

WHY BE A HONEYLESS HONEY MESQUITE? REPRODUCTION AND MATING SYSTEM OF NECTARFUL AND NECTARLESS INDIVIDUALS¹

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Populations of *Prosopis glandulosa* var. *torreyana* in the Chihuahuan desert have a fixed dimorphic system of nectar production in which half the individuals produce nectar (are nectarful) and the other half are nectarless. We analyzed the impact of nectar production on different estimates of fitness, comparing nectarful against nectarless individuals in size, mating system, seed traits, and fruit set in a 1-ha scrubland. Of the reproductive individuals (358), 46% were nectarful and 54% were nectarless. Neither tree size nor flowering phenology differed between nectar morphs. Fixation indices (F) for both progeny ($F = -0.2$) and adults ($F = -0.45$) were negative, and high heterozygosities were found in adults and progeny ($H = 0.45$). No differences were found between nectar morphs for F , H , and single ($t_s = 1.1$) and multilocus ($t_m = 1.03$) outcrossing rates. Controlled pollinations showed differences between selfing and control treatments with no differences between nectar morphs. Nectarless individuals produced significantly more pollen grains than did nectar producers, but all other measured floral traits showed no differences. Nectarful trees were visited by pollinators 21 times more often and had a significantly higher overall fruit set than did nectarless trees. No differences between nectar morphs in seed mass or in percentage seed germination were found, but heavier seeds tended to have higher heterozygosities. Both morphs had similar success as females, but nectarless trees had ~7% higher male function. We discuss three possible scenarios for the evolution of the fixed dimorphism in nectar production, two involving unstable phases (substitution of one morph by the other, and evolution towards dioecy) and one stable scenario (maintenance of the dimorphic system).

Key words: floral traits; Leguminosae; mating systems; nectar production; outcrossing rates; pollination; polymorphism; *Prosopis glandulosa* var. *torreyana*; seed traits.

Pollination of most angiosperms depends on visitation by animals seeking rewards found within flowers (Kevan and Baker, 1983). Of all floral rewards, nectar is the most commonly found in angiosperms (Kevan and Baker, 1983; Simpson and Neff, 1983). Its provision within flowers influences components of pollinator visitation, including plant choice, foraging time, and foraging movements (Zimmerman, 1983, 1988; Hodges, 1993). Plants might be expected to evolve optimal allocation towards floral rewards to attract visitors, as increased visitation rates might prove beneficial in two ways: (1) the probabilities of pollen import and export may increase with visitation rates (Zimmerman, 1988; Harder and Barrett, 1996), and (2) the plant can become selective as to which

pollen grains fertilize the ovules, leading to higher seed quality (Stanton, Snow, and Handel, 1986). However, increased visitation rate does not always increase seed set (Klinkhamer, De Jong, and Metz, 1994; Mitchell, 1994). Excess visitation may lead to lower seed set, because of higher levels of selfing, clogging of stigmatic surfaces with self pollen, and an overall decrease of pollen export (de Jong et al., 1992; de Jong, Waser, and Klinkhamer, 1993; Klinkhamer, De Jong, and Metz, 1994). In addition, greater allocation to nectar involves an energy investment (Pleasants and Chaplin, 1983; Southwick, 1984), which can entail a reproductive cost (Pyke, 1991). Some plants, however, show strategies to avoid some of the costs associated with floral displays. These include food deception mimics. Within a population in which most individuals produce a food reward such as nectar, nectarless individuals would have an advantage if they received floral visitors attracted by nectarful individuals while avoiding the costs of nectar production (Little, 1983; Gilbert, Haines, and Dickson, 1991; Dafni, 1992). The consequence of such deceptive pollination is conspecific deceit mimicry (automimicry) or mimicry based on naiveté, as described by Little (1983). In such a case, the fitness of nectarful and nectarless plants would be frequency dependent: if nectarless individuals increased in number, pollination rates for all individuals in the population would be expected to diminish significantly (Bell, 1986; Gilbert, Haines, and Dickson, 1991). However, few food deception mimics have yet been examined; in

¹ Manuscript received 30 September 1997; revision accepted 7 December 1998.

The authors especially thank N. Waser for suggesting improvements to the manuscript, S. F. and A. Herrera, A. Rojas, A. Martínez, Y. Verhulst, A. Silva, and S. Pérez for their help in various aspects during our long stays at MBR; R. López and L. Godínez at the Museo de Zoología, UNAM, for identifying bee specimens; and J. Bronstein, C. Domínguez, D. Piñero, P. Kevan, and R. Mitchell for reading a previous version of the manuscript and making helpful suggestions. This work is in partial fulfillment of a Ph.D. by J. G. at the Facultad de Ciencias, UNAM. Field work was partially funded by CONACyT grant to C. M., PAPIIT, D.G.A.P.A. grant IN 205894 to L. E. E. and a CONACyT scholarship to J. G. who also thanks Fundación UNAM and New Mexico State University for financial and logistic support.

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particular studies that quantify the effects of deception mimics are not common.

