

## Reproductive Biology and Mating System Estimates of Two Andean Melocacti, *Melocactus schatzlii* and *M. andinus* (Cactaceae)

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• **Background and Aims** The genus *Melocactus* comprises 36 species of globose cacti with the most derived traits in the Cereeae tribe. It is the proper study system to examine what are the most derived reproductive strategies within that tribe. This study aims to characterize the reproductive biology and to estimate the mating system parameters of two Andean melocacti, *Melocactus schatzlii* and *M. andinus*.

• **Methods** The reproductive attributes of the two species were described, including floral morphology, anthesis patterns, floral rewards, floral visitors and visitation patterns. Levels of self-compatibility and autonomous self-pollination were estimated by hand-pollination experiments. Mating system estimates were obtained by conducting progeny array analyses using isozymes.

• **Key Results** The flowers of the two species present the typical hummingbird-pollination syndrome. Despite their morphological resemblance, the two species differ in flower size, pollen and ovule production and anthesis pattern. Their main pollinator agents are hummingbirds, four species in *M. schatzlii* and one species in *M. andinus*. Both cacti are self-compatible and capable of self-pollination without the aid of pollen vectors. Population-level outcrossing rate was higher for *M. schatzlii* ( $t_m = 0.9$ ) than for *M. andinus* ( $t_m = 0.4$ ). At the family level, outcrossing rates for most mothers of *M. schatzlii* were higher ( $t_m > 0.8$ ) than for *M. andinus* ( $t_m < 0.5$ ).

• **Conclusions** Although the two cacti are capable of selfing, *M. schatzlii* is a predominantly outcrossing species, while *M. andinus* behaves as a mixed-mating cactus. Hummingbirds are the only pollinators responsible for outcrossing and gene flow events in these species. In their absence, both melocacti set seeds by selfing. Based on its low population size, restricted distribution in Venezuela, low rates of floral visits, and high levels of inbreeding, *M. andinus* is considered to be an endangered species deserving further study to define its conservation status.

**Key words:** Andes, Cactaceae, hummingbird, isozymes, mating system, *Melocactus andinus*, *Melocactus schatzlii*, reproductive biology.

### INTRODUCTION

Even though the mating system is one of the most influential life history traits on the patterns of genetic variation and evolution in flowering plants (Hamrick and Godt, 1989, 1996; Hamrick *et al.*, 1992), information about the reproductive biology of many angiosperm groups is still quite limited. For the Cactaceae, a distinctive family native to the Americas with approx. 1600 species, our knowledge on reproductive biology is scarce compared with other fields of study such as morphology, physiology, biochemistry, biomechanics and ethnobotany (Nobel, 2002). Besides their importance as food and water sources to animal communities in arid and semiarid environments, cacti are gaining agricultural and ornamental value in countries where climatic and soil conditions favour cultivation of these plants (Nobel, 2002). More research is necessary to assess their regeneration potentials and evaluate how their reproductive attributes can be used for commercial purposes. In addition, several cacti species are currently threatened (Oldfield, 1997). A thorough understanding of how mating systems can affect their

microevolution and population dynamics is fundamental to design conservation strategies for these plants.

The genus *Melocactus* comprises 36 species of cacti with some of the most derived traits in the Cereeae tribe: a single short globe-like stem, reproductive parts enclosed within a specialized protective structure called the ‘cephalium’, pollen grains with simple tectal perforations, hummingbird pollination and elongate clavate fruits (Taylor and Zappi, 1989). The derived condition of *Melocactus* within its tribe is supported by a recent molecular phylogenetic study by M. Machado *et al.* (University of Zurich, unpubl. res.). In contrast to *Melocactus*, the majority of the other genera in Cereeae are multibranching columnar cacti, one to several metres tall, with fully exposed large flowers, bat- or hawkmoth-pollinated and have depressed-globose to ovoid fruits. Other more complex reproductive attributes in the tribe indicative of the mating system, such as levels of self-incompatibility and capacity for self-pollination, have not been investigated. Taylor (1991) suggested that most melocacti are self-compatible and autogamous; however, these attributes have not been rigorously examined in most *Melocactus* species. As far as is known, reproductive studies in this genus have been

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conducted only in two taxa, *M. zehntneri* and *M. curvispinus* (Locatelli and Machado, 1999; Nassar and Ramírez, 2004). The high levels of fruit set (>60%) obtained from automatic and hand self-pollination experiments indicate that these species are self-compatible and autogamous. Estimates of selfing rates based on progeny array analyses indicate that *M. curvispinus* is facultatively xenogamous (Nassar, 1999). In order to have a comprehensive knowledge of the range of reproductive strategies within the *Melocactus* clade, reproductive studies combining classic pollination tests with genetic estimations of mating system parameters need to be conducted.

*Melocactus schatzlii* and *M. andinus* are among the few melocacti that inhabit Andean xeric patches. These species are appropriate to illustrate the reproductive attributes of the Andean representatives of this genus. Here, based on pollination tests and progeny array analyses using isozymes, the reproductive biology of the two species is described and the relative importance of autogamy and xenogamy in each cactus evaluated.

## MATERIALS AND METHODS

### Study sites and species

*Melocactus schatzlii* Till & Gruber and *M. andinus* Gruber ex Taylor are two melocacti circumscribed within the ‘*Melocactus curvispinus* group’, which includes species from Mexico, Cuba, Central America, northern Brazil and the Andes (Taylor, 1991). The two species have restricted distributions in the Venezuelan and Colombian Andes. Their habitats are characterized by steep and rocky valley slopes within xeric patches. Their altitudinal ranges suggest little overlap, *M. schatzlii* is found between 500 and 1050 m and *M. andinus* between 1500 and 3000 m, which is the altitudinal record for the genus (Taylor, 1991). *Melocactus schatzlii* was studied at Laguna de Caparú (08°30′10″N, 71°21′53″W), 3 km south-east of San Juan de Lagunillas, Mérida State, at 760 m a.s.l. The population of this species comprises several hundreds of individuals per hectare (J. Nassar, personal observation). *Melocactus andinus* was studied at Mesa de Esnujaque (09°03′22″N, 70°42′37″W), 1 km north of La Mesa, Trujillo State, at 1710 m a.s.l. The population of this species consists of 20–30 individuals per hectare (J. Nassar, personal observation), a low population density probably associated with illegal extractions of wild specimens (R. Caravalló, personal communication). The two sites are characterized by a mean annual precipitation below 600 mm and mean annual temperatures around 27 °C (MARNR, 1995). The vegetation type characteristic of these locations corresponds to the ‘xerophilous spiny scrubs’ and ‘intra-Andean cardonales’ categories of Huber and Alarcón (1988).

