Reproductive biology of the melon cactus, *Melocactus curvispinus* (Cactaceae)

J. M. Nassar¹ and N. Ramírez²

¹Instituto Venezolano de Investigaciones Científicas, Centro de Ecología, Caracas, Venezuela ²Universidad Central de Venezuela, Facultad de Ciencias, Centro de Botánica Tropical, Caracas, Venezuela

Received April 15, 2003; accepted April 28, 2004 Published online: August 27, 2004 © Springer-Verlag 2004

Abstract. We examined the reproductive biology of Melocactus curvispinus Pfeiffer (Cereeae, Cactoideae) in xeric areas of northern Venezuela. Floral traits correspond to a classic hummingbirdpollination syndrome; however, pollination is shared between hummingbirds, Leucippus fallax (Bourcier 1843), and anthophorid bees, Ceratina sp. Reproduction occurs during most of the year. Anthesis and nectar secretion occur between noon and sunset. Average daily nectar production per flower was 163.1 µl, nectar sugar concentration between 29.1 and 30.2% (w/w). Hummingbirds promoted inter-plant pollen movement and were relatively more reliable floral visitors than anthophorid bees, but these insects had a higher frequency of floral visits (28.75/day) than hummingbirds (4.96/day). M. curvispinus is self-compatible and autogamous. By combining extended reproductive activity, frequent animal-mediated pollination, and selfing capabilities, this cactus possesses a mating strategy that guarantees reproduction under variable environmental conditions. We argue that based on its reproductive biology, M. curvispinus should be considered an example of evolutionary transition towards selfing within tribe Cereeae.

Key words: Autogamous, breeding system, Cactaceae, anthophorid bees, hummingbird,

Melocactus curvispinus, pollination, self-compatibility.

The genus Melocactus comprises a very homogeneous group of small globose cacti, which are very common in arid and semiarid lands of tropical and subtropical zones in the Western Hemisphere. They are highly appreciated among cactus growers all over the world (Taylor 1991, Anderson 2001). In recent years, they have been the subject of studies addressing their potential medicinal properties (da Silva and Parente 2002). The taxonomy of Melocactus has been studied in considerable detail (Taylor 1991). Including three recently described species from Colombia (Fernández-Alonso and Xhonneux 2002), this genus contains a total of 36 species (Anderson 2001). Despite of the great taxonomic effort that has been invested in describing and classifying melocacti, other aspects of their biology remain poorly understood. One of these aspects is the reproductive biology. Research on the reproductive biology of cacti, and particularly on their breeding systems, is considered a matter of agricultural importance (Boyle and Idnurm 2001). Information on the

reproductive biology of this group of plants is not evenly distributed across the numerous genera and growth forms observed in the family. Species in genus *Echinocereus* (Grant and Grant 1979, Breckenridge and Miller 1982, Hoffman 1992, Scobell and Scott 2002), *Opuntia* (Grant and Grant 1979, Grant and Hurd 1979, Osborn et al. 1988, McFarland et al. 1989), and several columnar cacti (Fleming et al. 1996, Sahley 1996, Nassar et al. 1997, Valiente-Banuet et al. 1996), have received much more research attention in terms of their reproductive ecology than other cactus groups and life forms.

Taylor (1991) summarized general information on the floral biology, pollination, seed dispersal, and breeding systems of Melocactus species. Despite their morphological homogeneity, it appears that different members of this genus have important differences in their floral characteristics, time of anthesis, pollination, and mating systems. For example, some species have pronounced herkogamy while others are presumed to be cleistogamous. Some species bloom in the morning, whereas others bloom in the afternoon or at night (J. Nassar and N. Ramírez, unpublished data). Most species have open bright-colored flowers, typical of hummingbird pollination. Although hummingbirds seem to be important pollinators of Melocactus, other taxa including small bees and butterflies have been proposed as alternative pollen vectors (Taylor 1991). Although most species appear to be selfcompatible, some taxa appear to be selfincompatible under greenhouse conditions. As a whole, interspecific differences detected in this genus may represent different reproductive strategies that can have important and diverging ecological and evolutionary consequences for the members that form the Melocactus clade.

An adequate understanding of the reproductive strategies adopted by *Melocactus* species requires detailed studies of their floral biology, reproductive phenology, pollination, and breeding systems. A starting point for this is the reproductive characterization of *Melo*- *cactus curvispinus* Pfeiffer (Cereeae, Cactoideae). This species has probably the widest geographic range in the genus (Taylor 1991). It is very common in arid and semiarid lands and possesses the typical open bright-colored flowers and hummingbird pollination described for the genus. The objective of this study was to characterize the reproductive biology and phenology of *M. curvispinus* in two populations in mainland and coastal Venezuela.

Materials and methods

Study areas and species. Two study sites were selected to conduct field observations and obtain flowers for morphological analyses: (1) a coastal population in "Pueblo de Mamo," 10 km west of "Simón Bolívar" International Airport (10°53' N and 67°02' W), Vargas State; and (2) a mainland population in "Padre Diego" Sector (10°08' N and 69°32' W), at the edge of "Cerro Saroche" National Park, Lara State, in western Venezuela. The two sites are located within arid regions, characterized by mean annual precipitation below 600 mm, with most rainfall occurring in July and from October to November; mean annual temperatures is ~27°C (MARNR 1995). The topography of the coastal location is characterized by low to moderate hills, 1000 m a.s.l.. The mainland location is dominated by hillocks, plains, and mountains that range in altitude from 500 to 1280 m a.s.l.. Vegetation corresponded to the 'xerophilous littoral shrubs' and 'xerophilous spiny scrubs and shrubs' categories of Huber and Alarcón (1988).

Melocactus curvispinus, commonly known as "melón" or "buche," ranges from Mexico to northern South America, including part of the Caribbean. It is found from sea level to 1250 m a.s.l. and is always associated with arid and semiarid environments on a variety of soil types. In Venezuela, M. curvispinus is distributed along the coast and nearby islands, in western drylands and farther south on the Colombian border (Ponce 1989). Population densities can be as high as 1290 individuals/ha (Nassar et al. 2001). Allozyme segregation data obtained from Venezuelan populations (Nassar et al. 2001) suggest that M. curvispinus is a diploid cactus. Cytological evidence offered by Das et al. (1998) supports the diploid (2n = 22)condition of the species. M. curvispinus is characterized by a depressed-globose body < 30 cm tall.

