination of the contributions of individual treatments to the overall *G* statistic indicates that in 1993, only hummingbird- and diurnal-insect-visited flowers (nocturnal exclusion) resulted in a significantly higher proportion (76%) of fruits initiated ($G = 4.927$, df = 1, $P < 0.05$) relative to expected values. Pairwise comparison to the hummingbird and nocturnal exclusion treatment did not reveal a significant difference ($\chi^2 = 0.086$, $P = 0.768$), indicating that in addition to hummingbirds, diurnal insects may have played a role in pollination. As in 1991, a relatively high proportion of flowers (48%) developed fruits in the absence of flower visitors.

When data from 1991 and 1993 are combined, differences among treatments were significant, with the nocturnal exclusion treatment (hummingbird and diurnal insect pollination) contributing a significantly greater proportion of developing fruits ($G = 3.861$, df = 1, $P < 0.05$) and the total exclusion treatment contributing a significantly smaller proportion of fruits ($G = 5.1099$, df = 1, $P < 0.05$).

Mature fruit set—In 1991, the diurnal exclusion treatment (bat pollination) resulted in the largest proportion of mature fruits (48%), while in 1993, the nocturnal exclusion treatment (hummingbird and diurnal insect pollination) yielded the largest proportion (35%) of mature fruits (Table 5). However, differences in mature fruit set among treatments in both 1991 and 1993 were not significant at the experimentwise or individual level, nor were they significant when data for 1991 and 1993 were combined.

Total seed mass per fruit was highly correlated with the total number of seeds ($r = 0.915$, $P < 0.001$). However, differences in total seed mass for mature experimental fruits collected in 1991 were not significant among treatments (one-way ANOVA, $F = 0.727$, $P = 0.539$; Table 6).

Between-year comparisons—In 1993, fruit initiation frequencies were significantly higher for the nocturnal exclusion treatment than in 1991 ($G = 3.920$; df = 1, $P < 0.05$; Table 7). However, differences in mature fruit set between years for the nocturnal exclusion treatment were not significant. The most marked difference between years was the decrease in mature fruit set by bat-

TABLE 6. Total seed mass per fruit by pollinator exclusion treatment in 1991. Data from the total exclusion treatment were pooled with bat and hummingbird exclusion treatments.

Pollinator exclusion treatment	No. of fruits	Mean total seed mass per fruit (g)	SD
Open	22	0.838	0.460
Total exclusion	23	0.842	0.550
Diurnal exclusion	28	1.00	0.406
Nocturnal exclusion	15	0.789	0.292

TABLE 7. Fruit initiation and mature fruit set compared between years for *Weberbauerocereus weberbaueri*. Numbers indicate *G* statistics.

Treatment	1991 vs. 1993		
	Flowers	Initiated fruits	Mature fruits
Open	95	0.747	1.190
Total exclusion	80	0.457	0.065
Diurnal exclusion	83	0.832	9.243**
Nocturnal exclusion	87	3.920*	0.010
Bat and diurnal exclusion	71	1.236	0.273
Hummingbird and nocturnal exclusion	72	1.166	0.077

* $P < 0.05$; ** $P < 0.01$ (likelihood ratio χ^2 for deviation of expected ratio of 1:1 for each pollination treatment).

visited flowers (diurnal exclusion) in 1993 relative to 1991 ($G = 9.243$, $P < 0.001$). Despite the variability in bat and hummingbird pollination effectiveness, both fruit initiation frequencies and mature fruit set for open-pollinated flowers did not differ significantly between years. The proportion of both autogamous initiated and mature fruits was also constant from year to year.

Larval infestation of developing fruits—Infestation of developing fruits by unidentified lepidopteran larvae occurred in both 1991 and 1993 and had a pronounced effect on fruit survivorship. Fruit mortality in developing, open-pollinated fruits ranged from 36% in 1991 to 61% in 1993; of these, 11% and 39%, respectively, were positively identified as being due to larval infestation. This difference in fruit mortality due to larval infestation between years was statistically significant ($G = 7.74$, df = 1, $P = 0.005$). Differences in fruit mortality among pollinator exclusion treatments were not significantly different in 1991 or in 1993 ($G = 5.051$, df = 5, $P = 0.410$; $G = 3.689$, df = 5, $P = 0.595$, respectively).

DISCUSSION

Flower shape, color, and nectar production of *W. weberbaueri* at the study site are consistent with bat and hummingbird pollination syndromes. Results of this study indicate that bats, hummingbirds, and diurnal insects visit and pollinate flowers of *W. weberbaueri*. Nevertheless, a large proportion (26%) of flowers from which all visitors were excluded set fruit, indicating the presence of self-compatibility and autogamy (or possibly apomixis) in this cactus. Mating system analysis, however, suggests that if flowers are visited, they are highly outcrossed.