Populations of honey mesquite, *Prosopis glandulosa* var. *torreyana* Benson (Mimosoideae), in the Mapimi Biosphere Reserve of Mexico have a fixed intrapopulation dimorphic system of nectar production (López-Portillo, Eguiarte, and Montaña, 1993). Half of the individuals within a mesquite population are nectarful, producing on average 0.51 mg of sucrose equivalents per millilitre of nectar, while the other half produce no nectar at all (López-Portillo, Eguiarte, and Montaña, 1993). Nectarful flowers are visited between 2 and 4 times more often by a larger diversity of pollinators (29 vs. 5, respectively) compared to nectarless individuals. Both nectar morphs have been shown to be self-compatible, but both display a high degree of inbreeding depression (López-Portillo, Eguiarte, and Montaña, 1993). Two observations suggest that nectar production in mesquites has a genetic basis. (1) Nectar production appears to be uncorrelated with environmental factors. During 4 yr (1994–1997), none of the studied plants has changed nectar morph (Golubov et al., unpublished data), even under varying rainfall regimes. This suggests genetic determination of nectar morph. (2) Nectarful and nectarless trees at various Chihuahuan desert sites are randomly distributed in space; even when found surrounding water supplies, proportions do not differ from 1:1 (López-Portillo, Eguiarte, and Montaña, 1993; López-Portillo, unpublished data). This species, therefore, provides a system in which the relative fitness values of traits associated with nectar production can be assessed under natural conditions.

Our study examines the relative costs and benefits of nectar production by comparing traits of nectarful vs. nectarless plants, specifically addressing the following question: (1) How is nectar production related with plant size, outcrossing rates, and seed traits? (2) What is the relative importance of floral traits and pollinator visitation on the reproductive success of each nectar morph? and (3) Do plants that provide nectar have higher fitness than those that do not?

MATERIALS AND METHODS

Study site—The study was made in the Mapimi Biosphere Reserve (MBR), southern Chihuahuan Desert (26°N104° W, 1100 m altitude, 264 mm yearly average rainfall mainly in summer, 21°C mean annual temperature, Mexico). Yearly rainfall during the study period was 138 mm for 1994, 198.4 mm for 1995, and 234.7 mm for 1996 (MBR climatic station). Fieldwork was done on a 1-ha area of desert scrubland dominated by *Prosopis glandulosa* var. *torreyana* surrounding a seasonal water-catchment site.

Study species—The honey mesquite, *Prosopis glandulosa* var. *torreyana* Benson (Mimosoideae) (hereafter *P. glandulosa*), is a woody perennial tree widely distributed in northern Mexico and southeastern United States (Rzedowski, 1988). This species is a dominant element in different communities of the Chihuahuan Desert. At MBR, *P. glandulosa* is found in the bajadas, playas, and arroyos and is the dominant species that surrounds all water catchments (López-Portillo, Eguiarte, and Montaña, 1993). Flowering at the study site starts in mid-March (before the summer rains) and ends in mid-May. *Prosopis glandulosa* is mainly pollinated by bees (López-Portillo, Eguiarte, and Montaña,

1993), like other species of *Prosopis* (e.g., Simpson, Neff, and Moldenke, 1977; Keys, Buchmann, and Smith, 1995).

Study population—In 1994, we censused, tagged, measured, and mapped all individuals of *P. glandulosa* within the 1-ha area of desert scrubland. Tree dimensions were taken as two canopy widths and height. Spatial distribution was analyzed with contingency tables (100 squares, each 10 × 10 m). Within the study site, the density of individuals was 0.13 ind./m², and of these 0.035 ind./m² were reproductive. The study site was subdivided into three plots of contrasting plant densities (low density [LD] 0.07 ind./m², medium density [MD] 0.22 ind./m², and high density [HD] 0.48 ind./m²).

During the flowering seasons of 1994 and 1995, we measured nectar presence of all reproductive individuals ($N = 358$). In each plant, we bagged five first-day open inflorescences of two separate branches at dawn. Nectar presence-absence was determined at dusk after collecting and probing five flowers per inflorescence with graduated 2- μ L micropipettes.

The following traits were measured to assess any possible difference between nectar morphs other than the production of nectar. Tree size was calculated as the volume of the tree (using a spherical model of plant size) and cube-root transformed for further analyses (Sokal and Rohlf, 1981). Tree size was ranked into five categories, and possible differences in size between nectar morphs were analyzed by means of a 2 × 5 contingency table (Everitt, 1977). To assess differences between morphs in flowering phenology, we recorded the presence-absence of mature inflorescences every 2 d between 14 and 30 March 1994 on nine consecutive observation dates and differences were tested with a 2 × 9 contingency table (Everitt, 1977). Finally, to characterize water availability between nectar morphs, in each of the three plots with different plant densities, we measured predawn water potentials (Scholander et al., 1965) for five randomly selected trees from each morph (5 trees × 2 morphs × 3 plots, $N = 30$). During March 1994, predawn water potentials were measured on two randomly selected shoots per tree using a pressure chamber (PMS model 1000, Corvallis, Oregon).

Mating system—Outcrossing and population genetics—We estimated heterozygosity and outcrossing rate of each nectar morph using starch-gel electrophoresis of progeny arrays. Fresh cotyledons of 10-d-old emerging seedlings ($N = 10$ seeds per tree of 30 trees per nectar morph) from the germination experiments (see Seed traits below) were ground on Tris-citrate buffer (pH 8.3) (Keys and Smith, 1994) and placed on a 10% starch gel Tris-citrate pH 8.3 substrate (Soltis et al., 1983). Specific staining and interpretation were done on glutamate oxaloacetate transaminase (E.C. [Enzyme Commission number] 2.6.1.1.; Saidman, 1986), leucine aminopeptidase (E.C. 3.4.11.1.; Solbrig and Bawa, 1975), esterase (E.C. 3.1.1.1.1.; Saidman and Naranjo, 1982), and the malic enzyme (E.C. 1.1.1.40.; Murphy et al., 1990). Single (t_s) and multiloci (t_m) outcrossing rates, maternal genotypes, and maternal and progeny fixation indices were obtained using the multilocus estimation program (MLT; Ritland, 1990).