Sexually mature individuals develop a distinctive and conspicuous *cephalium*, made of white woolly fibers intermixed with tightly clustered reddish bristles. Flowers are located in the *cephalium* and are visible only when mature. The magenta flowers open during the afternoon and close the same day by sunset (Taylor 1991, this study). Pollination has been attributed to hummingbirds and, to a lesser extent, solitary bees and butterflies (Taylor 1991). Fruits are conical fleshy berries, magenta or red, and multiple-seeded. Individuals are solitary or occur in clusters. No vegetative reproduction occurs in the genus except for secondary growing of small stems with cephalia after structural damage is inflicted on the plant's apical area (J. Nassar, personal observation).

Floral traits. Thirty three open flowers, one flower per plant, were collected from the coastal and mainland populations and preserved in 70% ethanol for morphological measurements. We selected fully open flowers 3-4 hours after anthesis. In addition, ten open flowers from each population (one flower per plant) were collected and preserved for biomass allocation analysis. Floral shape, odor, and perianth color were described from fresh material. Using a digital calliper, we measured total external length of flowers (from the base of the ovary to the base of tepals), internal perianth diameter, and distance between base of the stigma and the distal most anthers (Fig. 1). We counted number of stamens/flower and number of ovules/ flower. The number of pollen grains per anther was estimated indirectly using Neubauer chambers with known dilution volumes of pollen grains obtained from five anthers of five flowers (Lloyd 1965). The number of pollen grains/flower was estimated as the product of pollen grains per anther times average number of anthers/flower. Pollen/ovule ratios were estimated by dividing mean number of pollen grains/flower by average number of ovules/flower. Flowers for biomass allocation were dissected into vegetative tissue, female organs (gynoecium), and male organs (androecium), and parts were dried in a laboratory oven at 45-50 °C during 15 days (Precision, Jouan Inc., Winchester, Virginia, US). Dry weights of parts were determined and proportions of floral biomass allocated into vegetative, gynoecium, and androecium tissues were estimated. In addition, we determined male/female and reproductive/vegetative biomass ratios. Forty-five fruits were collected from different individuals per



Fig. 1. Flower of melon cactus, *Melocactus curvispinus*, in longitudinal section. Figure abbreviations: *a* anthers ring; *s* stigma; *n* nectar chamber; *o* ovary

population. We determined number of seeds per flower and proportion of aborted seeds per flower. All floral variables were compared between localities using two-tailed *t*-tests. The following transformations were conducted on the variables prior to comparisons: square root of x for floral measures and proportions of biomass allocation and aborted seeds, 1 + x square root for stigma-anthers distance (herkogamy), and logarithm for number of stamens, ovules, and seeds per flower.

Reproductive phenology. Flower and fruit production was monitored monthly in 40 reproductive melocacti in the mainland population for two years (Sep. 1996 - Sep. 1998). Plants were labeled and total number of mature floral buds and mature fruits were recorded each month. For each phenophase, we estimated monthly percentage (%) of occurrence ([number of plants in phenophase/total plants] \times 100) and monthly phenophase intensity, as number of flowers or fruits produced by an individual divided by maximum number recorded in a month for that individual during the two years of observations. Phenophase intensity is a relative measure that describes how intense or strong a phenophase is in each individual in a given period of time. We used the maximum number of floral or fruit units produced by each individual in a month as a

referential value to estimate phenophase intensity. We determined this monthly maximum for each individual once the phenophase monitoring process had concluded (two years for this study). Phenophase intensity reaches its maximum value (PI = 1.0), when an individual produces the maximum number of floral of fruit units recorded for that individual in a month. In addition, number of flowers produced per plant per day was estimated.

Anthesis and floral rewards. The process of anthesis was recorded in 108 flowers from 40 different plants on 21 days. Flower monitoring began at noon and lasted until flowers closed. We assigned flowers to two anthesis categories, closed and open. Changes in stigma morphology and position from anthesis to senescence were monitored hourly in 23 flowers from 10 different individuals. In these flowers we measured the distance between the base of the stigma and the distal most anthers with a digital calliper. We also looked for changes in the arrangement of the stigmatic lobes from anthesis to senescense. To estimate nectar secretion amount (µl), 30 flowers from 30 different plants were covered with fine wedding veil and a small cap of aluminum foil before anthesis. Nectar secretion was measured at two stages during the entire anthesis period, middle and end. Nectar produced in the nectar chamber was extracted using microcapillary tubes. We then measured length of nectar extracted in microcapillary tubes and converted length to volume. Extracted nectar was used to estimate sugar concentration (percentage sucrose equivalence by weight/total weight of solution) using a hand refractometer (Model BRIX50, Leica Corporate, Buffalo, New York, USA). Solute quantity (assumed to be sugars, expressed in sucrose-equivalents) was calculated following Bolten et al. (1979).

Floral visitors and visitation patterns. Floral visitors were observed, photographed or captured (insects) and preserved in 70% ethanol and identified. Flower visitation behavior of the main visitor types was observed and described in detail by observing visitation with binoculars about 4 m from the plants in the case of hummingbird and lizard visits, and 1 m from the plants in the case of insect visits. Pollinators were distinguished from floral visitors using the following criteria: (1) presence of pollen on the body, (2) effective contact between the area covered with pollen in the visitor and sexual

organs in the flower, and (3) relative abundance of visits. We recorded number of floral visits and visitors using binoculars, observing from inconspicuous locations behind bushes ≥ 4 m from the target plants. Data recording was conducted at alternate intervals of 30 min duration, from beginning of anthesis until flowers closed. Normally, between two and three melocacti in bloom were monitored in a given day. A total of 123 melocacti in flower were monitored over one year at an approximate rate of one day of observation/week. On each day we recorded visits, we estimated percentage of visits per plant attributed to each visitor type for each observation period; we then averaged these estimates over the total number of plants monitored during the entire year. Percentages of floral visitation were compared between the two most important floral visitors for each observation interval using a t- test on transformed values (arcsine square root). We also determined the rate of floral visitation during the entire anthesis period for the main visitor types. Pearson correlation analyses were conducted to test for association between number of flowers/plant and rate of floral visits.