Both melocacti have depressed-globose bodies (Fig. 1). *Melocactus schatzlii* is usually shorter (<25 cm) than *M. andinus* (<40 cm). The two species develop a distinctive cephalium made of white or grey very compact wool. In *M. andinus*, the cephalium has exerted red bristles, while in *M. schatzlii* bristles are usually hidden in the

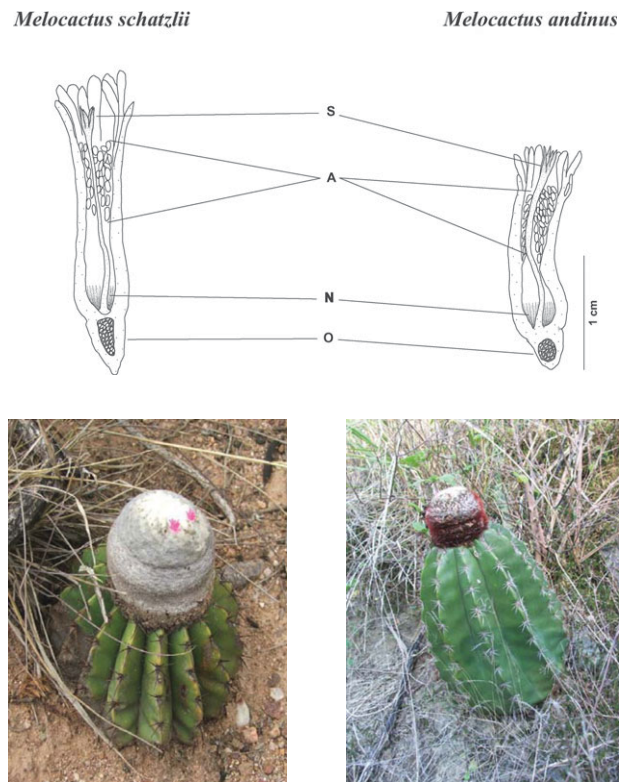


FIG. 1. Flowers of *Melocactus schatzlii* and *M. andinus* in longitudinal section and below sexually mature individuals of each species. Abbreviations: A, anthers ring; S, stigma; N, nectar chamber; O, ovary. Photos: J. A. González and J. M. Nassar.

wool. In both species flowers are pale to deep magenta and are located in the cephalium, barely exerted from it 1 d before anthesis and while flowers remain open. Although flowers can be observed during most of the year, flowering intensifies during periods of rainfall. Individuals are solitary or occur in clusters. Fruits of both species are conical fleshy berries, magenta or red, and multiple-seeded. No clonal reproduction occurs in these species, except for secondary growing of stems with cephalia after physical damage has occurred in the plant’s apical area.

### Floral traits

The number of flowers produced per plant per day was estimated for *M. schatzlii* ( $n = 89$ ) and *M. andinus* ( $n = 66$ ) during 2–3 weeks of field observations through the rainy season (July–September). Twenty open flowers, one flower per plant, were collected from each species and preserved in 70% ethanol for morphological measurements. In addition, 30 open flowers from each species (one flower per plant) were preserved for biomass allocation analysis. Floral shape, odour and perianth colour were described from fresh material. Using a digital calliper, measurements were taken of total external length of flowers, internal perianth diameter and distance between stigma base and top anthers (Fig. 1). The number of stamens and ovules per flower ( $n = 45–97$  per species) was counted. The number of pollen grains per anther was

estimated from five anthers of five flowers using Neubauer chambers (Lloyd, 1965). The number of pollen grains per flower was estimated as the product of pollen grains per anther times the average number of anthers per flower. Flowers for biomass allocation were dissected and divided into vegetative tissue, female organs (gynoecium) and male organs (androecium). Floral parts were dried in a laboratory oven (Precision, Jouan Inc., Winchester, VA, USA) at 45–50 °C for 15 d and weighed using an analytical balance (Metler Toledo, Model AG104, resolution 0.1 mg; Greifensee, Switzerland). Forty to fifty fruits were collected from different individuals of each species. The number of seeds per flower and estimated proportion of aborted seeds was counted. Aborted seeds were distinguished by their corrugated and amorphous surface, flattened and reduced body, reddish coloration and, also, by the fact that they float on water. All floral variables were compared between species using two-tailed *t*-tests. The following transformations were conducted on the variables prior to comparisons: square root of  $x$  for floral measures, arcsine of square root of  $x$  for 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.

#### Anthesis, floral longevity and rewards

Anthesis and floral longevity were monitored for about 25 d in 138 flowers of *M. schatzlii* and 217 flowers of *M. andinus* obtained from 24 and 20 different individuals, respectively. Floral buds were observed every hour starting at noon until flowers closed. In *M. andinus*, it was assumed that flowers remained open the entire night after 2 d of hourly nocturnal monitoring. For this species, observations were resumed at 0730 h the next morning until the flowers closed. For both species, changes over time in stigma morphology and stigma–anther distance were monitored in 30 flowers obtained from 12 different individuals each. These observations were conducted at 1500 h and 1900 h in *M. schatzlii* and at 1500 h, 1900 h, 0800 h and 1300 h for *M. andinus*. The distance between the stigma base and the top of the anthers was measured with a digital calliper and changes were looked for in the arrangement of the stigmatic lobes from anthesis to senescence. To estimate nectar secretion volume ( $\mu\text{L}$ ), flowers from different plants were covered with fine wedding veil and a small cap of aluminium foil before anthesis. Flowers of *M. schatzlii* were bagged at noon and nectar secretion was measured at 1900 h by removing the flowers, dissecting them in longitudinal section, and extracting nectar with microcapillary tubes. Flowers of *M. andinus* were bagged at noon and then divided into three groups according to time at which nectar production had to be measured (1900 h, 0800 h and 1300 h), following the same procedure. The 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, NY, US). Solute quantity (assumed to be sugars,

expressed in sucrose-equivalents) was calculated following Bolten *et al.* (1979).

