ORIGINAL ARTICLE

# Trends in the reproductive biology of Venezuelan *Melochia* (Malvaceae) species

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**Abstract** The breeding system, floral morphology, morph frequency, biomass allocation to floral whorls, pollination and reproductive efficiency were examined in four distylous and three monomorphic *Melochia* species. Monomorphic species were self-compatible and distylous species were self-incompatible. Flowers of homomorphic species were longer than those of distylous species. Herkogamy was significantly higher in pin than in thrum morphs of the two distylous species, and monomorphic species exhibited the lowest values of herkogamy. Pollen/ovule ratios were similar between monomorphic and dimorphic species, irrespective of the self-incompatibility level. Biomass allocation to flowers was biased toward non-sexual structures, attraction and support. Androecium biomass and androecium/gynoecium biomass ratio were larger in thrum than in pin flowers of distylous species indicating maleness in the thrum morph and femaleness in the pin morph. There was no clear difference between fruit set of monomorphic and dimorphic species; however, the greatest fruit set was found in the monomorphic species, *M. pyramidata*, which is a self-compatible species. Fruit set was significantly higher in pin than in thrum morphs in three out four distylous species and fecundity was only significantly higher in pin

morphs of *M. caracasana* and *M. parvifolia*. *Melochia* species have generalist pollination systems. According to the taxonomic classes and number of pollinator species, *M. caracasana* and *M. parvifolia* have similar generalist pollination systems. Our comparative analyses of the characters between floral morphs of distylous species and the relationship with these characters in monomorphic species allow divergences and similarities to be established and different evolutionary trends to be postulated in the breeding systems of *Melochia* species. Specifically, *M. parvifolia* and *M. caracasana* are apparently biased toward monomorphy and dioecy, respectively.

## Introduction

Distyly is an attribute frequently present in the genus *Melochia*, but pin and thrum morphs do not always occur in all *Melochia* species, and in some cases the level of self-incompatibility may vary widely (Martin 1967; Ramírez and Brito 1990; Lemus-Jiménez and Ramírez 2005; Machado and Sazima 2008). The populations of distylous species are frequently self-incompatible at equilibrium, and morph frequency is close to equal numbers, and isoplethy is expected (Ornelas et al. 2004; Coelho and Barbosa 2004; Teixeira and Machado 2004b). Deviations from a 1:1 ratio in the frequency of pin and thrum individuals at the population level have been reported for plant species which show some degree of self-compatibility (Mulcany 1964; Martin 1967; Ganders 1975; Sobrevila et al. 1983; Coelho and Barbosa 2004;

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Rossi et al. 2005). Stability of distylous self-incompatible species may be associated with a compensatory function in morphological and functional traits between pin and thrum morphs in the population. On the contrary, the superiority of some characters could differentially increase the male and female fitness, and consequently could promote differential advantages for one of the two morphs under selective pressure.

Distylous self-compatible populations may change into homostylous populations (i.e. Sobrevila et al. 1983; Consolaro et al. 2005) or alternatively into dioecious populations (Beach and Bawa 1980; Bawa and Beach 1981). Instability of the distylous condition and the origin of homostyly from a distylic ancestor seem to be promoted under different selective pressures. First, the levels of pollination specialization, generalist or specialist, have been considered an important factor promoting changes in the morph frequency and compatibility level in distylous species (Beach and Bawa 1980; Sobrevila et al. 1983; Arroyo and Dafni 1995). Changes in pollination service may stimulate transitions to gender dimorphisms (Charlesworth and Charlesworth 1979). Second, floral morphology and pollinators have a particular importance in the stability of distyly. For example, open or short tube flowers may allow pollination by generalist pollinators with short mouth parts, and self-pollination is promoted due to unspecialized pollen transference (Barrett et al. 1996; Arroyo et al. 2002). Third, changes of populations from distylous to homostylous may be associated with the decreasing size of flowers in self-compatible compared to self-incompatible species (Lloyd 1965; Sobrevila et al. 1983; Case and Barrett 2004). Fourth, self-compatible species would have a comparatively low biomass allocation to flowers and flower parts than self-incompatible species or morphs. Sex-allocation models have predicted that allocation to both male and attractive

structures decreases as the selfing rate increases (Charlesworth and Charlesworth 1987; Lloyd 1987). In this context, biomass allocation to male function will be lower than biomass allocation to female function in plant species or morphs where self-compatibility potentially may allow selfing compared to xenogamous (self-incompatible) species or morphs.