Breeding system. Four hand pollination treatments were performed to 20-59 flowers of M. curvispinus in coastal and mainland populations to determine its breeding system: (1) agamospermy (bagged flowers with stigma blocked with Tanglefoot ®, The Tanglefoot Company, Grand Rapids, Michigan, USA), (2) automatic pollination (undisturbed bagged flowers), (3) hand-self pollination (bagged flowers hand-pollinated with their own pollen), and (4) hand cross-pollination (bagged flowers hand-pollinated with pollen from plants separated from focal females by at least 20 m). Flowers were bagged using doubly folded extra-fine wedding veil. Hand-self pollination was performed by rubbing vigorously a cluster of anthers against the stigma of the same flower. Hand cross-pollination was performed by rubbing a cluster of anthers from three different plants against the stigma of a focal plant. Pollen donor plants were bagged before anthesis to avoid pollen contamination. For this treatment, each cluster of anthers was used only on flowers of one focal plant. Self- and cross-pollination treatments were repeated at least twice (at two and four hours after anthesis) in each flower to guarantee stigma receptivity and pollen viability. No more than two flowers of the same individual were used for each test. Fruit/flower ratio and total

number of seeds were determined for each pollination treatment. Indexes of self-incompatibility (ISI) and autogamy (IA) were calculated at two levels, fruit set (Ruiz-Zapata and Arroyo 1978) and seed set (Jaimes and Ramírez 1999). ISI was calculated by dividing ratios of fruit/flower and total seeds/ total ovules via hand self-pollination by fruit/flower and total seeds/total ovules ratios via hand-crosspollination, respectively. IA was calculated as ratios of fruit/flower and total seeds/total ovules via automatic pollination divided by ratios of fruit/ flower and total seeds/total ovules via hand crosspollination. Based on Ruiz-Zapata and Arroyo (1978), ISI and IA values above 0.2 indicate selfcompatibility and autogamy, respectively.

Results

Floral traits. Flowers have a narrow floral tube with a nectar chamber limited by the base

of the stamen ring and an inferior ovary (Fig. 1). The perianth and upper part of the floral tube is bright magenta. No distinctive odor was detected from open flowers. Floral traits analyzed for the two populations surveyed are summarized in Table 1. Despite their close resemblance, mainland flowers were slightly larger (t = 3.36, P < 0.01, d.f. = 64)and had a narrower (t = 5.01, P < 0.0001,d.f. = 64) corolla than coastal flowers. Flowers were hermaphroditic in both populations. Total flower biomass is partitioned into 65.9 (2.1 SE) - 77.9 (1.6) % vegetative tissue, 13.2 (0.8) - 20.9 (1.9) % gynoecium, and 8.9 (0.8) -13.1 (1.2) % androecium. In each flower, a single central style ended in a multilobed stigma with 12 to 20 lobes surrounded by a ring of numerous stamens (coastland: 151.9 (3.0) stamens/flower; mainland: 145.9 (3.1)

Table 1. Comparison of floral traits between two populations of Melocactus curvispinus from Venezuela

Character	Ν	Coastal mean (SE) range	Mainland mean (SE) range	Test	
External length (mm)	33	24.48 (0.28) 20.9–27.7	25.89 (0.31) 22.3–29.3	t = 3.357 P < 0.01, d.f. = 64	
Corolla width (mm)	33	3.17 (0.04) 2.8–3.8	2.76 (0.07) 2.1–3.9	t = 5.012 P < 0.0001, d.f. = 64	
Herkogamy (mm)	33	1.78 (0.24) -1.8-4.7	0.58 (0.20) -1.9-2.6	t = 3.850 P < 0.001, d.f. = 64	
Number of stamens/flower	33	151.9 (3.04) 121–192	145 (3.07) 117–179	t = 1.644 P = 0.105, d.f. = 64	
Number of pollen grains/anther	5	1096	967	_	
Number of pollen grains/flower	_	166482.4	140215.0	-	
Number of ovules/flower	33	394.3 (14.56) 220–522	343.7 (14.1) 204–488	t = 2.420 P = 0.018 d f = 64	
Pollen:ovule ratio	_	422.2	408.0	_	
Number of seeds/flower	45	290.4 (11.7) 109–457	280.3 (11.8) 83–492	t = 0.617 P = 0.539, d.f. = 88	
Seed:ovule ratio	_	0.736	0.816	-	
Aborted seeds (%)	45	1.49 (0.53) 0–23.7	3.88 (0.72) 0–22.8	t = 3.918 P < 0.001, d.f. = 88	
Androecium biomass (%)	10	8.9 (0.8) 5.2–12.6	13.2 (0.8) 8.8–18.0	t = 3.868 P = 0.01, d.f. = 18	
Gynoecium biomass (%)	10	13.1 (1.2) 6 5–17 2	20.9 (1.9) 14 2–34 4	t = 3.458 P < 0.01 d f = 18	
Vegetative biomass (%)	10	77.9 (1.6) 70 4–87 6	65.9 (2.1) 50 7–73 6	t = 4.494 P < 0.001 d f = 18	
Androceum:Gynoecium ratio Reproductive:vegetative ratio	_	0.679 0.282	0.632 0.517		

stamens/flower). Stigma-anthers separation (herkogamy) was significantly different from zero (95% confidence interval) in the two populations, with the stigma normally above the ring of stamens. The stigma-anthers separation in the coastal population (1.78 \pm 0.24 SE mm) was greater than in the mainland population (0.58 \pm 0.20 mm); however, within each population, herkogamy was quite variable. In the coastal population, the stigma-anthers distance ranged from 1.8 mm below the distal most anthers to 4.7 mm above them. In the mainland population it ranged from 1.9 mm below the distal most anthers to 2.6 mm above them. Estimated pollen production per flower was >140,000 pollen grains. On average, flowers produced between 343.7 (14.1) and 394.3 (14.6) ovules. About 400 pollen grains are produced per each ovule. Mature fruits contained numerous black seeds embedded in the pulp. Number of seeds produced per flower was quite similar in the mainland $(280.3 \pm 11.8 \text{ SE})$ and coastal (290.4 ± 11.7) populations. Percentage of aborted seeds per flower was below 4%, but average values significantly differed between populations (Table 1). Overall, above 73% of total ovules per flower were successfully fertilized and yielded viable seeds.