#### Floral visitors and visitation patterns

Floral visitors of the two species were observed, photographed or captured (insects), and preserved in 70 % ethanol. Pollinators were distinguished from floral visitors using the following criteria: (a) presence of pollen on the body; (b) effective contact between areas covered with pollen in the visitor and sexual organs in the flower; and (c) relative abundance of visits. The number of floral visits and visitors was recorded using binoculars, observing from inconspicuous locations behind bushes close to target plants. Data were recorded at alternate intervals of 30-min duration. Observations of *M. schatzlii* started at the beginning of anthesis and ended when flowers closed. Observations of *M. andinus* started at the beginning of anthesis, stopped at sunset, resumed the next morning at 0730 h and ended when flowers closed. At the beginning of the study, nocturnal observations were conducted from 1900 h to 2100 h for 1 week. Two or three melocacti in bloom were monitored on a given day. For 1 month floral visits were monitored in 106 and 36 flowering individuals of *M. schatzlii* and *M. andinus*, respectively. Each day, visits were recorded and the rate of visits per plant attributed to each visitor type for each time interval of observation was determined. Then these estimates were averaged over the total number of plants monitored for each species. Also the rate of floral visits per plant was estimated 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.

#### Self-incompatibility and autogamy tests

Four hand-pollination treatments were performed on each species: (1) agamospermy (bagged, emasculated flowers with stigma blocked with Tanglefoot®; Tanglefoot Co., Grand Rapids, MI, USA); (2) autonomous self-pollination (undisturbed bagged flowers); (3) hand self-pollination (bagged, emasculated flowers hand pollinated with their own pollen or pollen from other flowers of the same individual); and (4) hand cross-pollination (bagged, emasculated flowers hand pollinated with pollen from plants separated from focal females by at least 20 m). Each pollination treatment was performed on 30–61 flowers of 25 individuals of each species. Each individual received each pollination treatment at least once and a maximum of three times. Flowers were bagged using doubly folded extra-fine wedding veil. Pollen donor plants were bagged before anthesis to avoid pollen contamination. Self- and cross-pollination treatments were applied at least twice in each flower to guarantee stigma receptivity and pollen viability. Pollinations were performed at 1500 h and 1800 h in *M. schatzlii*, and at 1800 h and 0900 h in *M. andinus*. In addition, between 30 and 70 flowers of the two species were marked and left exposed to natural pollinators as a control. Fruit : flower ratio and



total number of seeds produced were determined for each pollination treatment and the control. Indices of self-incompatibility (ISI) and autogamy or capacity for autonomous self-pollination (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 fruit : flower and total seeds : total ovules ratios via hand self-pollination by fruit : flower and total seeds : total ovules ratios via hand cross-pollination, respectively. IA was calculated as fruit : flower and total seeds : total ovules ratios via autonomous self-pollination divided by fruit : flower and total seeds : total ovules ratios via hand cross-pollination, respectively. ISI and IA values above 0.2 indicate self-compatibility and autogamy, respectively (Ruiz-Zapata and Arroyo, 1978; Jaimes and Ramírez, 1999).

#### *Mating system inferred from progeny array analysis*

Population- and family-level outcrossing rates were estimated for each species using progeny array analysis based on a mixed-mating model (Brown and Allard, 1970; Ritland, 2002). The mating system of a single population of each species was examined, with 13 families per population, each family including 10–13 progeny obtained from five to six ripe fruits. The 13 adults (mothers) of each species were selected from their respective study sites in June 2002. All fruits used in these analyses were sampled within 20 d to guarantee that they belonged to the same flowering interval. The seeds were germinated and seedlings kept in greenhouse facilities of Centro de Ecología, Instituto Venezolano de Investigaciones Científicas. To extract enzymes, 15 mg of fresh tissue was placed in a microcentrifuge tube containing 120  $\mu$ L of cold PVP-phosphate (Wendel and Weeden, 1989) and crushed. Crude homogenates were centrifuged at 10 060 g for 10 min at 4 °C. Approximately 60  $\mu$ L of the supernatant was mixed with 10  $\mu$ L of cold sample loading buffer composed of 50 % (v/v) glycerol and 0.1 % (w/v) bromophenol blue (Shields *et al.*, 1983). Polyacrylamide gel electrophoresis was conducted on 10 % gels following procedures by Boyle and Idnurm (2001). The following enzyme systems were assayed for the two species: phosphoglucoisomerase (Pgi, EC 5.3.1.9), malate dehydrogenase (Mdh, EC 1.1.1.37), phosphoglucosmutase (Pgm, EC 5.4.2.2), isocitrate dehydrogenase (Idh, EC 1.1.1.42) and malic enzyme (Me, EC 1.1.1.40). In addition, for *M. schatzlii*, gels were assayed for glutamate dehydrogenase (Gdh, EC 1.4.1.2) and triosephosphate isomerase (Tpi, EC 5.3.1.1). Gels were stained following procedures modified from Soltis *et al.* (1983). The program MLTR version 3.0 (Ritland, 2002) was used to estimate population- and family-level multilocus outcrossing rates ( $t_m$ ), single-locus outcrossing rates ( $t_s$ ), parental inbreeding coefficient ( $F$ ) and rates of biparental inbreeding ( $t_m - \bar{t}_s$ ). If mating occurs between relatives, the multilocus outcrossing rate should be higher than the single-locus outcrossing rate, and the difference should be interpreted as a minimum estimate of the apparent selfing due to biparental inbreeding. The expected inbreeding coefficient at equilibrium

( $F_e$ ) was calculated from the multilocus outcrossing rate  $t_m$  using  $F_e = (1 - t_m)/(1 + t_m)$  (Wright, 1965; Hedrick, 1985). By comparing parental  $F$  with  $F_e$  it is possible to see if inbreeding is having a negative effect (inbreeding depression) on the survivorship of selfed progeny in the populations. Under significant inbreeding depression, survivorship of selfed progeny should be lower than survivorship of outcrossed progeny, and therefore parental  $F$  should be lower than  $F_e$ . Standard errors of these estimates were obtained based on 500 bootstraps.