Controlled pollination experiments—We tested the importance of selfing and the effectiveness of floral visitors through hand-pollination, using a completely randomized partial hierarchical design (Kirk, 1995). Hand-pollinations were done on ten randomly selected trees (main plot) per nectar morph in March 1996, during the peak flowering period. On each tree, 15 24 × 12 cm cotton bags (to avoid insect and wind pollination) were used to cover selected branches having immature inflorescences. When inflorescences reached maturity, we randomly assigned five bagged branches to each of the following treatments: (1) automatic self-pollination—bagged inflorescences with no further manipulation, (2) forced self-pollination—inflorescences manually self-pollinated every 2 d, and (3) open-pollination (control)—mature inflorescences left open for 5 d and then bagged. As the number of inflorescences varied between samples, each bagged branch assigned to a pollination treat-

ment was considered the subplot experimental unit. Bags were sprayed with insecticide every 2nd d to avoid flower loss from various larvae that consume flower parts.

We assessed fruit set (pods/inflorescence) on 15 May 1996, once pods were almost fully developed. Fruit set was arcsine-transformed (Sokal and Rohlf, 1981), and data were analyzed using nested ANOVA for completely randomized partial hierarchical designs (Kirk, 1995). The experimental model is nectar morph as the main treatment, trees (nested in nectar morph), and the pollination treatment crossed with nectar morph and tree (nested in morph). Differences between selfing and control pollination treatments were analyzed with orthogonal contrasts (Sokal and Rohlf, 1981).

Floral traits and visitors—To address the possibility of differences in flower traits between nectar morphs, we selected ten nectarful and ten nectarless trees of similar sizes within the study site during the 1996 flowering season. From these, we collected ten first-day inflorescences per tree. Total rachis length was measured, and total number of flowers per inflorescence were counted. Then, one flower per inflorescence was selected randomly, and we measured style and stamen length and counted the number of ovules and pollen grains. Given the symmetry of the two-parted anther, pollen counts were recorded from half an anther (Cruden, 1977). We stained ovules and pollen grains with methylene blue (0.01 g/mL) and counted them under a stereomicroscope at 80 \times . Floral measures that did not conform to Shapiro-Wilk normality tests (for $N < 1000$) were log transformed (Sokal and Rohlf, 1981). All floral measurements were analyzed using nested ANOVA (JMP statistical package, version 3.1.2; SAS, 1995), considering nectar morph and tree as factors (Kirk, 1995). Finally, as further indirect evidence of the mating system, we calculated pollen:ovule ratios according to Cruden (1977).

We evaluated the abundance and diversity of floral visitors on both nectarful and nectarless individuals during the 1994 flowering peak (21 March–6 April). Every 2nd d, on six randomly selected trees per nectar morph, 5-min fixed-capture efforts were made three times a day (0800, 1200, and 1600). Visitors were captured by using plastic bags (10 \times 20 cm), as plant architecture and thorns prevent the use of mesh nets and visitors to this species are easily captured. Plants were randomized on a daily basis, to avoid temporal and observer-biased errors. Captured specimens were identified, sexed, and measured (head to tip of abdomen), using four size classes: <2, 2–3, 3–4 and >4 cm. Data sets include only bees (Apoidea, Hymenoptera), as they are the main pollinators of *Prosopis* (Simpson, Neff, and Moldenke, 1977; López-Portillo, Eguiarte, and Montaña, 1993; Golubov et al., unpublished data). Differential activity of bees with respect to time of day was analyzed using contingency tables (Everitt, 1977).

Flowering and fruit set—To determine differences and variability in pod production between nectar morphs, we tagged three widely spaced branches around the canopy of each reproductive tree in 1994. Total numbers of flowers, nodes, and fruits were recorded for each branch during two consecutive years. To avoid losses in counts from dehiscence and predispersal predation, pods were counted before fully mature (May). Fruit set was calculated as the total number of developed pods per total number of inflorescences. To obtain a single estimate of reproductive success per tree, we averaged and arcsine transformed (Sokal and Rohlf, 1981) flower and pod counts of all three branches. Fruit set was analyzed through multivariate analysis of variance (MANOVA) with the 3 yr as repeated measures (von Ende, 1993), taking nectar morph, site, and tree (nested in morph) as factors (JMP, version 3.1.2; SAS, 1995). Statistical significance was assessed using exact F contrast tests, and F adjusted with the Greenhouse-Geisser (G-G) method (SAS, 1995).

Seed traits—To assess differences in seed mass and germination success between nectar morphs, we collected pods from all reproductive

individuals from the site in June 1994 (plants having <20 pods were excluded from further manipulations). Ten pods from 30 nectarful and 30 nectarless trees were randomly chosen. To reduce the probability of sampling consanguineous progeny, only one seed (also randomly selected) per pod was used ($N = 300$ seeds for each morph). Selected seeds were weighed to the nearest 0.001 g, scarified mechanically, sown in soil from the original site, and grown in a greenhouse at the Instituto de Ecología, UNAM, Mexico. Germination was considered successful when the seedlings had a developed radicle and fully expanded cotyledons.

Differences in seed mass were analyzed using nested ANCOVA with three factors: nectar morph, plot, and parent tree (nested in plot) and one covariate (tree size; Kirk, 1995). Differences in germination success associated with nectar morph and parent tree were analyzed using contingency tables (Everitt, 1977). The relationship between seed mass and germination was calculated using a logistic regression model (Sokal and Rohlf, 1981; JMP version 3.1.2; SAS, 1995).