Reproductive phenology. Flower production occurs during most of the year. At least 20% of the plants in the population had mature flowers in all monitored intervals during two years of observations (Fig. 2). Flowering peaks corresponded fairly well with peaks of precipitation for Venezuelan arid lands (May-July and September-October). During those peaks, up to 70% of plants monitored produced flowers. Percentage and intensity of fruit set were relatively low (< 20%) over the two years monitored, with small fruiting peaks also located during the same time periods in which precipitation is important. Overall, these results indicate that M. curvispinus is reproductively active during most of the year, although a seasonal pattern occurs in the flower phenophase.



Fig. 2. Flowering and fruiting phenology of *Melocactus curvispinus* over a two-year period measured as percentage of occurrence and intensity of each phenophase. See text for explanation

Anthesis and floral rewards. On average, sexually mature individual produces а 1.44 ± 0.064 SE (N = 123) flowers/plant/day. Overall, flower duration lasted less than 7 hours. Floral buds partially emerged from the *cephalium* during the morning (08:00 - 12:00). Anthesis started around noon (\sim 12:30). Anther dehiscence was observed to occur before anthesis (after 09:00). All flowers had reached complete anthesis by 15:00. Stigma lobes separated from each other around the time of floral opening. They did not reflex nor their apices extended below the base of the stigma as the time progressed until senescence. During the first three hours after anthesis the stigma-anthers separation remained quite stable 2.84 (0.32 SE) mm. At the fourth hour after anthesis the stigma of 52.1% (N = 23) of the flowers monitored started retracting towards the anthers, and the stigma-anthers separation reduced to 1.95 (0.38) mm. Percentage of flowers with stigma retraction increased in the fifth (73.9%) and sixth (87.0%) hour after anthesis. By the time of senescence, the stigma-anthers separation had decreased to an average of 1.30 (0.33) mm. For a substantial proportion (47.8%) of the flowers monitored for changes at the stigma level, the base of the stigma and the stigma lobes made contact with the anthers before senescence. Based on these results, herkogamy in M. curvispinus should be considered a variable and dynamic process, which decreases in most flowers as the time progresses. Flowers senescence started on the same day at sunset $(\sim 18:00)$, with 89% of them closed at 19:00, and the rest closed before 22:00. When flowers close the anthers collapse against the stigma, a process that could promote pollen deposition on the latter. Senesced flowers remained dry on the cephalium for several weeks and even months after anthesis.

Nectar secretion began at anthesis $(\sim 12:30)$, but measurable amounts of nectar were only available about 2 h after anthesis began. Average total volume of nectar produced in flowers isolated from floral visitors was 163.1 \pm 15.0 SE µl. Nectar production for the first three hours (12:00–15:00) after anthesis $(77.74 \pm 5.69 \mu l)$ was six times higher than nectar production (12.26 \pm 3.12 µl) during the next three hours (15:00-18:00). No nectar production was detected after 1800. Nectar sugar concentration remained relatively stable during the entire period of nectar secretion $(30.2 \pm 0.21 \text{ SE \%} \text{ at } 15:00 \text{ and } 29.1 \pm 0.28\%$ at 18:00). Flowers produced an average of 62.31 ± 3.92 SE mg sugar during their entire flowering duration.

Floral visitors and visitation patterns. The Buffy Hummingbird, *Leucippus fallax* (Bourcier 1843), and small bees in the genus *Ceratina*, subgenus *Ceratinula* (Anthophoridae) were the main floral visitors of

M. curvispinus during one year of observations. Both visitor types effectively contacted anthers and stigmas during their visits. Hummingbirds hovered for 1-3 seconds above flowers, or more rarely, perched briefly on the *cephalium* during their visits. These birds visited flowers of several individuals (1-5) within a patch, switching among different individuals and returning to them after variable time intervals (5–50 min). While visiting, hummingbirds introduced their bills partially or totally into the floral tube. On numerous occasions the bird's crown contacted the flower perianth and stigma. In their visits, anthophorid bees hovered at flowers and landed on the perianth before entering the floral tube. They emerged by climbing through the floral tube or using the style. Some individuals remained inside the floral tube for up to 2 min. Bees tended to concentrate their visits on flowers of a few adjacent plants. Besides hummingbirds and anthophorid bees, other less frequent floral visitors included birds such as the Bananaquit, Coereba flaveola (Linnaeus 1758), honeybees, Apis mellifera Linnaeus, butterflies (Phoebis sp.), and lizards, Cnemidophorus arenivagus Markezich, Cole & Dessauer, the latter mostly acting as flower predators. During the first four hours of anthesis, percentages of floral visits by hummingbirds and anthophorid bees remained relatively stable, between 35 and 50% of total visits, and did not differ significantly between the two visitor types (Fig. 3). After 16:00, percentages of floral visits differed significantly between hummingbirds and bees. Floral visitation by anthophorid bees decreased gradually until sunset, while percentage of floral visits by hummingbirds increased gradually, reaching values above 90% by sunset (Fig. 3). Floral visitation by other visitor types never surpassed 20%. In a given day, one plant in bloom received in average 28.75 ± 3.75 SE visits by anthophorid bees and 4.96 ± 0.21 visits by hummingbirds. Despite a higher average number of visits by bees, many more melocacti in the population were visited by hummingbirds (98%, n=123) than by these insects (54%, n=123)n = 123). Finally, total number of floral visits



Fig. 3. Percentage of visits (mean ± 1 SE) to flowers of *Melocactus curvispinus* by five floral visitor types during floral anthesis. Percentages were calculated based on total number of visits at each time interval and averaged over total number of observations (n = 123). Statistical comparisons (two-tailed *t*-test) were conducted between hummingbird and anthophorid bee visits. **ns** = non significant, * = P < 0.05, ** = P < 0.001

was positively correlated with number of flowers open per plant (r = 0.471, P < 0.0001, N = 123) as were number of floral visits by hummingbirds (r = 0.504, P < 0.0001,

N = 123), and by anthophorid bees (r = 0.436, P < 0.0001, N = 123).

