

Functional Gynodioecy in *Opuntia quimilo* (Cactaceae), a Tree Cactus Pollinated by Bees and Hummingbirds

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Abstract: The tree cactus *Opuntia quimilo* is one of three known gynodioecious cacti. Its flowers deviate from most *Opuntias* in features that are attributable to ornithophily: petals are shiny red in colour, and fleshy in consistency, a nectar chamber is present, and stamen seismonasty is lacking. Pollinators include large matinal bees (predominantly *Ptilothrix tricolor* and *Megachile* sp.) and hummingbirds (*Chlorostilbon aureoventris* and *Helio-master furcifer*). Hummingbirds rarely visit other local *Opuntias*. Hummingbirds, which are more common in the afternoon, prefer female flowers whereas bees prefer hermaphroditic flowers. Female flowers have more dilute nectar than hermaphroditic flowers. Under experimental conditions female fertility is as high as that of hermaphrodites, however, seeds from females always result from cross-pollination and from more severe ovule selection (ovule number is higher in female flowers). Under natural conditions female plants are reproductively more successful than hermaphrodites. Known cases of bird pollination in *Opuntia* and the incidence of ornithophilic features in *Opuntia* and related genera are discussed.

Key words: Argentine, *Megachile*, *Ptilothrix*, *Chlorostilbon*, *Helio-master*, breeding system, self-compatibility.

Introduction

Any kind of dioecy (gynodioecy, androdioecy, and dioecy proper) is uncommon in Cactaceae. Dioecy proper has been described for four species of *Opuntia*, one of *Echinocereus* and one of *Gymnocalycium*, gynodioecy (bearing hermaphroditic or female flowers) has been described in two *Mammillaria* species, and androdioecy is found in one species of *Neobuxbaumia* (Ganders and Kennedy, 1978; Parfitt, 1985; Valiente-Banuet et al., 1997). One trioecious species (bearing hermaphroditic, female and male flowers), *Pachycereus pringlei*, is also known (Fleming et al., 1994).

Dioecy, like other structural polymorphisms (heterostyly, enantiostyly) promotes or ensures outbreeding. However, increased outcrossing is not taken as the sole selective pressure

leading to dioecy. Sexual selection may promote differential resource partitioning (Bawa, 1980): according to the Bateman's (1949) model of sexual selection extended to plants (Willson, 1979), males are limited in their reproductive success more by their access to females, while females are limited more by resource availability. Thus, males optimise quantity of matings whereas females favour the maturation of higher quality seeds. It is expected from the model that males increase flower number and that females produce more fruits and seeds.

Gynodioecy is assumed to be either a stable condition or just a stage in evolution to dioecy (Darwin, 1877; Lloyd, 1973; Bawa, 1980). If cytoplasm genes determine sex, as is the case in some *Lamiaceae*, evolution to dioecy does not take place because female sterility genes would not be inherited (Lewis, 1942). Gynodioecy is believed to be the most likely stage leading to inherited dioecy (Charlesworth and Charlesworth, 1979; Lewis, 1942). In fact, gynodioecious species commonly have dioecious relatives (Charlesworth and Charlesworth, 1979). Species series even exist within genera in which hermaphroditism merges into gynomonoeicy, and from gynomonoeicy into dioecy (Lloyd, 1973; Webb, 1979). In such a pathway it is assumed that the evolution of dioecy from gynodioecy must be gradual, via subhermaphrodites with greater pollen production at the expense of ovule production (Lloyd, 1974; Bawa, 1980). Male sterility would spread if it resulted in higher fertility than in hermaphrodites or, even though having the same fertility, because its progeny is always outcrossed and inbreeding depression is avoided. Sex expression may also be labile and vary in response to environmental conditions (Richards, 1997).

Gynodioecy appears to be more common in temperate zones and less common in tropical zones and oceanic islands (Richards, 1997). The incidence of pollination syndromes in gynodioecy has not been explored to our knowledge. But if pollination syndromes are related to gynodioecy in the same way as they are to dioecy, one should expect gynodioecious plants to be rarely bird pollinated and more frequently insect pollinated (Bawa, 1980). Moreover, in some families (Loranthaceae and Onagraceae) bird pollination has apparently prevented or strongly hindered the development of any kind of dioecy (see Bawa, 1980). There is no obvious reason for this pattern but in these families ornithophily has a significantly lower incidence among dioecious than hermaphroditic species.

Here we report gynodioecy in *O. quimilo*, a fact that has passed unnoticed probably because the female flowers still have stamens with anthers. The case is exceptional for the rareness of any kind of dioecy in the Cactaceae, for the scarcity of gynodioecy in tropical zones and in bird pollinated plants. We explore the possible effect of its pollination biology in maintaining this unusual case of gynodioecy. *O. quimilo* occurs with congeneric hermaphroditic species exhibiting a variety of life forms (trees, erect or prostrate herbs and shrubs) and flower traits in several ways deviating from *O. quimilo*. In addition, these other species are pollinated mainly by bees, which may or may not be shared with *O. quimilo*. This circumstance provides an opportunity to test how different biotypes and pollinator behaviours correlate to sexual systems (gynodioecy vs. hermaphroditism) in the Cactaceae. We also discuss other possible cases of bird pollination in *Opuntia* and related genera in relation to traits associated with bird pollination.