## RESULTS

### *Floral traits*

The flowers of *M. schatzlii* and *M. andinus* have narrow floral tubes and nectar chambers limited by the base of the stamen ring and an inferior ovary (Fig. 1). No distinctive odours were detected from open flowers. Floral traits examined and results of statistical comparisons are summarized in Table 1. *Melocactus schatzlii* has flowers significantly larger than *M. andinus* ( $t = 4.13$ , d.f. = 38,  $P < 0.001$ ). The flowers of both species are hermaphroditic and biomass partitioning was very similar between species. Most of the floral biomass corresponded to vegetative tissue (70–77 %). In both species, a single central style ends in a multilobed stigma with four to seven lobes surrounded by a ring of numerous stamens. Stigma–anthers distance (herkogamy) was similar in the two species (1.1–1.4 mm). *Melocactus schatzlii* has significantly more stamens per flower than *M. andinus*, but pollen production per flower was similar in the two species. The flowers of *M. schatzlii* have twice the number of ovules as *M. andinus*. Pollen : ovule ratios were 220 in *M. schatzlii* and 550 in *M. andinus*. On average, *M. schatzlii* produced twice the number of seeds per fruit as *M. andinus*. About 60 % of the ovules produced per flower in both species were successfully fertilized under natural conditions and became viable seeds. About 9 % of the seeds produced per fruit aborted.

### *Anthesis, floral longevity and rewards*

Sexually mature individuals of *M. schatzlii* and *M. andinus* produce 1.5 (s.e. = 0.09,  $n = 89$ ) and 1.8 (s.e. = 0.09,  $n = 166$ ) flowers per plant per day, respectively. Floral buds of *M. schatzlii* initiated anthesis at 1400 h. By 1600 h, nearly all flowers (approx. 98 %) were fully open and with stigma lobes separated. Flower senescence started after 1800 h and concluded before 2000 h. No significant changes in stigma–anthers distance were observed from anthesis to senescence. *Melocactus andinus* showed a quite contrasting anthesis pattern. Flowers initiated anthesis at a variable time in the afternoon (1400–1700 h). Around 1900 h, 85 % of the flowers were open. Flowers remained open all night long and for a variable number of hours the following morning. Flowers closed between noon and 1500 h. Depending on the relative humidity of the environment, flowers closed earlier (dry sunny days) or later (wet cloudy days). In both

TABLE 1. Comparison of floral traits between *Melocactus schatzlii* and *M. andinus* in the Venezuelan Andes

Character	n	Mean (s.e.) and range		Test
		<i>M. schatzlii</i>	<i>M. andinus</i>	
External length (mm)	20	17.8 (1.1) 13.3–29.5	12.9 (0.4) 7.3–16.4	$t = 4.130$ $P < 0.001$ d.f. = 38
Corolla width (mm)	20	2.7 (0.1) 2.0–3.9	2.6 (0.1) 1.9–3.2	$t = 0.812$ $P < 0.001$ d.f. = 38
Herkogamy (mm)	30–37	1.1 (0.1) 0.0–2.6	1.4 (0.2) 0.5–4.0	$t = 1.422$ $P = 0.159$ d.f. = 65
No. of stamens/flower	45–91	113.0 (1.3) 88–152	98.4 (1.0) 75–112	$t = 7.69$ $P < 0.0001$ d.f. = 132
No. of pollen grains/anther	5	627	867	–
No. of pollen grain/flower	–	70851	85313	–
No. of ovules/flower	45–97	320.1 (8.7) 184–496	154.9 (4.8) 97–256	$t = 15.60$ $P < 0.0001$ d.f. = 140
Pollen : ovule ratio	–	221.2	550.2	–
No. of seeds/flower	40–50	189.9 (8.2) 19–282	96.0(5.3) 36–163	$t = 7.65$ $P < 0.0001$ d.f. = 88
Seed : ovule ratio	–	0.594	0.620	–
Aborted seeds (%)	40–50	8.6 (1.1) 0.0–31.6	9.6 (2.3) 0.0–46.2	$t = 0.568$ $P = 0.571$ d.f. = 88
Androecium biomass (%)	20–30	12.8 (1.2) 3.8–24.6	10.0 (0.6) 5.6–14.1	$t = 1.46$ $P = 0.151$ d.f. = 48
Gynoecium biomass (%)	30	16.6 (0.7) 8.7–24.6	13.4 (0.8) 5.0–18.0	$t = 2.93$ $P < 0.01$ d.f. = 48
Vegetative biomass (%)	30	70.6 (1.5) 55.1–79.5	76.6 (0.8) 69.8–81.9	$t = 2.998$ $P < 0.01$ d.f. = 48
Androecium : gynoecium ratio	–	0.628	0.747	–
Reproductive : vegetative ratio	–	0.373	0.308	–

species, when flowers close the anthers collapse against the stigma, promoting pollen deposition on the latter.