Breeding system. M. curvispinus is a selfcompatible $(ISI_{[fruit]} = 0.728 - 0.865,$ cactus $ISI_{[seed]} = 0.702 - 0.929$; Table 2). Results of the undisturbed cloth-bagged flowers indicate that this species is also autogamous $(IA_{[fruit]} = 0.718 - 0.755, IA_{[seed]} = 0.575 - 0.785;$ Table 2). No agamospermy was detected in this species. In open-pollinated flowers, fruit set was > 75% and seed set was > 56%(Table 2). Highest levels of fruit and seed set occurred in the mainland population, where the highest pollen/ovule ratio was observed (Tables 1 and 2). In the mainland population, fruit set via open pollination (96%) slightly surpassed $(X^2 = 4.39, P < 0.05, d.f. = 1)$ fruit set via hand cross-pollination (85%), suggesting that pollen limitation did not occur in that location. For the coastal population, fruit set via hand cross-pollination (88%) surpassed $(X^2 = 43.4, P < 0.0001, d.f. = 1)$ fruit set via open pollination (76%), suggesting a small but significant degree of pollen limitation for this population. Overall, the relatively high levels of seed set observed in the two populations suggest high effectiveness in the process of pollen deposition on floral stigmas,

Table 2. Fruit set results of pollination treatments conducted on *M. curvispinus* in the mainland and coastal populations examined in Venezuela. In addition, self-incompatibility (ISI) and autogamy (IA) indexes are included. Index values below 0.2 are indicative of self-incompatibility and no autogamy, respectively (Ruiz-Zapata and Arroyo 1978)

Population	Treatment	No. of flowers	Fruit: flower ratio	Index	No. of Seeds	Seeds: ovules ratio	Index
Coastal	Agamospermy	10	0		0	0	
	Cloth bagging	38	0.684	IA 0.755	7547	0.5037	IA 0.785
	Hand self-pollination	38	0.763		8934	0.5963	
	Hand cross-pollination	34	0.882	ISI 0.865	8601	0.6416	ISI0 0.929
	Open pollination	112	0.759		24854	0.5628	
Mainland	Agamospermy	11	0		0	0	
	Cloth bagging	59	0.610	IA 0.718	7331	0.3615	IA 0.575
	Hand self-pollination	42	0.619		6367	0.4411	
	Hand cross-pollination	40	0.850	ISI 0.728	8643	0.6287	ISI 0.7016
	Open pollination	50	0.960		13361	0.7775	

either via pollinator action or through automatic pollination.

Discussion

The results of this study support several of the generalizations formulated by Taylor (1991) for the genus Melocactus, but also add specific new information on the pollination biology and reproductive phenology of one of the most widely distributed representatives of the genus. Melocactus curvispinus has several floral traits, including diurnal anthesis, bright reddish perianth color, narrow tubular shape, basal nectar chambers, and odorless flowers which suggest that this cactus is bird-pollinated (Faegri and van der Pijl 1979, Proctor et al. 1996). Floral morphology was quite homogeneous across populations, although slight but significant inter-population differences in a few morphological and biomass attributes were detected. From these differences, herkogamy is the only one that could have some impact on the reproductive traits of the populations. Herkogamy seems more prominent in the coastal population, thus a higher proportion of outcrossing events could be expected in this population, at least during the first hours after anthesis. Narrow floral tubes restrict the spectrum of potential avian pollinators mainly to hummingbirds (Brown and Kodric-Brown 1979). This was confirmed in our study by the great importance that these birds had as assiduous floral visitors of this cactus. Altogether, this body of evidence is congruent with the hummingbird-pollination syndrome attributed by Taylor (1991) to species within Melocactus. M. curvispinus, and probably many other congeneric species with similar floral traits, form part of the numerous examples of ornithophilous cacti mentioned in the literature (Porsch 1924, 1929; Cota 1993; Rose and Barthlott 1994; Anderson 2001). But, despite its hummingbird adaptations, pollination of the melon cactus can not be attributed exclusively to these birds. As we discuss below, small bees might play also an important role in the sexual reproduction of this plant.

Contrary to the marked reproductive seasonality reported for Venezuelan semiarid vegetation (Guevara de Lampe et al. 1992, Poulin et al. 1992), M. curvispinus produces flowers and fruits during most of the year; however, flowering intensifies during rainfall periods. This reproductive pattern seems stable during the two-year period we monitored reproductive phenology. Other Venezuelan cacti that show year-round reproductive activity include the columnar cactus Stenocereus griseus (Nassar et al. 1997). This longterm reproductive strategy tends to maximize the chances for sexual reproduction in the species. As shown in Fig. 2, M. curvispinus produced fruits in 21 of the 25 months monitored. From the perspective of floral visitors and fruit consumers, blooming and fruiting periods that extend over most of the year are an evidence for a guarantee of water, nutrients, and energy supplies, even during times of low food resource productivity in arid zones.