Materials and Methods

Study site

Observations on *Opuntia quimilo* were carried out near Quilino (Córdoba Province, Argentina) between the years 1998 to 2000. The plant community represents a western drier variant of the Chaco Vegetation Province. The vegetation is dominated by small trees (up to 10 m) and shrubs such as *Aspidosperma quebracho-blanco*, *Acacia praecox*, *Zizyphus mistol*, *Prosopis pugi-nata*, *Cercidium australe*, *Larrea divaricata*, *Celtis pallida*, *Condalia microphylla*, *Senna aphylla*, among others (Cabido and Zak, 1999).

To a lesser or greater extent the community may be subjected to human modification (logging, goat herding and occasional fire) (Sayago, 1969). Less modified spots were chosen for the study. A seasonal summer rain climate with a mean annual temperature of 19.9°C prevails (Sayago 1969). Soils are sandy and alkaline.

Other local *Opuntia* species are: *O. anacantha* Speg. var. *utkilio* (Speg.) R. Kiesling, *O. ficus-indica* (L.) Mill, *O. glomerata* Haw. f. *oligacantha* (Speg.) A. Cast., *O. prasina* Speg., and *O. sulphurea* Gillies ex Salm-Dyck var. *hildmannii* (Fric.) Backeberg.

Flowering and anthesis

Over a three-year period 57 plants were checked weekly for beginning and ending of flowering. Flower opening and changes during anthesis were checked hourly in flowers of both sexes.

Sex ratio

The sexual condition (female or hermaphroditic) was determined in the same 57 plants. Possible change of sex of the plants was also checked from year to year.

Sexual differences

For each sexual morph, the following traits were measured: number of flowers/plant during flowering peak, duration of flower, flower biomass, nectar concentration (% Brix in sucrose equivalents), sepal and petal length, sepal and petal width,

number of stamens, number of pollen grains/anther, style length, stigma length, number of stigma lobes, ovary length and ovule number.

Breeding system

Some hours before opening, flower buds were individually enclosed in a modified paper cup, which had a removable lid made out of cloth to allow manipulations. The paper cups were removed after the corolla and stamens had been shed. Treatments were carried out, when applicable, to flowers of both sexual morphs and consisted of: 1) enclosed flowers not manipulated (spontaneous autogamy), 2) enclosed flowers hand-pollinated with pollen of the same plant (geitonogamy), 3) hand cross pollination with foreign pollen (allogamy), and 4) flowers not enclosed and subject to open pollination by the assemblage of pollinators present at the sites.

Pollinator behaviour

The species of visitors arriving, entering, and touching fertile flower parts were recorded on sets of 4 to 40 flowers that could be observed simultaneously. Each set was observed continuously for 20–60 min. These observation periods were arranged from 9:00 to 18:30 in the same 4 days for female and hermaphroditic morphs. The total observation effort amounted to 4593 flowers × minute for the female morph and 4229 flowers × minute for the hermaphroditic morph. For these observation efforts, the frequency of visitation (number of visits observed) was determined for each species of visitor and for both flower morphs. Visitation frequencies were tested for homogeneity (χ^2 -test) using plant sex, pollinator species, and class (birds vs. bees) as classifying variables. In the tables, frequencies are given in percentages. Handling time of the visitors on the flowers was also recorded.

For comparisons with other *Opuntia* species, a similar procedure was carried out on other days to determine the frequencies of visitation. Observation effort varied between species according to the availability of plants and flowers, from 255 to 7115 flower × minute (see Table 3). To capture hummingbirds, a 6 m wide mist net was erected for 31 h at 4 m height and at a distance of about 20 m from an *O. quimilo* tree. Pollen on the hummingbirds was removed by lightly pressing the glue side of clear plastic tape pieces against the sides, dorsal and ventral parts of the head. The tape pieces were then glued onto glass slides to be viewed later by epifluorescence microscopy.

Results

Flowering and anthesis

O. quimilo starts flowering before the beginning of the rainy season, that is, from September to March. Open flowers are found from the end of August to the end of January. Female plants start flowering about one month earlier than hermaphroditic plants, and males finish flowering about one month after the females. Flowers last two to three days. Opening begins at about 8:00 and all flowers are completely open before 14:00. No further petal movements are evident until the second or third day, when they wilt and are shed. After wilting, the whole androecium is also shed. Flowers of the female plants last on average 12 h longer than those of hermaphro-

dites (Table 1). In hermaphroditic flowers pollen is presented from the beginning of anthesis. Seismonastic movements, which are evident in all other local *Opuntia* species, were not detected in either hermaphroditic or in female flowers of *O. quimilo*.