In addition, we related genetic information (see section outcrossing rates and population genetics) to seed mass by means of multiway ANOVAs, considering the number of heterozygous loci (0–7; pooling loci 0 and 1 as well as 6 and 7), nectar morph, and tree as factors. Contrast t tests were performed to analyze the effect of heterozygous loci on seed mass (Sokal and Rohlf, 1981).

Cumulative effects of nectar production on plant fitness—To obtain an approximate and relative value of female and male function for each nectar morph, we multiplied all characters contributing to each function (Arnold and Wade, 1984). Among the values measured, we considered number of flowers, number of ovules, fruit set (1994–1996, see Flowering and fruit set above), and seed set to be components of female function. Numbers of flowers and pollen grains were considered as the components of male function. Numbers of flowers, ovules, and pollen grains were recorded from one flower per inflorescence ($N = 100$, ten trees per nectar morph). Given the symmetry of the two-parted anther, pollen counts were recorded from half an anther. Fruit sets were taken from tagged branches around the canopy of nectar and nectarless trees during the 3-yr period (1994–1996). Seed sets were taken as number of viable seeds from 20 pods of five trees per nectar morph. All components are multiplicative and, therefore, the error associated to relative fitness was calculated as: $Z = A_0 B_0 \pm (A_0 \Delta B_0 + B_0 \Delta A_0 + \Delta A_0 \Delta B_0)$, where Δ 's are SE associated to each mean value. This estimate of male fitness is approximate, because pollen flow and the proportion of seeds fathered were not estimated.

RESULTS

Of all reproductive individuals at the study site (358), 46% were nectarful and 54% nectarless; these numbers were not significantly different from a 1:1 proportion ($\chi^2 = 2.51$; $P = 0.113$). Neither tree size ($P = 0.211$; Fig. 1A) nor flowering phenology ($P = 0.7$; Fig. 1B) differed between morphs.

Water availability (measured as predawn water potentials) did not differ between nectar morphs ($P = 0.1259$) but was significantly different among plots ($F = 5.69$, $P = 0.009$), being lowest in the LD ($\bar{x} = -0.82 \pm 0.08$ MPa), as compared to MD ($\bar{x} = -0.58 \pm 0.046$ MPa) and HD plots ($\bar{x} = -0.55 \pm 0.046$ MPa). Nectarful and nectarless trees were distributed randomly throughout the whole 1-ha site ($\chi^2 = 23.2$, $df = 99$, $P > 0.05$).

Mating system—Outcrossing and population genetics—Seven polymorphic loci were resolved: LAP 1 (three alleles), EST1 and EST2 (three alleles each), EST3 (two alleles), ME (two alleles), and GOT1 and GOT2

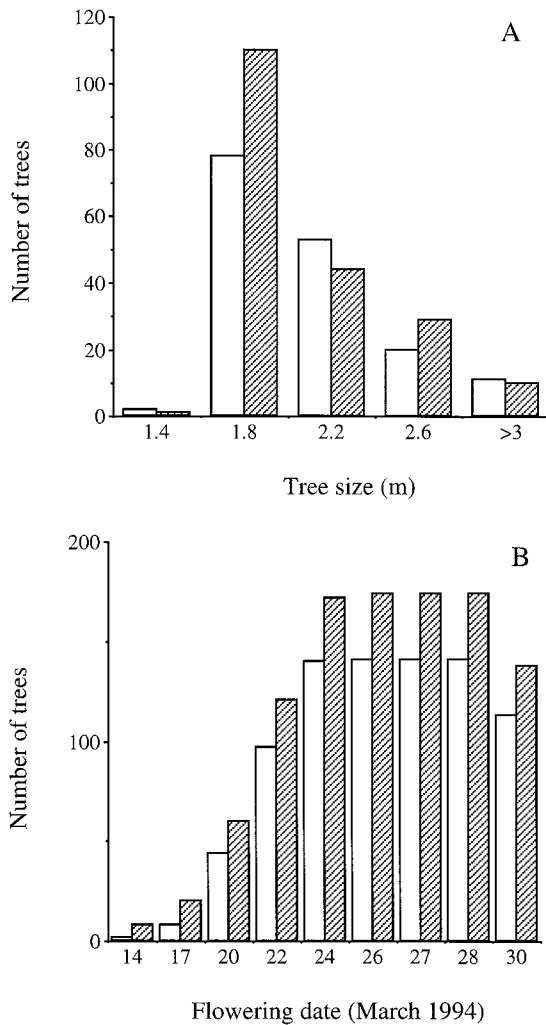


Fig. 1. (A) Size (as a radius in metres, $N = 358$) and (B) flowering phenology of adult ($N = 310$) individuals of nectarful (empty bars) and nectarless (hatched bars) trees during the 1994 reproductive season.

(two alleles), while GOT3 resulted in a monomorphic band. The fixation indices (F), excluding monomorphic loci, were negative in progeny and adults, indicating an excess of heterozygous individuals at both stages. F was lower in parents compared to their progeny, but we found no significant differences in F between morphs at either

stage. Heterozygosities were high in both stages, and no difference were found either (Table 1).

Single and multiloci outcrossing rate estimates (Table 1) indicate no detectable selfing, no biparental inbreeding (no positive differences between t_m and t_s ; Ritland, 1984), and especially no differences between nectar morphs. This indicates that both nectar morphs are outcrossers in spite of the unrealistic values of t (i.e., >1) obtained, probably caused by the multiple heterozygosity of parental genotypes.