In *M. schatzlii*, average nectar production at the end of the day (at approx. 1900 h) was 4.4  $\mu\text{L}$  per flower (s.e. = 0.7,  $n = 16$ ). In *M. andinus*, nectar production was similar at the three time periods considered, 5.3  $\mu\text{L}$  per flower (s.e. = 1.3,  $n = 31$ ) at 1900 h, 8.9  $\mu\text{L}$  per flower (s.e. = 1.3,  $n = 40$ ) at 0800 h the next morning, and 6.5  $\mu\text{L}$  per flower (s.e. = 1.1,  $n = 47$ ) at 1300 h. Average nectar sugar concentration in *M. schatzlii* was 23.3 % (s.e. = 0.5,  $n = 14$ ). In *M. andinus*, nectar sugar concentration did not vary much over time, 22.8 % (s.e. = 1.1,  $n = 12$ ) at 1900 h, 24.0 % (s.e. = 0.5,  $n = 26$ ) at 0800 h, and 23.9 % (s.e. = 0.3,  $n = 25$ ) at 1300 h. Based on nectar volumes obtained per flower, *M. schatzlii* produced an average of 1.3 mg sugar per flower (s.e. = 0.2, range = 0.5–2.7,  $n = 14$ ). *Melocactus andinus* produced between 3.2 mg sugar per flower (s.e. = 0.3, range = 0.7–6.0,  $n = 25$ ) and 3.5 mg sugar per flower (s.e. = 0.4, range = 0.8–8.2,  $n = 26$ ), depending on the time at which nectar was measured.

#### Floral visitors and visitation patterns

Hummingbirds are the main floral visitors and effective pollinators of *M. schatzlii* and *M. andinus*. These birds visited flowers of several individuals within a patch, switching among different individuals and returning to them in a few minutes to several hours later. The hummingbirds hovered above flowers, or less frequently, perched on the *cephalia*. Flowers of *M. schatzlii* were

visited by four species of hummingbirds, the steely-vented hummingbird (*Amazilia saucerrottei*), the rufous-tailed hummingbird (*A. tzacatl*), the narrow-tailed emerald (*Chlorostilbon stenura*) and the ruby-topaz hummingbird (*Chrysolampis mosquitus*). Flowers of *M. andinus* were only visited by the speckled hummingbird (*Adelomyia melanogenys*). Ants were frequently observed visiting flowers of both cacti, eight morphospecies (Polichodrinae, Formicinae, Ponerinae and Myrmicinae) in *M. schatzlii* and four morphospecies (Formicinae and Ponerinae) in *M. andinus*. In most cases, ants remained on the perianth feeding on floral parts. Besides hummingbirds and ants, other less frequent floral visitors of these cacti included bees and small beetles.

In *M. schatzlii*, floral visits by hummingbirds increased gradually after 1500 h and reached a peak ( $1.8 \pm 0.15$  s.e. visits  $0.5 \text{ h}^{-1}$ ) close to sunset (Fig. 2A). *Amazilia saucerrottei* was the most assiduous floral visitor among hummingbirds from 1500 h to 1900 h. This species made between 60 % and 78 % of all hummingbird visits during that time interval. Only after 1900 h did *A. tzacatl* replace *A. saucerrottei* in importance, performing 80 % of all hummingbird visits. *Chlorostilbon stenura* and *C. mosquitus* were sporadic visitors, with <14 % and 2 % of the visits, respectively. Ants were observed in the flowers for most of the afternoon, but their activity decreased gradually until sunset. Floral visits by bees and beetles were negligible (<1 %). Overall, on a given day, one individual of *M. schatzlii* in bloom received on average 3.6 visits by hummingbirds (s.e. = 0.3, range = 0–13,  $n = 106$ ) and 9.6 visits by ants (s.e. = 1.3,

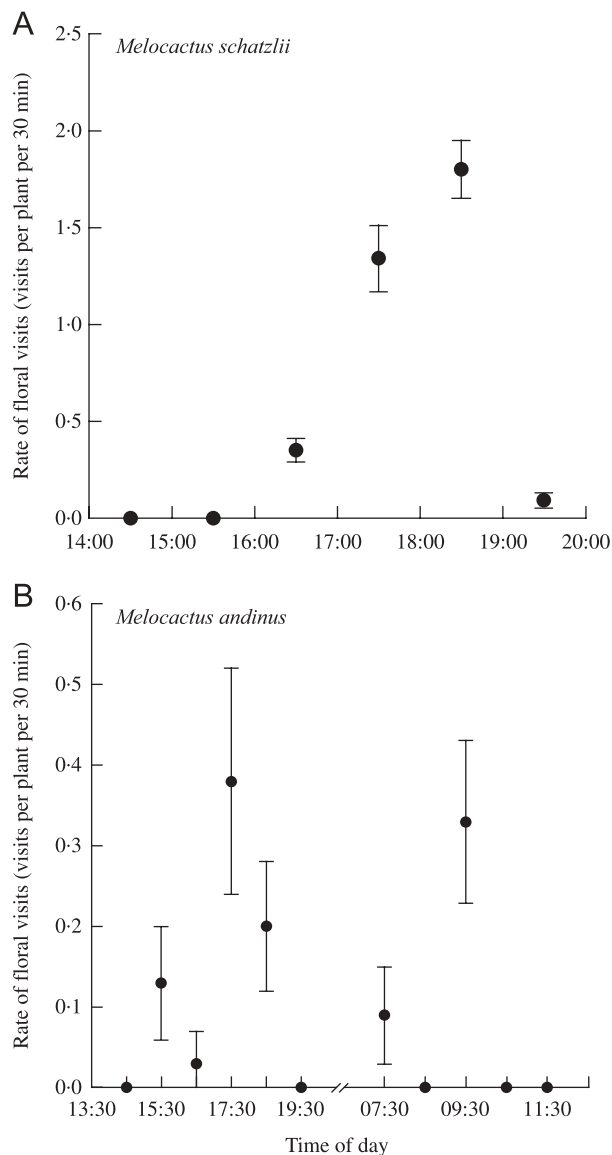


FIG. 2. Rates of floral visits (mean  $\pm$  1 s.e.) by hummingbirds in *Melocactus schatzlii* and *M. andinus* in the Venezuelan Andes. Rates were calculated based on total number of visits at each time interval of 30 min and averaged over total number of observations for each species ( $n = 106$  for *M. schatzlii*,  $n = 36$  for *M. andinus*).