Sex ratio and sexual differences

The sex ratio (27 ♀/30 = 0.47♂) did not significantly deviate from 1 : 1 ($\chi^2 = 0.0702$; n.s.). Sexes do not differ in the number of flowers/plant (Table 1A). Sexual condition of a given plant did not change from year to year. Female flowers have stamens with filaments and anthers. However, when opening, anthers expose a white powder that does not contain pollen, only spherical crystals, as revealed by the optical microscope. Sexes do differ significantly in flower weight, nectar concentration, sepal and petal length and width, number of stamens/flower, ovule/ovary, flower biomass and nectar concentration (Tables 1A,B). For other morphological differences between flower types see Figs. 1A–D.

Breeding system

O. quimilo is self-compatible. Neither fruit set nor seed set are significantly different between self-pollinated (autogamy and geitonogamy) and hand cross-pollinated hermaphroditic plants (Table 2). Female plants had significantly higher fertility when subjected to open pollination (Table 2). However, fruit set and seed set in manually cross-pollinated plants was not significantly different between sexes (Table 2).

Pollinator behaviour

Bees

Bees alight either on stigmas or, less frequently, on the petals and then move to the stamens, which they walk over to reach the nectar chamber (Fig. 1E). To leave the flower, they climb up to the stigma. For *Ptilothrix tricolor* and *Megachile* sp. we observed that the bees gathered the stamens towards their body to collect pollen. Pollen was found mainly on the bees' ventral parts and legs.

The longest stays of bees on the flowers were measured on the hermaphroditic flowers. However, on average, there are no significant differences in the time the bees stay on female or hermaphroditic flowers (Table 4). Bees are sometimes found more or less immobilized in the flowers during cool days. In other local *Opuntia* species, some were even found dead in the flowers.

Hummingbirds

Chlorostilbon aureoventris is the most common hummingbird visitor to this cactus (Fig. 1F). It is a territorial hummingbird, males controlling one or a few nearby plants and feeding regularly on them while chasing away other hummingbirds. Hummingbirds introduce their beaks between the style and the androecium. They touch the stigma and the stamens only with the beak. Occasional visits were recorded of *Helioaster furcifer*, a silent hummingbird with a trap-lining habit. Only one specimen of this species was captured and it carried *O. quimilo* pollen on its beak, with none found on other body parts.

Table 1 Gender differences in *Opuntia quimilo*

A	Morphs	Flowers/ind.	Days of flower life	Flower weight (g)	Nectar (%)	Petal length (ccm)	Petal width (cm)	Sepal length (cm)	Sepal width (cm)
	Hermaphroditic	12.2 ± 14.33 (n = 30)	2.80 ± 0.63 (n = 10)	14.13 ± 3.46 (n = 22)	35.21 ± 4.47 (n = 7)	1.82 ± 0.32 (n = 62)	1.07 ± 0.22 (n = 62)	0.78 ± 0.23 (n = 50)	0.85 ± 0.29 (n = 50)
	Female	21.4 ± 20.65 (n = 27)	2.25 ± 0.45 (n = 12)	7.92 ± 2.60 (n = 20)	31.61 ± 3.15 (n = 8)	2.31 ± 0.36 (n = 76)	1.52 ± 0.42 (n = 76)	1.06 ± 0.42 (n = 59)	1.42 ± 0.35 (n = 59)
	Tests	F(1;55) = 3.89, n.s.	t = 3.82, df = 20, p < 0.001	F(1;40) = 42.55, p < 0.05	F(1;20) = 4.62, p < 0.05	F(1;136) = 74.9, p < 0.05	F(1;136) = 112.65, p < 0.05	F(1;13) = 2.32, n.s.	F(1;107) = 12.51, p < 0.05
B	Morphs	Style length (cm)	Nectary diam. (cm)	Stamen number	Pollen grains/anther	Stigma length (cm)	Stigma branches	Ovary length (cm)	Ovule number
	Hermaphroditic	1.53 ± 0.34 (n = 7)	2.42 ± 0.21 (n = 7)	944.86 ± 70.00 (n = 7)	913.78 ± 213.93 (n = 6)	0.63 ± 0.11 (n = 7)	8.71 ± 1.11 (n = 7)	4.34 ± 0.55 (n = 7)	283 ± 32.8 (n = 7)
	Female	1.64 ± 0.18 (n = 8)	2.71 ± 0.36 (n = 8)	771.00 ± 37.31 (n = 8)	0.00	0.53 ± 0.07 (n = 8)	8.25 ± 1.39 (n = 8)	4.59 ± 0.68 (n = 8)	488 ± 56.88 (n = 7)
	Tests	F(1;13) = 0.64, n.s.	F(1;13) = 3.60, n.s.	F(1;13) = 32.48, p < 0.05	–	F(1;13) = 47.6, p < 0.05	F(1;13) = 0.50, n.s.	F(1;13) = 0.58, n.s.	F(1;12) = 68.12, p < 0.05