Melon cactus flowers and their associated rewards (pollen, nectar, and tepals) become accessible only to diurnal floral visitors that are active from early afternoon to sunset. Average daily nectar production per flower (163.1 µl) is comparable to average values reported for the claret cup cactus, *Echinocereus* coccineus (94.3 µl), another globose cactus pollinated by hummingbirds in southwestern US (Scobell and Scott 2002). These levels of nectar secretion are higher than values reported for hummingbird-pollinated flowers from temperate plant communities, not including cactus species, in southwestern US (Brown and Kodric-Brown 1979, Lange et al. 2000) and in tropical forests (Grases and Ramírez 1998). Nectar sugar concentration in flowers of M. curvispinus (~ 30% weight/weight) also resembled the estimated value reported for the claret cup cactus (29%; Scobell and Scott 2002), but was above average concentrations reported for other hummingbird-pollinated cacti (25%, n = 11; Scogin 1985) and hummingbird flowers in general (25%, n=202;Pyke and Waser 1981). The relatively high

levels of nectar sugar concentration found in M. curvispinus could be interpreted as evidence of an intermediate state between a hummingbird-pollinated and a bee-pollinated flower. The fact that both hummingbirds and anthophorid bees are frequent effective floral visitors of this species supports this hypothesis. Flowers of M. curvispinus produced on average twice as much sugar as flowers of the claret cup cactus (30 mg/flower/day; Scobell and Scott 2002), even though flowers of the latter species are three times larger and ten times wider than those of Melocactus. Altogether, our results place M. curvispinus as a hummingbird-pollinated cactus with highly rewarding diurnal floral resources in Venezuelan arid zones. The high energetic value of these floral resources is evidenced by the territorial displays performed by hummingbirds that feed on flowers of several Melocactus species (Taylor 1991, Raw 1996). Preliminary observations on the behavior displayed by the Buffy Hummingbird when using patches of *M. curvispinus* suggest that in some circumstances this bird defends the resource (J. Nassar, unpublished data).

Hummingbirds and small anthophorid bees were the main floral visitors of M. curvispinus in the populations we studied. Although we do not discount the possibility that other species of hummingbirds from Venezuelan arid habitats (e.g. Amazilia tobaci (Gmelin 1788) and Chrysolampis mosquitus (Linnaeus 1758); Poulin et al. 1992, Hilty 2002, respectively) may visit flowers of melon cactus, our observations suggest that the Buffy Hummingbird, Leucippus fallax, is probably the most important species visiting flowers of this species in Venezuela. This was the only hummingbird we observed feeding on melon cactus flowers in the two locations studied. The Buffy Hummingbird is an effective and reliable pollinator of M. curvispinus. During floral visits, hummingbirds contact anthers and stigmas mainly with their bills and sometimes crowns. These birds were always present in the study sites. They visited 98% of total plants monitored and they remained actively searching for nectar for the entire anthesis period. But despite always being present, their relative importance as visitors increased from midafternoon towards sunset, suggesting that pollination events occurring during the first hours of floral anthesis are shared with other floral visitors.

Anthophorid bees were the other important visitors of melon cactus flowers. During their visits, they effectively contacted anthers and stigma, therefore functioning as pollinators. These insects visited 54% of the plants we monitored. Although they were less assiduous visitors than hummingbirds, their average rate of visits/plant/day was almost six-fold (~29) higher than the values estimated for hummingbird visits. Contrasting with hummingbirds, their visiting role is concentrated during the first four hours of anthesis after which visitation decreased gradually until sunset. Even though anthophorid bees displayed a higher rate of floral visitation than hummingbirds, we can not attribute higher pollination efficiency to these insects, because we do not know how much pollen is deposited on the stigma by either of these floral visitors.

It is widely recognized that, despite displaying a distinctive pollination syndrome, a flowering plant can have alternative co-pollinators that do not fit that syndrome but which also play a significant role as alternative pollen vectors (Ollerton 1996, Waser et al. 1996, Johnson and Steiner 2000). Among plants pollinated by hummingbirds in general, examples of this include Macleania bullata (Ericaceae), in which nocturnal co-pollinators were responsible for at least half the total fruit set produced in open-pollinated flowers (Navarro 1999); Delphinium nelsonii (Ranunculaceae), in which hummingbirds and bumblebees are equally effective pollinators (Waser and Price 1990); and Ipomopsis aggregata (Polemoniaceae), in which bumblebees, on average, deposited much more outcross pollen per visit to virgin flowers and elicited much more seed set than hummingbirds (Mayfield et al. 2001). Among hummingbird-pollinated cacti, examples of generalized pollination include the claret cup cactus, in which several species of halictid bees are effective co-pollinators (Scobell and Scott 2002). Our observations suggest that *M. curvispinus*, despite of showing a distinctive hummingbird pollination syndrome, can also be effectively pollinated by small insects such as anthophorid bees. However, this conclusion is based only on results of visitation frequencies and observations of how floral visitors contact reproductive organs in the flower. Only by conducting pollination exclusion experiments we will be able to determine the relative importance of hummingbirds and small bees for sexual reproduction of this cactus.

Assuming that these two floral visitors function as pollinators, there are differences in the way they approach flowers that allow us to assume the consequences of their visits. In the case of anthophorid bees, by remaining on relatively few and adjacent flowers and spending appreciable time contacting reproductive organs within a given flower, this pollen vector could be promoting inbreeding and population subdivision. On the other hand, the Buffy Hummingbird is a relatively large hummingbird (total length = 8.9 cm, Hilty 2002), solitary, and aggressive, that was observed on several occasions chasing other birds out of melon cactus patches. As a result of its pollination and territorial behavior, this hummingbird can move pollen among plants within a patch, but at the same time can restrict mating to individuals belonging to a given group. The overall effect would be a substantial level of outcrossing within the patch but also genetic differentiation among patches and populations. In support of these expected effects, Nassar (1999) found that M. curvispinus has a mixed-mating system, with a population outcrossing rate of 76% and individual outcrossing rates varying between 18 and 100%. In addition to this, Nassar et al. (2001) reported that Venezuelan populations of *M. curvispinus* have relatively higher levels of genetic structure ($F_{\rm ST} = 0.193$) than other Venezuelan cacti pollinated by insects $(G_{ST}=0.112, Nassar et al. 2002)$ and bats $(G_{\rm ST} = 0.092 - 0.126, \text{ Nassar et al. 2003}).$

Cacti are predominantly outcrossers and self-incompatibility is considered to be widespread in the family (Ganders 1975, Gibson and Nobel 1986, Boyle 1997, Boyle and Idnurm 2001). In his monograph on the genus Melocactus, Taylor (1991) indicated that even though the breeding system of Melocactus has not been investigated, some observations conducted on cultivated and wild plants suggested that most species are self-compatible and capable of selfing. Our hand-pollination results confirm that *M. curvispinus* is self-compatible and autogamous. Pollen/ovule ratios recorded for M. *curvispinus* in this study place this species at the interface between autogamous and facultatively xenogamous species (Cruden 2000). In addition, the androecium:gynoecium biomass ratio observed for this cactus indicates that the female function receives a comparatively higher biomass investment than the male function. Female-biased biomass investment is typical of self-compatible and autogamous species (Charnov 1982, McKone 1987).