range = 0–75,  $n = 106$ ). No significant correlation was detected between number of flowers per plant and number of floral visits received. In *M. andinus*, floral visits by hummingbirds were less frequent than in *M. schatzlii*. Two peaks of floral visits were observed (Fig. 2B), one in the afternoon ( $0.38 \pm 0.14$  s.e. visits  $0.5 \text{ h}^{-1}$ ) and another at mid morning ( $0.33 \pm 0.10$  s.e. visits  $0.5 \text{ h}^{-1}$ ). Presence of small beetles in the flowers comprised  $<10\%$  of total visits, on average. Ant activity in the flowers was relatively high most of the time, but reached a peak in the afternoon (1600–1700 h). Overall, on a given day, one individual of *M. andinus* in bloom received on average 0.9 visits by hummingbirds (s.e. = 0.2, range = 0–3,  $n = 36$ ) and 3.1 visits by ants (s.e. = 0.7, range = 0–15,  $n = 36$ ). No

significant correlation was detected between number of flowers per plant and number of floral visits received.

#### Self-incompatibility and autogamy tests

The two species are fully self-compatible (*M. schatzlii*:  $\text{ISI}_{[\text{fruit}]} = 1.0$ ,  $\text{ISI}_{[\text{seed}]} = 1.1$ ; *M. andinus*:  $\text{ISI}_{[\text{fruit}]} = 0.93$ ,  $\text{ISI}_{[\text{seed}]} = 1.8$ ; Table 2) and capable of autonomous self-pollination (*M. schatzlii*:  $\text{IA}_{[\text{fruit}]} = 0.91$ ,  $\text{IA}_{[\text{seed}]} = 0.83$ ; *M. andinus*:  $\text{IA}_{[\text{fruit}]} = 0.24$ ,  $\text{IA}_{[\text{seed}]} = 0.57$ ). No agamospermy was detected. Compared with *M. schatzlii*, seed set results in *M. andinus* were much lower for all pollination tests except for open pollination. In open-pollinated flowers, fruit set and seed set varied between 60% and 67% in the two species (Table 2). In *M. schatzlii*, fruit set via open pollination (64%) slightly exceeded but did not differ significantly ( $\chi^2 = 1.29$ , d.f. = 1,  $P = 0.26$ ) from fruit set via hand cross-pollination (54%). In *M. andinus*, fruit set via open pollination (66%) significantly exceeded ( $\chi^2 = 8.28$ , d.f. = 1,  $P < 0.01$ ) fruit set via hand cross-pollination (45%).

#### Mating system estimates

It was possible to use seven polymorphic loci to estimate mating system parameters in *M. schatzlii* (number of alleles indicated in parenthesis): *Gdh-1* (2), *Idh-1* (2), *Mdh-1* (3), *Me-1* (3), *Pgi-1* (3), *Pgm-1* (4) and *Tpi-3* (3), and in *M. andinus*: *Idh-1* (2), *Mdh-2* (3), *Mdh-3* (3), *Me-2* (2), *Pgi-1* (2), *Pgm-1* (2) and *Pgm-2* (2). In *M. schatzlii*, the parental inbreeding coefficient was relatively low ( $F = -0.03$ ). At the population level, this species exhibited a high rate of outcrossing ( $t_m = 0.905 \pm 0.044$  s.e.;  $t_s = 0.900 \pm 0.047$  s.e.; Table 3). No biparental inbreeding was observed in the population ( $t_m - t_s = 0.005 \pm 0.022$  s.e.). The equilibrium inbreeding coefficient ( $F_e = 0.05$ ) was very similar to the parental inbreeding coefficient. The proportion of outcrossed progeny was high ( $t_m > 0.80$ ) for most families surveyed, but three out of 12 families had intermediate ( $s \sim 0.45$ ) to high ( $s = 0.75$ ) selfing rates (Table 3). The within population inbreeding coefficient for *M. andinus* ( $F = -0.112$ ) indicated a moderate deficiency of homozygotes among the adults examined. At the population level, this species had an intermediate level of outcrossing ( $t_m = 0.553 \pm 0.071$  s.e.;  $t_s = 0.533 \pm 0.007$  s.e.; Table 3). Very low biparental inbreeding was observed in the population ( $t_m - t_s = 0.020 \pm 0.031$  s.e.). The equilibrium inbreeding coefficient was comparatively higher ( $F_e = 0.29$ ) than the parental  $F$  obtained for the species. Family-level outcrossing rates were quite variable (Table 3). Four families were completely outcrossed ( $t_m > 1.0$ ), four had intermediate levels of outcrossing ( $t_m = 0.50$ – $0.68$ ) and five were mostly selfed ( $t_m = 0.0$ – $0.38$ ).

## DISCUSSION

All Andean species of *Melocactus* are circumscribed within the ‘MELOCACTUS CURVISPINUS GROUP’, an infrageneric clade proposed by Taylor (1991) based on a limited

TABLE 2. Fruit and seed set results of pollination treatments conducted on *Melocactus schatzlii* and *M. andinus* populations in the Venezuelan Andes and self-incompatibility (ISI) and autogamy (IA) indexes

Treatment	No. of flowers	Fruit : flower ratio	Index	No. of seeds	Seeds : ovules ratio	Index
<i>M. schatzlii</i>						
Agamospermy	40	0		0	0	
Cloth bagging	61	0.492	IA 0.91	4519	0.428	IA 0.83
Self-pollination	37	0.540		3679	0.575	
Cross-pollination	50	0.545	ISI 1.0	4444	0.514	ISI 1.11
Open pollination	70	0.643		9493	0.593	
<i>M. andinus</i>						
Agamospermy	47	0		0	0	
Cloth bagging	36	0.110	IA 0.24	207	0.037	IA 0.57
Self-pollination	45	0.420		779	0.112	
Cross-pollination	53	0.453	ISI 0.93	535	0.065	ISI 1.80
Open pollination	30	0.660		3105	0.668	

Index values below 0.2 are indicative of self-incompatibility and no autogamy, respectively (Ruiz-Zapata and Arroyo, 1978).