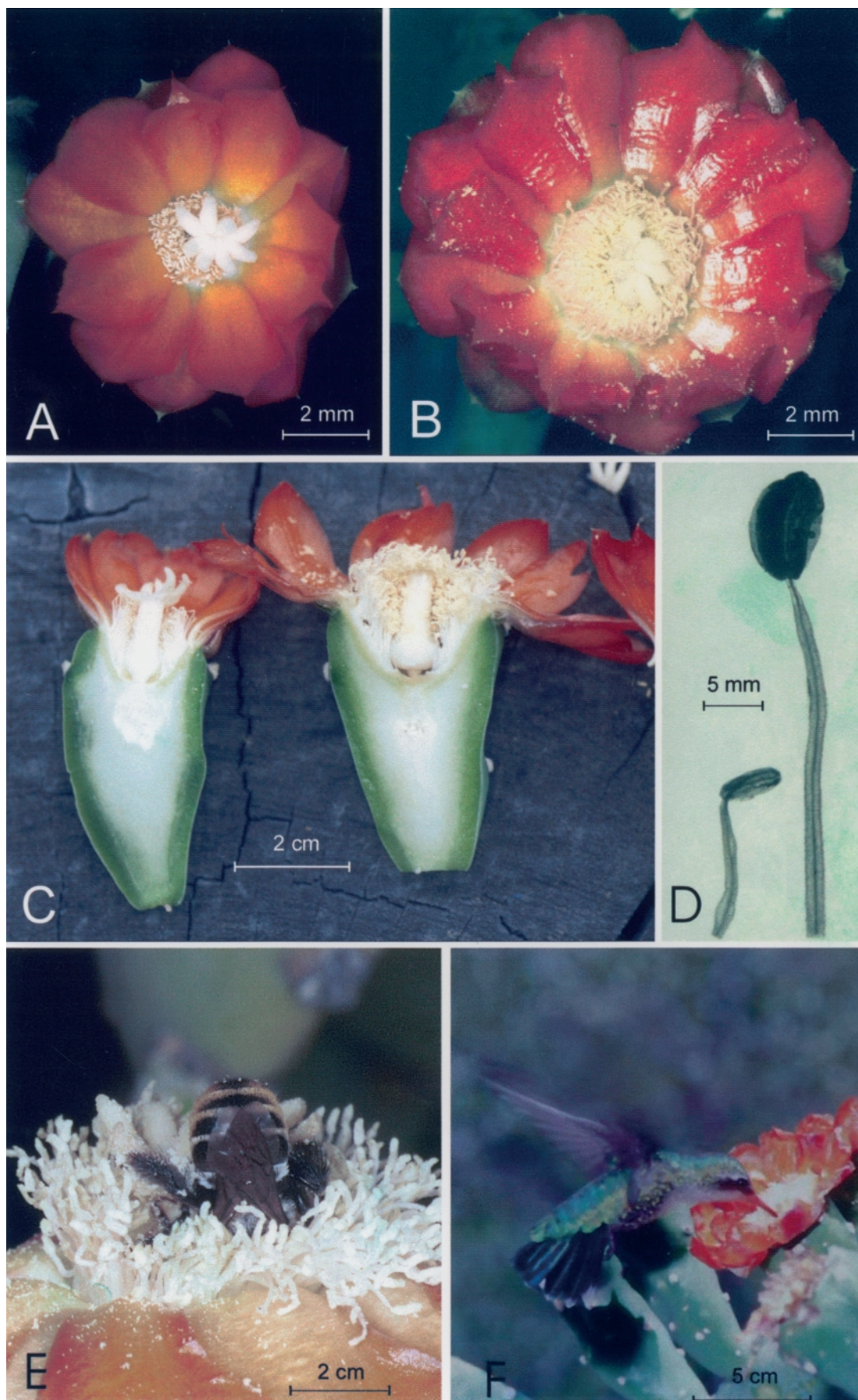


Fig. 1

Table 2 Reproductive differences between sexes of *O. quimilo*. Fruit is tested for independence between sexes for each treatment with two-way contingency tables (hermaphroditic or female vs. fruit or non-fruit) of which percentages of fruit produced relative to total number of flowers treated (n) are given. χ^2 : Difference in mean seed number/fruit between sexes was tested for each treatment with ANOVA

Morphs	Fruit set (%)				Seed number			
	Self pollin.	Geitonog pollin.	Cross pollin.	Open pollin.	Self pollin.	Geitonog pollin.	Cross pollin.	Open pollin.
Hermaphroditic	30.77 (n = 26)	36.00 (n = 25)	30.77 (n = 26)	38.46 (n = 26)	70.63 \pm 73.65 (n = 8)	56 \pm 52.59 (n = 7)	127.06 \pm 50.30 (n = 18)	116.00 \pm 60.26 (n = 10)
Female	0.00 (n = 17)	0.00 (n = 20)	50.00 (n = 30)	85.19 (n = 27)	–	–	101.75 \pm 68.38 (n = 8)	123.35 \pm 34.35 (n = 17)
Test	χ^2 , $p < 0.05$	χ^2 , $p < 0.01$	χ^2 , n.s.	χ^2 , $p < 0.01$			$F_{(1,24)} = 1.12$, n.s.	$F_{(1,25)} = 0.17$, n.s.