Controlled pollination experiments—We found differences between pollination treatments with respect to fruit set ($P < 0.001$; Table 2). The lowest fruit set was for automatic self-pollination ($\bar{x} = 0.0008 \pm 0.002$), followed by forced self-pollination ($\bar{x} = 0.015 \pm 0.012$), and finally open pollination ($\bar{x} = 0.058 \pm 0.023$). Both self-pollination treatments showed a marked decrease in fruit set with respect to the control ($P > 0.05$). On the other hand, fruit set did not differ between nectar morphs ($P = 0.2824$, Table 2). The significant treatment \times tree interactions indicated the high variability between trees ($P = 0.0278$, Table 2), but all other interactions were not significant.

Floral traits and visitors—Nectarless trees produced, on average, slightly more pollen grains than did nectarful trees ($P = 0.05$; Table 3). No other measured floral trait differed significantly between nectar morphs. However, for all floral traits, there was great variation between trees within each morph ($P < 0.0001$). Comparing pollen: ovule ratios (Table 3) to those reported by Cruden (1977), the breeding system of *P. glandulosa* falls between facultative autogamy and facultative xenogamy.

The most abundant bee genera visiting both nectar morphs (Table 4) included *Colletes*, *Ashmeadiella*, and *Megachile*, with smaller numbers of *Melissodes*, *Exomalopsis*, *Apis*, *Lasioglossum*, and *Perdita*. Of all genera, only one species of *Perdita* and one of *Lasioglossum* were only found on the nectarless morph, and then only in low frequencies (one recorded visit per bee species). Considering total bee visits to both nectar morphs, there were no significant differences in bee activity between different times of day ($\chi^2 = 4.61$, $df = 2$, $P = 0.1$) or between size classes of bees ($\chi^2 = 5.11$, $df = 3$, $P = 0.16$). Despite these similarities between nectar morphs, the number of visits differed dramatically, as the number of bees on nectarless trees comprised only 4.5% of total

TABLE 1. Levels of heterozygosity, fixation index (F), and outcrossing rates based on seven polymorphic loci (1 SE in parentheses). All estimates were calculated using MLT (Ritland, 1990).

		Overall ^a	Nectarful ^b	Nectarless ^b
Heterozygosity	Progeny	0.4507 (0.02)	0.4458 (0.03)	0.4533 (0.03)
	Parents	0.4507 (0.06)	0.4627 (0.1)	0.4577 (0.1)
Fixation index	Parents ^c	-0.4463 (0.2)	-0.4658 (0.1)	-0.4565 (0.1)
	Progeny	-0.2124 (0.048)	-0.2085 (0.003)	-0.2162 (0.004)
Outcrossing rate	t_m^d	1.033 (0.023)	1.101 (0.087)	1.023 (0.04)
	t_s^d	1.186 (0.028)	1.232 (0.050)	1.173 (0.044)

^a Progeny of 60 parents, ten seeds per parent ($N = 600$).

^b Progeny of 30 parents, ten seeds per parent ($N = 300$).

^c Inferred from MLT.

^d Based on 1000 bootstraps.

TABLE 2. Summary of F statistics and significance levels for ANOVA in a completely randomized partial hierarchical design testing the effect of different pollination treatments (open-pollinated control, forced self-pollination, and automatic self-pollination), nectar morph (nectarful or nectarless), and tree ($N = 20$) on fruit set. The pollination treatment factor was decomposed into orthogonal contrasts: (1) control (C) vs. both selfing treatments (FS and S), and (2) automatic self (S) vs. forced self-pollination (FS). Fruit set was arcsine transformed.

Source of variation	SS	df	F	P
Nectar morph	0.099	1	1.22	0.2824
Tree _[morph]	1.457	18	1.17	0.3304
Treatment	2.332	2	16.91	0.0001
C vs. SF + S	1.865	1	27.05	<0.001
S vs. SF	0.467	1	6.77	0.013
Nectar morph \times Treatment	0.0015	2	0.01	0.9890
Tree _[morph] \times Treatment	2.482	36	1.56	0.0278
Error	10.610	240		
Total	16.985	299		

captures. Nectarful trees were visited, on average, 21 times more often than were nectarless trees. Taking into account only the eight genera that are shared by both nectar morphs, the visiting ratio is reduced somewhat to 10:1 (nectarful: nectarless). Wasps and flies were seen constantly, but only on the nectarful morph. Both sexes of bees visited nectarful individuals, while females alone visited nectarless trees (Table 4).

Flowering and fruit set—Fruit set was very low in all years (range = 0.08–0.17 fruits per inflorescence; Table 5). Fruit set varied significantly between years (Tables 5, 6) and was highest in 1995. Nectar morph and plot had a significant effect on fruit set (Table 6), and there was a nonsignificant trend towards a tree effect (Table 6). Nectar producers had a higher overall fruit set than did nectarless trees (Tables 5, 6). The highest fruit set was in the plot MD ($\bar{x} = 0.1977 \pm 0.056$) followed by plot HD ($\bar{x} = 0.1662 \pm 0.053$), the plots with the highest water availability; plants in plot LD ($\bar{x} = 0.116 \pm 0.038$) had significantly lower fruit set ($P < 0.0001$). Variation in fruit set between plots and nectar morphs among years resulted in the only significant interaction (Table 6).

Seed traits—In 1994, we found no differences between nectarful and nectarless trees in seed mass (Table 7), but

TABLE 3. Mean values (1 SE) of measured floral traits for nectarful and nectarless trees. Significance levels correspond to a nested ANOVA with factors nectar morph and tree ($N = 100$ flowers per nectar morph). The pollen:ovule (P:O) ratio was calculated by dividing the estimate of pollen grains per flower by the number of ovules per flower.