According to variations observed in long- and short-style morph frequency in *Melochia* (Martin 1967; Ramírez and Brito 1990), and considering that self-incompatibility is not the rule among *Melochia* species (Martin 1967; Lemus-Jiménez and Ramírez 2005), the present study examined the reproductive biology of seven species of *Melochia* in Venezuela. The main goal was to describe the evolutionary trends in the breeding systems of *Melochia* species by: (1) describing the breeding systems of monomorphic and dimorphic species; (2) estimating the reciprocity of morphological and functional traits between floral morphs; (3) characterizing the flower-visiting insect community associated with floral morphology, and finally (4) determining how strongly the breeding system is related to morphological flower traits.

## Materials and methods

### Study area

A total of seven *Melochia* species were studied in the following four localities in Venezuela, with further details in Table 1 (vegetation types follow Huber and Alarcón 1988):

1. *Litoral Central*: xerophyllous, coastal thicket located along the Caribbean coast in Canes, State Vargas, and Distrito Federal.

**Table 1** *Melochia* species, floral colour, life form, and characteristics of the study areas

| Species | Floral colour | Life form | Locality | Coordinates | Elevation (m) | Mean temperature (°C) | Precipitation (mm) |
|---------|---------------|-----------|----------|-------------|---------------|----------------------|--------------------|
| *M. caracasana* Jacq. | White–yellow | Fruticose-shrub | Caracas | 10°30′N, 66°53′W | 1,100 | 18.0–24.0 | 1,000–2,200 |
| *M. crenata* Vahl | Pink | Fruticose | Falcón | 11°38′N, 69°44′W | 10 | 29.3 | 330 |
| *M. nodiflora* Sw. | Magenta | Fruticose | Caracas | 10°30′N, 66°53′W | 1,100 | 18.0–24.0 | 1,000–2,200 |
| *M. parvifolia* Kunth | White | Fruticose, annual or biannual | Calabozo | 8°56′N, 67°25′W | 75 | 27.0–28.0 | 1,200–1,300 |
| *M. pyramidata* L. var. *pyramidata* | Magenta | Fruticose-annual | Litoral Central | 10°36′N, 67°02′W | 5–100 | 26.3 | 558 |
| *M. tomentosa* L. | Pink | Fruticose | Litoral Central | 10°36′N, 67°02′W | 5–100 | 26.3 | 558 |
| *M. villosa* (Mill.) Fawc & Rendle var. *villosa* | Pink | Fruticose | Calabozo | 8°56′N, 67°25′W | 75 | 27.0–28.0 | 1,200–1,300 |

2. *Caracas*: Semideciduous, seasonal, ombrophilous, submontane forest in the Arboretum of the Instituto de Biología Experimental, Universidad Central de Venezuela, in Colinas de Bello Monte, Distrito Federal.
3. *Calabozo*: Shrub savannah with tree islands and gallery forest in the Estación Biológica de los Llanos, State Guarico.
4. *Falcón*: psamophilous vegetation, dominated by herbaceous species in the peninsula de Paraguaná, State Falcón.

## Floral morphology and morph frequency

Floral measurements were made on flowers fixed in 70% alcohol collected a few hours after opening when pollen presentation and stigma receptivity occurred. Flower length (from the base of the ovary to the top of the flower, stamens or stigma) and perianthum width (diameter delimited by the tips of the petals) were measured in 5–10 flowers from different individuals of each morph of dimorphic species and in 5–16 flowers from different individuals of monomorphic species. The spatial separation of pollen presentation and stigma receptivity (herkogamy) were determined in fully developed flowers. The frequency of each morph per plant species was recorded randomly in an area of approximately 1 ha for each dimorphic species.