Visitation frequencies

O. quimilo

The number of visits of both bees (all species) and hummingbirds (both species) differs markedly between sexes ($\chi^2 = 135.39$, $p < 0.001$), the percentage of bee visitation frequencies being much higher (more than six times higher) than hummingbird visits for the hermaphroditic flowers and hummingbird visits nearly as high as bee visits for the female flowers (Table 5). Bees visited both female and hermaphroditic flowers almost exclusively before 14:00. Visits decreased markedly after an 11:00 maximum visitation rate (Fig. 2). The bees more commonly visiting both sexes of *O. quimilo* are an undetermined species of *Megachile* sp. and *Ptilothrix tricolor*. No small-sized bees were seen visiting *O. quimilo*.

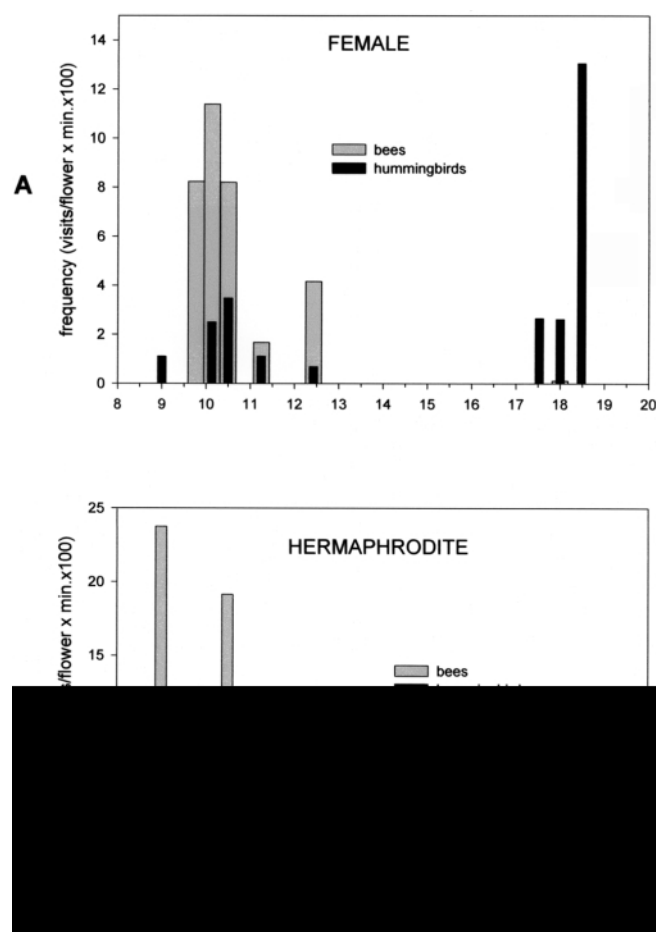
Co-existing *Opuntia* species

Among *Opuntia* species studied, other than *O. quimilo*, only in *O. prasina* did we find that the number of visits of hummingbirds is higher than that of bees. In other *Opuntia* species the number of visits by birds was either lower than 14% or no bird visits were recorded (Table 3). As in *O. quimilo*, most commonly visiting bees are *Megachile* sp. and *Ptilothrix tricolor*. Both of these two bees are the most common visitors in all local *Opuntia* species. These two species, along with *Lithurgus* sp., are the only bees that visited all the *Opuntia* species in the study area.

Discussion

Sexual differences

Differences in flower weight and number of ovules are in accordance with the idea that females can be better breeders than hermaphroditic plants. Despite these differences, the reproductive potentials are not significantly different between sexual morphs (equal fruit set with hand cross pollination). But the significantly higher ovule number in female flowers

**Fig. 2** Visitation frequencies of bees, hummingbirds and butterflies to female and hermaphroditic flowers of *Opuntia quimilo*.

implies more severe ovule selection to produce seeds. In addition, female plants always produce a cross-pollinated progeny, but self-pollination is unavoidable in hermaphroditic plants. Thus, fruits from female plants are expected to produce higher quality progeny. However, the performance of seedlings could not be tested here. Improved performance of the female sex morph is also suggested by its significantly higher fruit set when open-pollinated.

◀ **Fig. 1** Flower structure and flower visitors of *Opuntia quimilo*. (A,B) front view of female and hermaphroditic flowers, respectively. (C) Longitudinal section through female (left) and hermaphroditic (right) flowers. (D) Sterile stamen of a female flower (left) and fertile stamen (right). (E) Hermaphroditic flower with *Ptilothrix tricolor*. (F) Hermaphroditic flower with *Chlorostilbon aureoventris*.