Floral trait	Nectarful (SE)	Nectarless (SE)	P
Pollen grains ($\frac{1}{2}$ anther) ^a	391 (12)	420 (10)	0.05
Ovules per flower	17.88 (0.28)	18.34 (0.27)	0.578
Flowers per inflorescence	92.76 (0.12)	92.38 (0.26)	0.967
Length of anther (mm)	4.98 (0.12)	4.88 (0.12)	0.772
Length of style (mm)	4.75 (0.09)	4.89 (0.08)	0.647
Corolla diameter (mm)	2.3 (0.04)	2.28 (0.04)	0.838
Length of pedicel (mm)	39.59 (0.12)	38.07 (0.13)	0.661
P:O ratio	441 (18)	461 (33)	0.602

^a Each flower has ten stamens.

there was wide variation within morphs ($\bar{x} = 30.16 \pm 0.27$ mg, range = 12–58 mg). The main influence on seed mass related to maternal effects ($P < 0.0001$; 40% of total variance, Table 7). Seed mass of both nectar morphs did not vary among plots, but nectarless trees showed a slightly reduced seed mass in plot LD, giving a significant plot \times nectar morph interaction (Table 7). The orthogonal contrasts showed seed mass in plot LD to be different from those in plot MD and HD ($P < 0.001$), but plots MD and HD did not differ from each other ($P = 0.66$).

We found no significant differences in germination success between nectar morphs ($P > 0.05$). Both seed mass ($\chi^2 = 6.43$, $df = 1$, $P = 0.0112$) and parent tree ($\chi^2 = 73.944$, $df = 29$, $P < 0.001$) had significant effects on germination success. The mass of seeds was influenced by both seed heterozygosity (taken as the number of heterozygote loci over the six possible; $F = 3.2106$, $P = 0.0009$) and tree-related effects ($F = 8.395$, $P < 0.001$), but nectar morph had no significant effect ($F = 0.6525$, $P = 0.32$). Contrast t tests after a significant ANOVA divided the sampled population of seeds into two categories (Fig. 2), those having fewer than three heterozygote loci and those having more. There was a slight tendency for heavier seeds to have higher heterozygosities, but little total variation is explained by this factor ($r^2 = 0.03$, $df = 598$, $P < 0.001$).

Cumulative effects of nectar production on plant fitness—The analyses revealed little difference between nectar morphs. Thus, to include an overall measure of plant fitness through either female or male functions, the mean values of some characters important for plant fitness from both nectarful and nectarless morphs are described in Table 8. In terms of female function, there is a large variation associated with each trait; overall, nectarful and nectarless trees did not differ. In contrast, nectarless individuals had a higher relative male function (Table 8), and the error associated indicates the difference to be significant (Table 3).

DISCUSSION

Our results suggest there are benefits and costs associated with the lack of nectar production in *P. glandulosa*. Nectar production has been proposed to be expensive, both in terms of total energy investment (>30%; Pleasants and Chaplin, 1983; Southwick, 1984) and in reproductive costs (Pyke, 1991). We would expect these costs to be reflected in nectarful trees having decreased reproductive output (i.e., fewer flowers, shorter flowering times) and/or decreased plant growth (resulting in smaller plants). However, we found no differences in plant size, flower production, or phenology between nectar morphs. This could be, in part, because nectar production represents a low energy cost for *P. glandulosa*. Negligible costs of nectar production have also been found by Harder and Barrett (1992) in the bee-pollinated *Pontederia cordata*.

Nectar production variability has been shown to affect pollinator foraging bouts and patterns (Waddington, 1983). In *P. glandulosa*, nectar production clearly affects the number, species, and sex of bees that visit each nectar

TABLE 4. Number of bees and other floral visitors, bee sex ratios, and visitor densities on nectarful and nectarless plants. Data set obtained from nine census dates (21 March–6 April 1994) of six trees per nectar morph at three times of day (0800, 1200, and 1600).

Traits of floral visitors	Nectarful	Nectarless
Total no. of bee visits	423	20
Sex ratio (female : male)	2:1	1:0
No. of bee species	58	15
Andrenidae	<i>Andrena</i> 2 spp., <i>Perdita</i> 5 spp.	<i>Perdita</i> 2 sp.
Anthophoridae	<i>Epeolus</i> 2 spp., <i>Exomalopsis</i> 1 sp., <i>Melissodes</i> 2 spp., <i>Neopasites</i> 1 sp., <i>Nomada</i> 1 sp., <i>Triepeolus</i> 1 sp., <i>Zacosmia</i> 2 spp.	<i>Exomalopsis</i> 1 sp., <i>Melissodes</i> 1 sp.
Apidae	<i>Apis</i> 1 sp.	<i>Apis</i> 1 sp.
Colletidae	<i>Colletes</i> 8 spp., <i>Hylaeus</i> 3 spp.	<i>Colletes</i> 2 spp.
Halictidae	<i>Agapostemon</i> 1 sp., <i>Lasioglossum</i> 4 spp.	<i>Lasioglossum</i> 4 spp.
Megachilidae	<i>Anthidium</i> 3 spp., <i>Ashmeadiella</i> 12 spp., <i>Coelioxys</i> 2 spp., <i>Megachile</i> 5 spp., <i>Stelis</i> 2 spp.	<i>Ashmeadiella</i> 3 spp., <i>Megachile</i> 1 sp.
Other orders of visitors	Coleoptera 4 spp., Diptera 6 spp., Hemiptera 2 spp., Hymenoptera (Vespoidea) 3 spp. (Formicidae) 2 spp., Lepidoptera 4 spp., Thysanoptera 2 spp.	Diptera 1 sp., Thysanoptera 2 spp.