TABLE 3. Estimates of population-level multilocus outcrossing rates, average single-locus outcrossing rates, biparental inbreeding rates and family-level multilocus outcrossing rates in one population of *M. schatzlii* and one population of *M. andinus*

Mating system parameter	<i>M. schatzlii</i>	<i>M. andinus</i>
Population-level		
$t_m$	0.905 (0.044)	0.553 (0.071)
$t_s$	0.900 (0.047)	0.533 (0.007)
$t_m - t_s$	0.005 (0.022)	0.020 (0.031)
Family-level ( $t_m$ )		
Family 1	1.200 (0.106)	1.143 (0.373)
Family 2	1.200 (0.169)	1.200 (0.109)
Family 3	1.200 (0.002)	1.200 (0.203)
Family 4	1.200 (0.002)	1.200 (0.070)
Family 5	1.200 (0.002)	0.682 (0.336)
Family 6	1.155 (0.170)	0.562 (0.451)
Family 7	1.024 (0.163)	0.509 (0.416)
Family 8	1.009 (0.132)	0.504 (0.280)
Family 9	0.832 (0.194)	0.378 (0.233)
Family 10	0.559 (0.151)	0.270 (0.196)
Family 11	0.549 (0.252)	0.146 (0.137)
Family 12	0.251 (0.160)	0.186 (0.179)
Family 13	–	0.001 (0.000)

Standard errors based on 500 bootstraps are shown in parenthesis.

number of characters. Although not definitive, the grouping proposed by Taylor suggests a close evolutionary relationship between Andean melocacti. This relationship could help to explain the numerous reproductive attributes

shared between *M. schatzlii* and *M. andinus*: similar floral morphologies, comparable amounts and qualities of floral rewards, the same kind of pollinators, genetic self-compatibility and autonomous self-pollination. Nevertheless, differences observed in other reproductive traits, such as flower size, female and male investment, time of anthesis, and levels of outcrossing, are suggestive of divergent reproductive strategies in these cacti.

The floral traits of *M. schatzlii* and *M. andinus* are typical of ornithophilous plants (Porsch, 1939; Faegri and van der Pijl, 1979; Proctor *et al.*, 1996), and match floral descriptions offered by Taylor (1991) for the genus. Based on floral morphology only, these species seem to have an autogamous reproductive strategy. It is well known that resource investment per flower often decreases with increasing selfing (Ornduff, 1969; Sobrevila *et al.*, 1983; Cruden and Lyon, 1985; Sato and Yahara, 1999). The Andean melocacti have smaller flowers than other congeners, such as *M. curvispinus* in the arid flatlands of Venezuela (floral length = 25.0 mm; Nassar and Ramírez, 2004) and *M. zehntneri* in the Brazilian caatinga (floral length = 15.0–25.0 mm; Locatelli and Machado, 1999). They produce fewer ovules per flower and seeds per fruit than *M. curvispinus* (390 ovules per flower, 290 seeds per fruit; Nassar and Ramírez, 2004), but many more than *M. zehntneri* (90 ovules per flower, 30–50 seeds per fruit; Locatelli and Machado, 1999). In addition, the two Andean species have fewer stigma lobes than *M. curvispinus* ( $n = 12–20$ ), a difference that could affect the number of pollen grains that can germinate and reach the ovules in these cacti. Also, the low androecium : gynoecium biomass ratios observed indicate that resource investment in these species is greater for the female function. Female-biased biomass investment is typical of self-compatible and autogamous species (Charnov, 1982; McKone, 1987).

Differences observed in floral morphology suggest that *M. andinus* has a stronger tendency towards a selfing strategy than *M. schatzlii*. The flowers of *M. schatzlii* are larger, have higher biomass investment in stamens, and produce twice as many ovules as those of *M. andinus*. As a result, *M. schatzlii* produces more seeds per fruit.



Interestingly, *M. andinus* produces more pollen grains per anther and flower and allocates substantially more pollen grains per ovule than *M. schatzlii*; however, according to Cruden (2000), both pollen : ovule ratios fall within the limits of plants considered autogamous and facultatively xenogamous.

Like most species in *Melocactus*, *M. schatzlii* has flowers that open in the afternoon and close at sunset (Taylor, 1991; Locatelli and Machado, 1999; Nassar and Ramírez, 2004). On the other hand, the flowers of *M. andinus* remain open at least until the next morning. It is hypothesized that potential competition for pollinator visitors with *M. schatzlii* might be a selective pressure driving the anthesis period of *M. andinus* away from the flowering interval of *M. schatzlii*. Even though Taylor (1991) states that these two species are found at different altitudinal levels, it is possible that they could overlap or have overlapped in distribution in certain regions in the Andes. Under sympatry, the two melocacti could exploit the same pollen vectors at different hours during the day, reducing the chances of competition for pollinator services and hybridization.

The Andean melocacti invest fewer energetic resources in attracting pollinators than other species in the genus. Total nectar secretion per flower in the Andean species was considerably lower than in *M. zehntneri* (35–41  $\mu\text{L}$ ; Locatelli and Machado, 1999) and *M. curvispinus* (163.1  $\mu\text{L}$ ; Nassar and Ramírez, 2004). However, the present estimations of nectar secretion should be taken as underestimations, because nectar was not removed periodically, a process that sometimes stimulates nectar replenishment (Ordano and Ornelas, 2004). Furthermore, floral nectar can be reabsorbed if not removed (Búrquez and Corbet, 1991), which can also explain the relatively low volumes of nectar secreted in these flowers. On the other hand, it is not rare to find minute amounts of nectar secretion in globose cacti. Species in the genera *Echinomastus* and *Echinocereus* produce only one small drop of nectar (<1  $\mu\text{L}$ ) per flower at the beginning of anthesis (Johnson, 1992). Nectar sugar concentrations in the Andean melocacti resemble other values reported for hummingbird-pollinated cacti (25 %,  $n = 11$ ; Scogin, 1985) and hummingbird flowers in general (25 %,  $n = 202$ ; Pyke and Waser, 1981), but are lower than those reported for *M. zehntneri* (27 %  $\mu\text{L}$ ; Locatelli and Machado, 1999) and *M. curvispinus* (30 %; Nassar and Ramírez, 2004).