Interestingly, in neither year was the open pollination treatment the most successful. Open-pollinated flowers never demonstrated any form of damage due to flower visitors. Preliminary observations indicate that because open-pollinated flowers were never covered with netting, they may have been exposed to a higher degree of larval infestation than the other treatments. This may have caused the slightly lower fruit initiation frequencies, but since I did not want to unintentionally damage flowers by checking them for larvae, this possibility needs to be explored further. Nocturnal insect visitation to flowers did not significantly contribute to either fruit initiation or mature fruit set in either year, while diurnal insect visitation contributed to fruit initiation in 1993 only. Differences in mature fruit set among pollination treatments within a year were statistically undetectable, as were differences in seed production. The importance of bats as pollinators varied between years with the proportion of bat-pollinated flowers being greater in 1991 than in 1993. In 1993, hummingbirds and diurnal insects were the most important pollinators. Lepidopteran larval infestation and subsequent fruit mortality were greater in 1993 than in 1991. Despite variability in pollinator effectiveness as measured by fruit initiation frequencies, mature fruit set of open-pollinated flowers did not differ significantly between 1991 and 1993.

Genetic and morphological diversity—Polyploidy is frequent (35–47% of species) in angiosperms (Grant, 1971; Stebbins, 1971), and the Cactaceae are no exception. Pinkava et al. (1985) found that 27.9% of 551 cactus taxa analyzed were polyploid. Polyploidy has been hypothesized as having several advantages and/or consequences. For example, polyploidy enhances the capacity of individuals and populations to "store" genetic variation (Briggs and Walters, 1990). Several theoretical and empirical studies have shown that autotetraploids have higher heterozygosity relative to diploids (Ness, Soltis, and Soltis, 1989; Soltis and Soltis, 1989; Wolf, Soltis, and Soltis, 1990; Moody, Mueller, and Soltis, 1993). Genetic diversity of *W. weberbauerocereus* in the study population is high compared to diploid plants with similar mating systems, but is similar to estimates in other autotetraploid plants (Table 8).

A second advantage to polyploidy is the release of genetic variation and a consequent increase in ecological amplitude and geographical distribution relative to diploids (Briggs and Walters, 1990; Brochmann and Elven, 1992). Previous authors (Backeberg, 1976; Ritter 1981) have noted the morphological variation present within and among populations belonging to the genus *Weberbauerocereus* but have assumed that this variation was due to interspecific differences. This study suggests that only one species is present in the vicinity of Arequipa

TABLE 8. Levels of heterozygosity in *W. weberbaueri* and other autotetraploid and animal-outcrossing diploid plants.

Plant species	Heterozygosity	Source
Weberbauerocereus weberbaueri (Cactaceae)	0.257 (Autotetraploid)	This study
Pachycereus pringlei (Cactaceae)	0.209 (Autotetraploid)	J. Hamrick (University of Georgia) and T. Fleming (University of Miami, unpublished data)
Heuchera grossularifolia (Saxifragaceae)	0.159 (Autotetraploid)	Wolf, Soltis, and Soltis (1990)
Tolmiea menziesii (Saxifragaceae)	0.237 (Autotetraploid)	Soltis and Soltis (1989)
Mixed-animal outcrossing (mean value, $N = 85$)	0.120 (SD = 0.015) (Diploid species)	Hamrick and Godt (1990)

and that much of the intrapopulational variation may be because of high heterozygosity caused in part by autotetraploidy.

Mating system—Analysis of the mating system of *W. weberbaueri* based on fruits collected in 1991 indicated that most seedlings were outcrossed (t_m = 0.937); this was probably because both bats and hummingbirds were present that year and high visitation rates resulted in high levels of outcrossing. Unfortunately I did not estimate outcrossing rates in 1993, when the proportion of bat pollination was low. Because the increase in homozygosity due to selfing is far less for a tetraploid than for a diploid (Moody, Mueller, and Soltis, 1993), facultative autogamy and self-compatibility in *W. weberbaueri* may be an effective strategy in years when important pollinators such as bats are absent.