The self-compatible and autogamous condition observed for M. curvispinus agrees with the widely recognized evolutionary transition proposed for flowering plants, by which selfing races or species have evolved from outcrossing ancestors (Stebbins 1950, Grant 1981, Takebayashi and Morrell 2001). Within tribe Cereeae, species in genus Cereus represent the most plesiomorphic condition (Taylor and Zappi 1989), and some of their representatives such as Cereus repandus, C. horrispinus, and Pilosocereus lanuginosus are obligate outcrossers (Nassar et al. 1997). On the other hand, Melocactus contains the most derived characteristics in that tribe (Taylor and Zappi 1989), and it is within this genus where we find examples of an evolutionary transition towards selfing. In fact, there are documented cases of cleistogamous species, like M. lanssensianus (Taylor 1991), which suggest that selfing has reached an extreme stage in some members of this genus.

But far from being an extreme example of a selfing cactus, *M. curvispinus* behaves more like a mixed-mating species that successfully

achieves sexual reproduction either by outcrossing or selfing. Outcrossing can be mechanically promoted by herkogamy. Most flowers promote outcrossing during the first hours after anthesis, but during the last hours before senescence the stigma of many flowers can make contact with the anthers, therefore promoting self-pollination. Several floral traits in this cactus encourage floral visitation and pollination by hummingbirds. These birds normally transport pollen among multiple individuals within a patch. On the other hand, small bees appear to stimulate selfing by circulating pollen within flowers. Finally, if all types of animal-mediated pollination happen to fail, this species has the possibility of self-pollination by collapsing the anthers against the stigma when flowers close at sunset. Thus, by combining extended reproductive activity, reliable and frequent animal-mediated pollination, and selfing capabilities, this cactus has adopted a reproductive strategy that guarantees reproduction under variable environmental conditions in arid zones.

This research was funded by Instituto Venezolano de Investigaciones Científicas (to JMN) and Fondo Nacional de Ciencia Tecnología e Innovación (to NR). We are thankful to Janet Castro, Herber Briceño, Roberto Casado, José Antonio González, and William Durán for laboratory assistance. We thank Argenis Silva for field assistance. Marco Gaiani from Universidad Central de Venezuela identified the anthophorid bees. Theodore H. Fleming made valuable comments on the final draft of the manuscript.

References

- Anderson E. F. (2001) The Cactus Family. Timber Press, Portland, Oregon, US.
- Bolten A. B., Feinsinger P., Baker H. G., Baker I. (1979) On the calculation of sugar concentration in flower nectar. Oecologia 41: 301–304.
- Boyle T. H. (1997) The genetics of self-incompatibility in the genus *Schlumbergera* (Cactaceae).J. Heredity 88: 209–214.
- Boyle T. H., Idnurm A. (2001) Physiology and genetics of self-incompatibility in *Echinopsis*

chamaecereus (Cactaceae). Sex Plant Reprod. 13: 323–327.

- Breckenridge F. G., Miller J. M. (1982) Pollination biology, distribution, and chemotaxonomy of the *Echinocereus enneacanthus* complex (Cactaceae). Syst. Bot. 7: 365–378.
- Brown J. H., Kodric-Brown A. (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. Ecology 60: 1022–1035.
- Cota J. H. (1993) Pollination syndromes in the genus *Echinocereus*: a review. Cactus and Succulent J. (U.S.) 65: 19–26.
- Cruden R. W. (2000) Pollen grains: why so many? Plant Syst. Evol. 222: 143–165.
- Charnov E. L. (1982) The theory of sex allocation. Princeton University Press, Princeton, NJ, USA.
- Da Silva B. P., Parente J. P. (2002) Chemical properties and biological activity of a polysaccharide from *Melocactus depressus*. Planta Med. 68: 74–76.
- Das A. B., Mohanty S., Das P. (1998) Variation in karyotype and 4C DNA content in six species of *Melocactus* of the family Cactaceae. Cytologia 63: 9–16.
- Faegri K., Pijl L. van der (1979) The principles of pollination ecology. Pergamon Press, Oxford, USA.
- Fernández-Alonso J. L., Xhonneux G. (2002) Novedades taxonómicas y sinopsis del género *Melocactus* Link & Otto (Cactaceae) en Colombia. Rev. Acad. Colomb. Cienc. 26: 353–365.
- Fleming T. H., Tuttle M. D., Horner M. A. (1996) Pollination biology and the relative importance of nocturnal and diurnal pollinators in 3 species of Sonoran Desert columnar cacti. Southwest. Nat. 41: 257–269.
- Ganders F. R. (1975) Self-incompatibility in the Cactaceae. Incompatibility Newsletter 6: 5–9.
- Gibson A. C., Nobel P. S. (1986) The cactus primer. Harvard University Press, London, England.
- Grant V. (1981) Plant speciation, 2nd edn. Columbia University Press, New York, New York, USA.
- Grant V., Grant K. A. (1979) Pollination of *Echinocereus fasciculatus* and *Ferocactus wislizennii*. Plant Syst. Evol. 132: 85–90.
- Grant V., Hurd P. D. (1979) Pollination of southwestern *Opuntias*. Plant Syst. Evol. 133: 15–28.