Table 3 Percentage of visitations by bees and hummingbirds to *Opuntia* species in the study site

Order	Hymenoptera												Lepidoptera	Apodiformes	Number of visits	Observation min.	Number of flowers	min. × flowers	
Family	Andrenidae	Halictidae	Megachilidae		Apidae							Vespidae	Pteridae	Trochilidae					
Tribe	Calliopsini	Augochlorini	Megachilini	Lithurgini	Xylocopini	Tetrapedini	Emphorini		Eucerini	Bombini	Meliponini	Apini							
Species	Arhysosage sp.	Augochloropsis sp.	Megachile sp.	Lithurgus sp.	Xylocopa sp.	Tetrapedia sp.	Ptilothrix tricolor	Diadasia patagonica	Gen. spec.	Bombus morio	Melipona sp.	Apis mellifera	Polybia ignobilis	Gen. spec.	Heliomaster furcifer, Chlorostilbon aureoventris				
<i>O. glomerata</i>		3.42	39.04	2.74			34.93	10.27	9.59							146	95	8	255
<i>O. anacantha</i>			46.90	2.65		8.85	34.51								7.08	113	90	12	360
<i>O. ficus-indica</i>	0.98	1.39	16.60	9.48	1.95		35.98	15.90		5.58	0.28	0.56	1.81		9.48	717	625	221	7 115
<i>O. prasina</i>	0.63	1.06	9.09	1.27			20.93	0.85	2.33	0.63			0.85	0.63	61.73	473	404	253	8 307
<i>O. quimilo</i> (female)			22.50	1.67	5.00		18.89			1.67					50.28	500	355	117	4 229
<i>O. quimilo</i> (hermaph- roditic)			37.40	9.40			32.40	3.80		1.00			1.00	1.60	13.40	360	271	163	4 593
<i>O. sulphurea</i>	13.06		52.65	2.45		4.08	21.63	0.82	3.27	2.04						245	190	26	790

Table 4 Handling times in seconds of different bee species in hermaphroditic and females flowers of *Opuntia quimilo*

Bee	Hermaphroditic	Female	ANOVA (ln transformed)
<i>Arhysosage</i> sp.		87.33 ± 76.56 (n = 3)	
<i>Bombus</i> sp.	6.77 ± 4.53 (n = 22)	8.86 ± 7.47 (n = 7)	F(1, 27) = 0.57; n.s.
<i>Eucerini</i>	25.75 ± 15.84 (n = 4)		
<i>Lithurgus</i> sp.	62.75 ± 68.25 (n = 8)		
<i>Megachile</i> sp.	24.62 ± 27.53 (n = 77)	25.55 ± 17.51 (n = 9)	F(1, 84) = 0.02; n.s.
<i>Ptilothrix tricolor</i>	2.95 ± 0.92 (n = 60)	2.85 ± 1.00 (n = 48)	F(1, 106) = 0.56; n.s.
<i>Xylocopa</i> sp.		6.22 ± 1.78 (n = 9)	

Breeding system and gynodioecy

A previous indication that *O. quimilo* is self-incompatible (Aizen, 1994) may have resulted from using false pollen in manual cross pollination. The sexual condition appears to be determined genetically and the individual plants did not change sex during our present study. Sexuality is probably not depen-

dent on environmental conditions, as in some gynodioecious shrubby Chenopodiaceae (Freeman et al., 1984). In Central American *Fuchsias* studied by Arroyo and Raven (1975), that also include bird-pollinated species, gynodioecy is apparently an unstable condition because of the high proportion of male sterile plants among the morphologically hermaphroditic plants.

Table 5 Number of observed visits to sexes of *O. quimilo*

Visitors	Hermaphroditic	Female
Bees	425	179
Hummingbird	67	181
Butterfly	8	0
Total	500	360

Gynodioecy is probably a stable condition in *O. quimilo*, as is suggested by the scarcity of dioecy in the nearest relatives in the genus and the weak differences in female fitness between sexes (Richards, 1997). It must be noted, however, that in the neighbouring genus (or subgenus) *Brasilopuntia*, that includes tree-like plants that do not seem to be ornithophilous, subhermaphrodites are present which bear flowers with staminodes mixed with fertile stamens (Backeberg, 1958).

Possible inheritance modes of the male sterility genes should be limited to one of the known inheritance models, i.e. nuclear (♀ Mm-■ mm; ♀ mm-■ Mm; ♀ mm-■ MM+Mm), or cytoplasmic (Lloyd, 1974). Lloyd (1974) considered pollen availability to female and relative female/hermaphrodite fecundity (F), among other factors, to predict sex ratios in gynodioecious plants. In *O. quimilo* a female frequency of 0.5 can be at equilibrium in only two of the four possible models, if equal survival of hermaphroditic and female progeny is assumed (Lloyd, 1974): (1) If sex is nuclear inherited and hermaphrodites are heterozygous (♀ mm-■ Mm), (2) If sex is determined by cytoplasmic inheritance.