The high degree of stigma exsertion exhibited by most flowers likely decreased the possibility of self-pollination during visits by bats or hummingbirds in 1991. Motten and Antonovics (1992) examined outcrossing rates in *Datura stramonium* and found that the most important determinant of outcrossing rate was the degree of stigma exsertion exhibited by flowers. Flowers with stigmas positioned above the anthers had higher outcrossing rates than did those with overlapping stigmas and anthers. Results obtained for the bat-pollinated Mexican columnar cactus *P. pringlei* show that open-pollinated hermaphrodite flowers were found to have a low outcrossing rate (t_m = 0.301) even though high flower visitation rates were observed (Murawski et al., 1993; Fleming et al., 1994). Murawski et al. (1993) hypothesized that the high degree of selfing in the presence of flower visits was due to absence of stigma exsertion in flowers of *P. pringlei.*

Variation in pollinator effectiveness and larval infestation among years: the role of El Niño—As in the rest of western Peru, the arid western slopes of the Peruvian Andes are subject to dramatic climatic changes associated with the El Niño southern oscillation (Rasmusson and Wallace, 1983). Changes in sea level and temperature during this time cause considerable reduction in primary productivity of upwelling areas off the coast of Peru that result in the elevation of mortality rates and disruption of breeding schedules in seabirds, and marine mammals (Barber and Chavez, 1983; Schreiber and Schreiber, 1984; Castro, 1986), while having a beneficial effect on Andean condor populations (Wallace and Temple, 1988). However, the effects that such interyear variability in climate have on terrestrial ecological interactions in Peru have previously received little attention.

A major finding of this study was that mature fruit production attributable to bat pollination decreased significantly in 1993 relative to 1991 because of a decrease in bat populations near the study area. The 1991–1992 El Niño event led to 3 yr of low rainfall at the study site and a rainless period that spanned 17 mo. Phenology data obtained over the course of this study indicate that fruit and flower production of *W. weberbaueri* dropped considerably during this time, while census data obtained from 1990 to 1993 for *Platalina genovensium* demonstrated a substantial decrease in bat abundance in 1992 and 1993 (Sahley, 1995). Because data on the diet of *P. genovensium* indicates that in Arequipa, it specializes on cactus flowers and fruit, and no other species of cactus produced flowers or fruit during the drought, it is probable that bats emigrated from the study area during the drought (Sahley, 1995). While I did not census hummingbirds systematically, one of two species, *Rhodopis vesper,* was consistently observed throughout the entire study. Because of the small size of this bird (≈4.5 g), its total energetic requirements are lower than those of *P. genovensium* (average mass = 19 g; Sahley, 1995). Therefore, in addition to being able to subsist on fewer flowers than *P. genovensium* during times of low flower abundance, *R. vesper* was also able to feed from plants that produced small flowers that would have been unavailable to bats (C. T. Sahley, personal observation). If, as the data suggest, flower, fruit, and bat abundance decrease near Arequipa during drought conditions, then in some years bats are the most important pollinators, but in other years hummingbirds and diurnal insects may be more important.

Dramatic shifts in rainfall availability at the study site associated with El Niño events (Fig. 1) have implications not only for the pollination of columnar cacti, but likely have a large influence on variation in larval infestation frequency as well. Larval infestation and fruit mortality varied between years in this study, probably as a result of differences in lepidopteran abundance during and after the drought. After rains resumed in 1993 insects, especially moths and butterflies, were observed more frequently than they had been in late 1991 and 1992 (C. T. Sahley, personal observation). Thus, El Niño events, by influencing rainfall, plant phenology, and pollinator abundance, may play an important role in this pollination mutualism.

Maintenance of variation in W. weberbaueri—An important result of this study was that although bat and hummingbird/diurnal insect pollination treatments resulted in significantly higher fruit initiation frequencies than the other four treatments, differences in mature fruit set among treatments within a year were not significant. In addition to the small differences in mature fruit set among treatments, it is likely that autogamy or apomixis limited differences among treatments. Also, as fruits matured, many were subject to larval infestation and subsequent fruit death. This further reduced differences among treatments. These results suggest that even if bats and hummingbirds were to prefer different flower types and colors, in any given year selection for a bat vs. hummingbird flower morph would be weak.

I propose that interaction among several factors, including variation in pollinator importance among years, low within-year differences in pollinator importance, and high levels of genetic variation in part due to autotetraploidy, may have led to weak and/or shifting selection pressure for any one flower morph and have allowed the persistence of morphological variation in the population of *W. weberbaueri* that I studied. El Niño events may play an important role in influencing the pollination biology of this cactus by causing drought conditions and reducing the abundance of important pollinators such as nectar-feeding bats. Longer term studies that include observational data as well as experiments specifically designed to test the relationships among floral morphology, visitor preference, and pollination effectiveness at this and other sites within the range of *W. weberbaueri* would help evaluate this hypothesis.

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