morph. Nectar production enhanced visitation frequencies by at least one order of magnitude, as compared to nectarless trees. Similar patterns have been found in dioecious plants in which nectar is associated with female function (Pyke, Day, and Wale, 1988; Greco, Holland, and Kevan, 1996). However, the increase in visitation to nectarful individuals was not reflected in a corresponding increase in fruit set. Nectarful trees produced outcrossed progeny, and these did not differ from nectarless ones. Probably, nectar collection is increasing rates of selfing and consequently lowering both fruit and seed set. The possible cost of increased geitonogamy incurred by nectar production (Klinkhamer, de Jong, and Metz, 1994) seems to be high for nectarful *P. glandulosa* plants, especially when a single effective pollinator visit is sufficient to pollinate a flower, as has been found for the closely related velvet mesquite, *P. velutina* (Keys, Buchmann, and Smith, 1995). Few but effective pollinators (i.e., female bees that collect pollen to feed larvae) were the main visitors of nectarless trees providing a service by dispersing pollen and pollinating during their foraging bouts.

Nectar production does not seem to affect the mating system of the honey mesquite, even considering the differential visitation rates. *Prosopis glandulosa*, although capable of selfing, is mainly an outcrosser, producing a higher than expected proportion of heterozygote seeds. Previous pollination experiments on *P. glandulosa* (López-Portillo, Eguiarte, and Montaña, 1993) have also shown higher fruit set for cross- than for self-pollinations. Similar estimates of the mating system were found by Keys and Smith (1994) for the velvet mesquite, *P. velutina*. In *P. glandulosa*, high fruit abortion rates (up to four fruits/inflorescence) could mean selection against

homozygote progeny (<1% of seeds sampled were homozygous for all loci) or selection favoring the more heterozygous individuals (Eguiarte, Perez-Nasser, and Piñero, 1992), although these aspects must be fully explored. We found that fruit set, especially of nectarless morphs, is affected by environmental factors. Felker and Lee (1992) also found an environmental influence on fruit set of *P. glandulosa* var. *glandulosa*, although they did not describe a nectar dimorphism.

Seed mass is an important component of plant fitness, through its effects on time of germination, seedling establishment, competitive ability, survival, and fecundity (Stanton, 1984; Kalisz, 1989). In our study, germination and mass of *P. glandulosa* seeds were unaffected by nectar morph but seemed to be partially influenced by maternal and environmental effects. Maternal effects can be ascribed in part to environmental conditions that determine the amount of resource invested in the seeds by the parent (Roach and Wulff, 1987), such as those found for nectarless trees in the low water availability areas (plot LD). These maternal and environmental effects on seed traits (e.g., mass) have been found for other species as well (Kalisz, 1989; Waser, Shaw, and Price, 1995) and usually affect a large percentage of total variation (40% in this study). We also found negative F estimates (even

TABLE 5. Mean fruit set per inflorescence (1 SE) for three sampling years (1994–1996). *N* = number of trees sampled.

Year	Tree morph	
	Nectarful (<i>N</i> = 149)	Nectarless (<i>N</i> = 172)
1994	0.16 (0.106)	0.11 (0.08)
1995	0.17 (0.165)	0.17 (0.18)
1996	0.14 (0.143)	0.08 (0.10)
Average	0.156 (0.05)	0.12 (0.04)

TABLE 6. Multivariate nested analysis of variance with repeated measures in time (1994–1996) of the fruit set (arcsine transformed) of nectarful and nectarless individuals. Data correspond to three plots of contrasting densities in 1-ha scrubland of the Chihuahuan Desert.

Source of variation	df	<i>F</i>	<i>P</i>
Between subjects ^a			
Nectar morph	1, 146	4.82	0.0296
Plot	62, 146	8.12	0.0004
Tree _[plot]	169, 146	1.26	0.0703
Nectar morph × Plot	2, 146	2.08	0.1274
Within subjects ^b			
Year	1.8837, 275.02	6.04	0.0033
Year × Nectar morph	1.8837, 275.02	0.18	0.8207
Year × Plot	3.7674, 275.02	0.97	0.4167
Year × Tree _[plot]	311.41, 275.02	1.20	0.0558
Year × Nectar morph × Plot	3.7674, 275.02	3.23	0.0148

^a Exact *F*.

^b G-G adjusted *F*.

TABLE 7. Analysis of covariance of seed mass of nectarful and nectarless plants, using plant size as a covariate. Data correspond to three plots of contrasting densities.

Source of variation	SS	df	F	P
Nectar morph	1.805	1	0.0666	0.7965
Plot	309.758	2	0.6509	0.5282
Tree _[plot]	10 720.71	30	13.1869	0.00001
Tree size	4.926	1	0.1818	0.67
Plot × Morph	180.798	2	3.3358	0.0363
Error	15 256.941	563		
Total	26 476.212	599		

in seeds) and strong heterosis, correlated with seed mass, similar to those described in several long-lived perennials, mainly conifers (Ledig, 1986) and tropical palms (Eguiarte, Perez-Nasser, and Piñero, 1992; Eguiarte et al., 1993). The advantages of generating outcrossed progeny in relation to selfed progeny have been documented for many plant species (Charlesworth and Charlesworth, 1987; Mandujano, Montaña, and Eguiarte, 1996). This is especially important when heterosis is coupled to seed mass and the latter associated with higher germination rates (A. Martínez, Instituto de Ecología, A. C. unpublished data).