In the first case, if success of both kinds of male gametes is equal, the proportion of females would vary depending on the relative fertility of hermaphrodites/females between 0.4 and 0.5. Given the relative fecundity found in *O. quimilo* (from Table 2, fecundity is $F = 38.46/85.19 = 0.45$), the predicted female frequency (p) from the corresponding equation (Lloyd, 1974) would be $p = 0.45$.

$$p = \frac{2F - 1 \pm (1 + F^2)^{0.5}}{3F - 4}$$

A hypothesis of female frequency of 0.45 cannot be rejected with the obtained data ($\chi^2 = 0.0051$; n.s.), neither can a hypothesis of female frequency of 0.5 (see Results). In the second possible model, any proportions of sexes is possible if pollen availability to females is not limited. If, however, only a fraction of the female flowers are pollinated, female individuals are eliminated (Lloyd, 1974). Another important consequence predicted by the model is that hermaphrodites can become extinct if their ovules are fertilized equally frequently as females (Lloyd, 1974). The high fruit set of open-pollinated female flowers as compared with hand cross-pollinated flowers indicates that female plants are not limited by pollen availability.

Bee visitors

Only large bees visit the flowers of *O. quimilo*. In our study site *Lithurgus* sp. and *Ptilothrix tricolor* where seen on all species of *Opuntia* and on no other plants. *Ptilothrix fructifera* was found,

among 46 species of bee visitors, to be the most frequent pollinator of *O. brunneogemmia* and *O. viridirubra* of Southern Brazil (Schlindwein and Wittmann, 1997). Other *Ptilothrix* species are known as oligoleges of Malvaceae (Michener, 2000). *Lithurgus* is an important visitor of *Opuntia* species in North America (Cockerell, 1900; Grant and Grant, 1979; Grant et al., 1979; McFarland et al., 1989; Osborn et al., 1988) and is also recorded as an oligolege visitor of Malvaceae (Michener, 2000). Schlindwein and Wittmann (1997) found *Lithurgus rufiventris* to be the second most frequent pollinator of the above named south Brazilian *Opuntia* species.

The preference of *Ptilothrix* and *Lithurgus* for Cactaceae and Malvaceae pollen is related to their scopae being thinly covered by long and unbranched bristles adapted to large pollen grains (Schlindwein and Wittmann, 1997; Michener, 2000). The absence of visits by small bees in *O. quimilo* is surprising because of its lack of seismonasty, which acts to discourage visits by small bees in other species of *Opuntia* (Schlindwein and Wittmann, 1997).

Ornithophilous features of *O. quimilo*

Red, a particularly bright flower colour, is frequently associated with hummingbird pollination (Stiles, 1981; Proctor et al., 1996). Fleshy or robust flowers are also frequent in ornithophily and presumably protect them from nectar thieves and allow the relatively strong forces exerted by birds (Stiles, 1981; Proctor et al., 1996). The first explanation probably does not hold for *O. quimilo* because the petals do not help to contain nectar. The strength-support explanation may not hold either because humming birds are not supposed to exert stronger forces on the flowers than large bees (Stiles, 1981). Moreover, hummingbirds visiting *O. quimilo* were not observed to make contact with the petals. In *O. quimilo*, the greater petal fleshiness, as compared with other local *Opuntia* species, is more probably related to the longer flower lifespan. The other local *Opuntia* species have a one to two day period of anthesis, as is usual in *Opuntia*. The presence of a nectar chamber in the flowers of ornithophilous plants is often thought to hinder accidental loss of the abundant and low viscosity nectar and to prevent evaporation (Stiles, 1981; Proctor et al., 1996). In *O. quimilo* a chamber is built by the style dilatations that delimit a chamber just above the stamens. Such dilatations, which Backeberg (1958) refers to as "Manschetten" (cuffs), also build nectar chambers in the middle of the receptacle in *Consolea*, a genus related to *Opuntia*.

Stamen seismonasty is a common feature in *Opuntia* (Porsch, 1938) and has been associated with pollination by large bees (Schlindwein and Wittman, 1997). The lack of seismonasty in *O. quimilo* may indicate a weaker adaptation to bees. Nectar concentration lies near the upper limit expected for bird-pollinated plants (Baker, 1975).

Bird pollination and probable bird flowers in other species of *Opuntia* and related genera

There is one old record of bird pollination in *Opuntia cylindrica* D.C. (*Cylindropuntia*) (Lagerheim, 1895■), a species with no obvious ornithophilic features. The only other cases of bird visitation to *Opuntia* we are aware of were reported for four endemic species from the Galapagos Islands, *O. echios*, *O. megasperma*,

O. helleri and *O. galapageia* (Grant and Grant, 1981). These species are visited for pollen and nectar by finches, mockingbirds and doves. For *O. helleri* and *O. echios* it has been shown experimentally that birds act as additional pollinators to *Xylocopa darwini* or as major pollinators, where the carpenter bee is absent. Flowers are yellow and nectar concentration of *O. helleri* ($22.0 \pm 0.4\%$) was within the expected range for bird-pollinated flowers (Grant and Grant, 1981) and somewhat lower than that of *O. quimilo*. Otherwise the flowers do not appear to be particularly adapted for bird pollination. Birds even exert some distrophic activities whereby they reduced fruit set to some extent (Grant and Grant, 1981).