Outcrossing in *M. schatzlii* and *M. andinus* is mediated by hummingbirds. While visiting the flowers, these birds contact anthers and stigmas with the bill, behaving as effective pollinators; but their contributions to fruit and seed set depend on their frequencies of floral visitation. Hummingbirds were more diverse and abundant where *M. schatzlii* was studied. Only one species of these birds was observed visiting *M. andinus*. Pollinator reliability differed between the two cacti. Most individuals (92.5 %) observed of *M. schatzlii* were visited by hummingbirds, while only half (50 %) of the individuals observed of *M. andinus* received visits. Moreover, hummingbird visits to flowers of *M. schatzlii* were four times more frequent as visits to *M. andinus*. The present results suggest that

*M. schatzlii* at Laguna de Caparú receives a better pollination service than *M. andinus* at Mesa de Esnujaque. Based on these observations, it is predicted that seed set through outcrossing should be higher in *M. schatzlii* than in *M. andinus* at the study sites. Although ants were frequently observed in the flowers of both cacti, they do not pollinate them. Instead, they feed on their nectar and floral parts. Ants have also been reported visiting flowers of other melocacti (Locatelli and Machado, 1999).

The two Andean melocacti are self-compatible and capable of autonomous self-pollination. The relatively low levels of seed set obtained from *M. andinus* for all hand pollination treatments suggest a potential experimental limitation in the dosage of pollen on the stigmas. However, the results obtained from these tests are valid, because all flowers assigned to all treatments of this species were handled in the same way. The fact that ISI values slightly above 1.0 were observed suggests that these cacti have a high affinity for their own pollen, which in principle implies at least two things: (1) low dependence on foreign pollen to produce seeds; and (2) the possibility for occurrence of outbreeding events. In order to test these hypotheses, it would be necessary to estimate and compare relative fitness of progeny produced by self-fertilization and cross-fertilization events at different distances between the recipient and pollen donor populations.

*Melocactus schatzlii* is a predominantly outcrossing species, while *M. andinus* behaves as a mixed-mating cactus. Although some individuals of *M. schatzlii* had significant levels of selfing, this mating strategy was comparatively more important in *M. andinus*. Morphological, experimental and genetic evidence of a mixed-mating system have also been found in other melocacti (Locatelli and Machado, 1999; Nassar, 1999; Nassar and Ramírez, 2004). This emerging pattern is concordant with the suggestion of Taylor (1991) that most species in the genus are self-compatible and capable of selfing. However, this reproductive strategy seems to have a cost, at least in the case of *M. andinus*. The substantial difference observed between  $F$  and  $F_e$  in this species is indicative of inbreeding depression. It is suggested that the inbreeding coefficient of adult plants is much lower than expected based on the selfing rate, because selfed progeny have reduced survivorship at later life stages. However, this scenario seems incongruent with the ISI value of 1.81 obtained for *M. andinus* at the seed set level, which can be interpreted as indicating the possible occurrence of outbreeding in this species. The only explanation for this apparent discrepancy is that inbreeding depression in this cactus is not being expressed at the seed set level, but at later stages (seedling and sapling) not monitored in this study.

If all the evidence obtained in this study is combined, it is not clear whether one of the two Andean melocacti tends more to a selfing strategy than the other. From a morphological point of view, *M. andinus* has smaller flowers and produces fewer ovules than *M. schatzlii*, traits which are associated with selfing. However, *M. andinus* produces more pollen grains per ovule than *M. schatzlii*, a condition that favours xenogamy. *Melocactus andinus* produces lower fruit set via selfing without pollinator



intervention than *M. schatzlii*, which in principle makes the latter species less dependent on the aid of pollinators for self-pollination. On the other hand, the present progeny array analyses indicate that *M. schatzlii* outcrosses at a higher rate than *M. andinus* at both population and individual levels. The present mating system estimates are concordant with the population sizes of the two cacti at the study sites and also with the observations of floral visitations. At Laguna de Caparú, *M. schatzlii* is abundant and densely distributed. There, this cactus receives the pollination services of up to four different species of hummingbirds. High spatial availability of the floral resource stimulates floral visitations by hummingbirds and therefore promotes outcrossing. On the contrary, *M. andinus* is relatively scarce at Mesa de Esnujaque and floral visits by the only species of hummingbird observed in that location were 4-fold fewer than in *M. schatzlii*. Under these conditions, selfing becomes the most effective reproductive option for setting seeds.

Overall, the present results indicate that both intrinsic and extrinsic factors play important roles in the reproductive outputs of *M. schatzlii* and *M. andinus*. By being capable of selfing, the two cacti have the means to set viable seeds in the absence of pollinators. However, if selfing dominates as the mating strategy in the populations, a gradual decline would be expected in levels of genetic variation within populations and higher population differentiation among populations (Hamrick and Godt, 1996; Nassar *et al.*, 2001). This needs to be confirmed with surveys of genetic variability within and across populations. When present, hummingbirds are the only effective pollinators of these cacti. Most outcrossing and pollen-mediated gene flow events in these species should be mainly attributed to these birds. Finally, based on its low population size, its restricted distribution in Venezuela (<100 km<sup>2</sup>), illegal extraction of wild specimens, low local availability of pollinators, and high levels of inbreeding, it is proposed that *M. andinus* should be considered an endangered species. Currently, this cactus is not included in the Red Book of Venezuelan Flora (Llamozas *et al.*, 2003). A detailed demographic study of the species in the Venezuelan Andes will help to determine its precise risk status.

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