We found higher fruit set and lower pollen counts on nectarful morphs. These two components of fitness are pivotal for understanding total reproductive success in plants (Bell, 1985; Zimmerman, 1988). In relation to female function, the nectarful morph had higher success only in fruit set, but overall, female function did not differ between nectar morphs. Higher fruit sets have been found to be associated with higher nectar production (Pyke, Day, and Wale, 1988). Nonetheless, the nectarless morphs had higher pollen counts, which suggest a slightly (7%) higher male function. This “fitness accounting” is a crude approximation and must be interpreted carefully. Nevertheless, it is still surprising to find differences in male function between nectar morphs. Ongoing research is quantifying pollen flow, in order to estimate a more realistic component of male function (Golubov et al., unpublished data).

The evolutionary forces involved in the origin and maintenance of the nectar dimorphism in *P. glandulosa* are still unknown. We can rule out a possible relation with resource availability for several reasons. Firstly, if nectar production were associated with water condition, measured water potentials should be different between nectar morphs, and we found that the plots having different water availability still maintained a 1:1 proportion of nectarless to nectarful trees. Secondly, both nectar morphs have a spatially random distribution, and if the

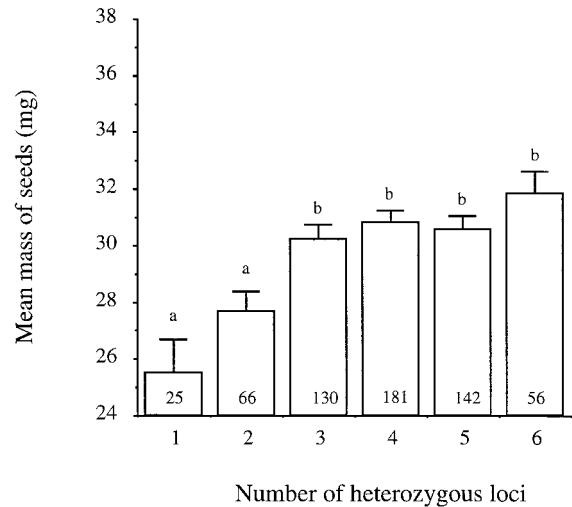


Fig. 2. Seed mass frequency with respect to heterozygote loci. Values having the same letter did not differ significantly in contrast comparison *t* tests ($P < 0.05$). Bars indicate 1 SE, and numbers within bars indicate number of seeds in each locus category ($N = 600$).

morph depended on microsite conditions, we would find different proportions at different sites and in different years. In all studied sites, the proportions of nectar morphs are the same, and individuals have not shifted between nectar morphs during the study period. We consider that there is enough evidence to conclude that the nectar dimorphism has a genetic basis. Three main sets of scenarios could be proposed for the evolution of the nectar dimorphism in *P. glandulosa*: (1) the population is an unstable phase in the evolution of nectar production. This scenario would result in populations with varying proportions of nectarful to nectarless trees. However, the empirical evidence presented here does not support the hypothesis that nectarful plants have higher fitness, so we would not expect them to displace nectarless trees. We could also consider that nectar production as a relatively cheap expense and this selectively neutral, however, this seems unlikely given the large difference in pollinator behavior. If we consider a case of floral automimicry, we would not expect the morphs to be in equal proportions (López-Portillo, Eguiarte, and Montaña, 1993). (2) The population is in an early stage of sexual differentiation. In this respect, nectarless plants would be evolving toward males (higher pollen) and nectarful trees towards females (higher fruit set). While we found differences in one component of female function (fruit set), there are no overall differences between morphs. Fruit set was higher for nectarful trees, but the overall female function

TABLE 8. Mean values (± 1 SE) for traits contributing to plant fitness through female and male functions in nectarful and nectarless individuals.

Morph	No. of flowers per inflorescence	No. of ovules or pollen grains per flower	Fruit set (3 yr) per inflorescence	Seed set per fruit	Relative fitness
Female function					
Nectarful	92.76 (0.12)	17.88 (0.28)	0.156 (0.05)	11.09 (0.3)	1 (0.38)
Nectarless	92.38 (0.26)	18.34 (0.27)	0.123 (0.04)	10.38 (0.3)	0.75 (0.29)
Male function					
Nectarful	92.76 (0.12)	391 (12)			0.93 (0.032)
Nectarless	92.38 (0.26)	420 (10)			1 (0.033)

did not differ between nectar morphs and even though male function did differ, the component we measured (pollen production) only represents a fraction of total male success; the proportion of pollen that reaches receptive stigmas has not been evaluated. In addition, nectar production is generally expected in males when there is sexual dimorphism, as a consequence of Bateman's principle, which states that female fitness is resource limited, while male fitness is limited by access to females (Wilson et al., 1994). Even though nectar production has been associated with female function in dioecious species as a means of attracting pollinators, it is associated with male fitness in monoecious species. Comparative studies of pollen flow and paternity analysis between nectar morphs will help estimate the relative contributions of male and female function of each nectar morph. (3) The populations are in a stable dimorphic state. This scenario would be the result of a complex balance between the different evolutionary forces mentioned in the above scenarios. This nectar dimorphism could be analogous to other sexual or morphological dimorphisms (dioecism and heteromorphy; Smith, 1963; Richards, 1986).

Additional experimental manipulation may help determine whether the differences found between nectarful and nectarless trees are directly associated with the presence of nectar, or are associated with correlated characters such as age (e.g., Zimmerman, 1988; Mitchell, 1994). Unfortunately, the heritability of nectar production is difficult to assess through progeny, as age at maturity is probably ~20 yr. However, ongoing studies measuring the costs of floral nectar production, monitoring pollinator activities, and quantifying pollen flow should help reveal how this dimorphism is maintained.

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