Flower traits in *O. quimilo* that are associated with bird pollination (see above) appear to be combined in other *Opuntia* species and in species of related Opuntioide genera. Some of these species may have additional features also associated with hummingbird pollination. Bird pollination in this plant group is perhaps not as rare as the scarcity of published reports would suggest. Red flowers and tree-like habit are traits that appear combined. The tree-like habit, which exposes flowers in a high position, has also been associated with bird pollination in plants with epiphytic and twinning habits (Porsch, 1929). For the 212 *Opuntia* species for which data on habit and flower colour are available, red flower colour has a significantly higher incidence among trees than among other growth forms, i.e. shrubs, herbs and cushion plants (Table 6).

Within *Opuntia*, five Mexican species that constitute the series *Stenopetalae* of section *Micranthae* (Backeberg, 1958) combine red or orange-red flowers and the presence, as in *O. quimilo*, of a nectar chamber built by a ring-like basal dilatation of the style. These species further resemble *O. quimilo* in exhibiting a bush-like habit and at least one species (*O. stenopetala*), and probably more, have male-sterile forms (Parfitt, 1985). Flowers of the male-fertile forms seem to have a reduced style and ovary chamber, and no ovules (Backeberg, 1958; Parfitt, 1985). So species with male sterility are truly dioecious.

Among other Opuntioide genera, bird pollination or features associated with bird pollination are noteworthy in *Nopalea* and *Conosolea*, two genera that, according to recent evidence (Griffith, 2002), can be included in *Opuntia*, and *Tacinga*, a sister group to *Opuntia* (Griffith, 2002). Their features associated with bird pollination are given in Table 7. In overall appearance the flowers have distinct flower forms, from more open funnel-shaped in *Conosolea* to a tubular form built by the exerted

Table 6 Incidence of the growth habit in flower colour among the species of *Opuntia*. Independence between growth form and flower colour (red or not red) is rejected with χ^2 and Irwin Fischer tests at $p < 0.001$ level. Data taken from (Backeberg, 1958)

Growth form	% of species with red flowers	Number of species
Trees	37.5	48
Other	12.8	164
Total	18.4	212

stamens arranged in a tight conical bundle above which the stigma emerges in *Nopalea* and *Tacinga*. Petals may be upright and appressed in *Nopalea* or turned backwards in *Tacinga*. The latter is a form of specialized flower architecture, well known in hummingbird-pollinated species, known as the Fuchsia-type (Delpino, 1868).

Differential pollination effects by hummingbirds and bees

Given the higher visitation intensity of bees to hermaphroditic flowers and of birds to female flowers, it must be assumed that pollinators can to some extent distinguish between flower types. Flowers do differ in size and in appearance of the androecium. Bees should visit hermaphroditic flowers more intensely because they contain pollen in addition to nectar. Hummingbirds should prefer females because of the lower nectar concentration.

Because bees usually have smaller flight ranges than trap-lining hummingbirds (and *Helimaster furcifer* is one), create mess, spoil pollination and prefer hermaphroditic flowers, they probably cause self-pollination and pollination among hermaphrodites. Whereas trap-lining hummingbirds have large flight ranges and, when visiting hermaphrodites, by precise handling of the flowers they most probably perform little or no self-pollination.

Visitation frequencies of bees and hummingbirds to female flowers are practically equal. But the probability of a pollinator visiting a female flower having first visited a hermaphroditic is much lower for hummingbirds than for bees, assuming equal patterns of interplant movement. Thus, if hummingbirds are a major component in the reproductive success of female plants, the patterns of interplant movement must be critical in the

Table 7 Genera related to *Opuntia* and their characters associated to bird pollination

Genus	<i>Conosolea</i>	<i>Nopalea</i>	<i>Tacinga</i>
Number of species	9	12	2
Distribution	West Indies	Mexico and Guatemala	Caatinga in Brazil
Life form	trees	bushes, trees	climbing
Dioecy	yes	no	no
Flower type	funnel-shaped	tubular	Fuchsia-type
Flower colour	orange-red, deep red	pink, red	green to dark violet
Nectar chamber	yes	yes	?
Flower size	1–3 cm in diameter	4–10 cm long	5.7–10 cm long
Bird pollination reports		Porsch, 1938; Knuth et al., 1904	

differential pollination effects. If hummingbirds visit several distant plants consecutively they can bring better pollen to the stigmas than bees which repeatedly visit the same plant.

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