## Experimental pollinations

Each distylous *(M. caracasana, M. parvifolia,* and *M. tomentosa)* and monomorphic *(M. pyramidata)* species was hand-pollinated separately by placing pollen on receptive stigmas with a tiny brush. Spontaneous self-pollination was tested in all *Melochia* species cited above by leaving bagged flowers from the bug stage until fruit development was finished. Flowers were bagged using double-folded extra-fine wedding veil. Self-compatibility was investigated by manually selfing bagged flowers by placing pollen of the same flower on receptive stigmas. Cross-pollination (bagged and emasculated flowers) was achieved in two ways for distylous species: (1) legitimate crosses were produced by placing pollen of one morph on receptive stigmas of the other morph, and (2) nonlegitimate crosses were produced using pollen of different plant individuals of the same morph species. The number of mature fruits and non-abortive seeds resulting from each cross were counted. Fruit set from experimental crosses was calculated as the total number of developed fruits divided by the total number of flowers pollinated. Seed set from experimental crosses was determined as the total number of non-abortive seeds produced by all fruits of each experimental pollination divided by the total number of ovules under experimental pollinations (flower number for each cross multiplied by the average number of ovules per flower; see below for counting methods) (Jaimes and Ramírez 1999). Plant species producing significantly fewer fruits/flower and seeds/ovule after hand self-pollination than after cross-pollination were considered self-incompatible, which was determined using the *G* test of independence, following the method of Jaimes and Ramírez (1999). Information on experimental crosses of *M. villosa* and *M. crenata* was obtained from previous studies (Ramírez and Brito 1990; Lemus-Jiménez and Ramírez 2005).

## Pollen/ovule ratio

Pollen/ovule ratios were estimated following the basic methodology described by Cruden (1977), with modified sample sizes applied to all *Melochia* species studied. The number of pollen grains per anther was estimated by suspending five anthers from five different plants in a solution of aniline-blue in lactophenol, and the number of pollen grains in a known volume of the suspension was counted using Neubauer chambers under an optical microscope (Lloyd 1965). This number was multiplied by the dilution factor, and then by the number of anthers per flower. This procedure was repeated three times, and from that we estimated the average number of pollen grains per flower. The number of ovules per flower was counted in 90–100 ovaries from at least five different plants.

## Biomass allocation to floral parts

Ten flowers from each of five dimorphic individuals and five monomorphic individuals were separated into their parts (sepals and receptacle, petals, androecium, and gynoecium) and then dried to constant weight at 40°C. Androecium/gynoecium dry biomass and sexual/nonsexual dry biomass ratios were estimated as measures of relative investment in male and sexual functions of the flowers, respectively.

## Reproductive efficiency

Four levels of reproductive efficiency were evaluated under open pollination with insects having access to flowers. First, fruit set was determined by the proportion of flowers per inflorescence that developed into mature fruits. The number of flowers and fruits per inflorescence was determined by direct counts of inflorescences, both at or prior to anthesis and during late stages of fruit maturation, in at least ten individuals per species in monomorphic populations, and ten individuals per morph in dimorphic populations. Second, the number of seeds per ovule was determined by dividing the average number of seeds per fruit by the average number of

ovules per flower. The number of ovules per flower was obtained by dissecting flowers at anthesis or in well-developed buds, and the number of seeds per fruit was determined by direct counts of mature fruits. Third, seed abortion was determined directly in mature fruits. Seeds that lacked an embryo, or were much reduced in size, malformed, or squashed were considered aborted. The number of aborted seeds was determined by dividing the average number of aborted seeds per fruit by the average number of seeds per fruit. Fourth, relative fecundity was defined as the proportion of well-developed seeds per ovule per inflorescence. It was determined by multiplying the average number of healthy seeds per fruit by the average number of fruits per inflorescence and dividing this by the total number of ovules per inflorescence (Ramírez 1992). This gives a lower but more accurate value for the overall reproductive success of the plant than fruit set alone.