- Grases C., Ramírez N. (1998) Biología reproductiva de cinco especies ornitófilas en un fragmento de bosque caducifolio secundario en Venezuela. Rev. Biol. Trop. 46: 1095–1108.
- Guevara de Lampe M., Bergeron Y., McNeil R., Leduc A. (1992) Seasonal flowering and fruiting patterns in tropical semi-arid vegetation of northeastern Venezuela. Biotropica 24: 64–76.
- Hilty S. L. (2002) Birds of Venezuela. 2nd edn. Princeton University Press, Princeton, NJ, USA.
- Hoffman M. T. (1992) Functional dioecy in *Echinocereus coccineus* (Cactaceae): breeding system, sex ratios, and geographic range of floral dimorphism. Amer. J. Bot. 79: 1382–1388.
- Huber O., Alarcón C. (1988) Mapa de Vegetación. M.A.R.N.R., Caracas, Venezuela.
- Jaimes I., Ramírez, N. (1999) Breeding systems in a secondary deciduous forest in Venezuela: The importance of life form, habitat, and pollination specificity. Plant Syst. Evol. 215: 23–36.
- Johnson S. D., Steiner K. E. (2000) Generalization versus specialization in plant pollination systems. Trends Ecol. Evol. 15: 140–143.
- Lange R. S., Scobell S. A., Scott P. E. (2000) Hummingbird – syndrome traits, breeding system, and pollinator effectiveness in two syntopic *Penstemon* species. Int. J. Plant Sci. 161: 253–263.
- Lloyd D. G. (1965) Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contr. Gray Herb. Harv. 195: 3–134.
- M.A.R.N.R. (1995) Balance ambiental de Venezuela 1994–1995. Ministerio del Ambiente y de los Recursos Naturales Renovables. Caracas, Venezuela.
- Mayfield M. M., Waser N. M., Price M. V. (2001) Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. Ann. Bot. 88: 591–596.
- McFarland J. D., Kevan P. G., Lane M. A. (1989) Pollination biology of *Opuntia imbricata* (Cactaceae) in southern Colorado. Canad. J. Bot. 67: 24–28.
- McKone M. J. (1987) Sex allocation and outcrossing rate: a test of theoretical predictions using bromegrasses (*Bromus*). Evolution 41: 591–598.
- Nassar J. M. (1999) Comparative population genetic structure of Venezuelan cacti and estimates of their mating systems (Ph.D. dissertation). University of Miami, Coral Gables, Florida, USA.

- Nassar J. M., Hamrick J. L., Fleming T. H. (2001) Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). Heredity 87: 69–79.
- Nassar J. M., Hamrick J. L., Fleming T. H. (2002) Allozyme diversity and genetic structure of the leafy cactus (*Pereskia guamacho* [Cactaceae]).
 J. Heredity 93: 193–200.
- Nassar J. M., Hamrick J. L., Fleming T. H. (2003) Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). Amer. J. Bot. 90: 1628–1637.
- Nassar J. M., Ramírez N., Linares O. (1997) Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. Amer. J. Bot. 84: 918–927.
- Navarro L. (1999) Pollination ecology and effect of nectar removal in *Macleania bullata* (Ericaceae). Biotropica 31: 618–625.
- Ollerton J. (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. J. Ecol. 84: 767–769.
- Osborn M. M., Kevan P. G., Lane M. A. (1988) Pollination biology of *Opuntia polyacantha* and *Opuntia phaeacantha* (Cactaceae) in southern Colorado. Plant Syst. Evol. 159: 5–94.
- Ponce M. (1989) Distribución de las cactáceas en Venezuela y su ámbito mundial (Trabajo Especial de Ascenso a Profesor Agregado). Universidad Central de Venezuela, Facultad de Agronomía, Maracay, Venezuela.
- Porsch O. (1924) Vogelblumenstudien I. Jahrbuch der wissenschaftlichen Botanik 63: 553–706.
- Porsch O. (1929) Vogelblumenstudien II. Jahrbuch der wissenschaftlichen Botanik 70: 181–277.
- Poulin B., Lefebvre G., McNeil R. (1992) Tropical avian phenology in relation to abundance and exploitation of food resources. Ecology 73: 2295–2309.
- Proctor M., Yeo P., Lack A. (1996) The natural history of pollination. Timber Press, Portland, Oregon, USA.
- Pyke G. H., Waser N. M. (1981) The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 13: 260–270.
- Raw A. (1996) Territories of the Ruby-Topaz hummingbird, *Chrysolampis mosquitus* at flowers of the "Turk's-Cap" cactus, *Melocactus salvadorensis* in the dry caatinga of north-eastern Brazil. Rev. Bras. Biol. 56: 581–584.

- Rose M. J., Barthlott W. (1994) Coloured pollen in Cactaceae: a mimetic adaptation to hummingbird-pollination? Bot. Acta 107: 402–406.
- Ruiz-Zapata T., Arroyo M. T. K. (1978) Plant reproductive biology of a secondary deciduous forest in Venezuela. Biotropica 10: 221–230.
- Sahley C. T. (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). Amer. J. Bot. 83: 1329–1336.
- Scobell S. A., Scott P. E. (2002) Visitors and floral traits of a hummingbird-adapted cactus (*Echinocereus coccineus*) show only minor variation along an elevational gradient. Am. Midl. Nat. 147: 1–15.
- Scogin R. (1985) Nectar constituents of the Cactaceae. Southwest. Nat. 30: 77–82.
- Stebbins G. L. (1950) Variation and evolution in plants. Columbia University Press, New York, NY, USA.
- Takebayashi N., Morrell P.L. (2001) Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. Amer. J. Bot. 88: 1143–1150.
- Taylor N. P. (1991) The genus *Melocactus* (Cactaceae) in Central and South America. Bradleya 9: 1–80.

- Taylor N. P., Zappi D. C. (1989) An alternative view of generic delimitation and relationships in tribe Cereeae (Cactaceae). Bradleya 7: 13–40.
- Valiente-Banuet A., Arizmendi M. C., Rojas-Martínez A., Domínguez-Canseco L. (1996) Ecological relationships between columnar cacti and nectar-feeding bats in México. J. Trop. Ecol. 12: 103–119
- Waser N. M., Chittka L., Price M. V., Williams N. M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. Ecology 77: 1043–1060.
- Waser N. M., Price M. V. (1990) Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*. Collect. Bot. (Barcelona) 19: 9–20.

Address of the authors: Jafet M. Nassar (e-mail: jnassar@oikos.ivic.ve), Instituto Venezolano de Investigaciones Científicas, Centro de Ecología, Apdo. Postal 21827, Caracas 1020-A, Venezuela. Nelson Ramírez, Universidad Central de Venezuela, Facultad de Ciencias, Centro de Botánica Tropical, Apartado 48312, Caracas 1041-A, Venezuela.