## Pollination

The activity of all types of floral visitors was described and then visitors were captured. This procedure was carried out over 30 min at intervals of 2 h during the life the flower. This procedure was repeated in different months of flowering phenology. Censuses were concluded when the visitor species number stayed constant over the observation periods. Pollinators were distinguished from floral visitors using four out of five criteria described by Ramírez (2004):

1. Presence and estimated abundance of pollen of the visited plant.
2. Part of the body where pollen was located and its relationship with the position or orientation of the sexual organs in the blossom.
3. Relationship between blossom size and a specific area of the blossom, and visitor size or specific part of the visitor body.
4. Relative abundance of each visiting species (number of visits per unit time).

Using these criteria each plant species might be associated with one or more than one class of pollinating agent. The pollination system was considered as generalist when pollination belonged to different taxonomic classes (flies, bees, wasps, butterflies, and sphingids), and specialist when pollination was by a genus or different genera belonging to a single family. Information on pollination for *M. villosa* and *M. crenata* was obtained from previous studies (Ramírez and Brito 1992; Lemus-Jiménez and Ramírez 2003).

## Statistical analysis

Mean values between pin and thrum morphs were statistically compared using the *t* test for unequal or equal sample size, and for dependent or independent variables according to each particular case. Similarly, mean values between monomorphic and dimorphic species were statistically compared. The following parameters were transformed before statistical comparison: number of flowers per inflorescence, number of fruits per infrutescence, number of ovules per flower, and number of seeds per fruit (square root), number of aborted seeds per fruit and dry biomass of floral parts (square root adding 0.5) (Sokal and Rohlf 1995). When the characters to be compared between morphs were values of frequency (fruit set, seed set, percentage of aborted seed, and fecundity), we used the *G* test of independence (Sokal and Rohlf 1995). The chi-squared test was used to determine if the observed values of pollen grains per anther, pollen grains per flower, and pollen/ovule ratio between morphs of each distylous species departed from expected equal values. For these comparisons, the data were transformed into square roots before analysis. The number and percentage of characters differing or not significantly different between pin and thrum morphs were estimated for each distylous species, and then each character of each plant species was determined as biased pin, biased thrum, or neutral when there was no statistically significant difference.

Two cluster analyses were performed to classify distylous species. The first used as grouping variables the pin/thrum ratios of all characters evaluated, which were transformed into the square roots plus 0.5 before analysis. The second cluster was analysed using as grouping variables the number of pollinator groups (bees, wasps, flies, butterflies, and sphingids) pollinating each plant species. The numbers of visitor species belonging to each group were transformed into the square roots plus 0.5 before analysis. In both analyses, linkage was measured using the method of single linkage (StatSoft 2001), which is determined as the distance of the two closest objects (nearest neighbours) in the different clusters. The distances were measured as Euclidean distances.

## Results

### Frequency of morphs

The pin individuals outnumbered the thrum individuals in *M. parvifolia* (pin:thrum 28:12), *M. tomentosa* (pin:thrum 19:12) *and M. villosa* (pin:thrum 24:13). In contrast, the thrum individuals outnumber the pin individuals in *M. caracasana* (pin:thrum 10:15). However, deviation from the expected 1:1 ratio was not significant in any case.

### Floral size and herkogamy

Flower length did not vary significantly between morphs in *M. parvifolia* and *M. tomentosa* (Table 2). In contrast,

thrum flowers of *M. caracasana* were longer than pin flowers, and the contrary was found in *M. villosa*. Similarly, flower width was not different between morphs of *M. parvifolia*, *M. tomentosa* and *M. villosa* (Table 2). However, the pin flowers were wider than thrum flowers in *M. caracasana*. Dimorphic species exhibited longer flowers than homomorphic species (Table 2).

The distance between stigmas and anthers was significantly higher in pin than in thrum morphs in *M. caracasana* and *M. parvifolia*, respectively (Table 2). Herkogamy did not show statistically significant differences between pin and thrum in *M. tomentosa* and *M. villosa*, respectively (Table 2). Homomorphic species of *Melochia* exhibited the lowest values of herkogamy (Table 2).

## Levels of self-incompatibility

Pollination experiments showed that pin and thrum morphs of distylous species did not produce fruits and seeds under spontaneous self-pollination (Table 3). However, some fruits and seeds were produced after hand self-pollination in some distylous species (Table 3): *M. tomentosa* with fruit and seed production under hand self-pollination in both morphs; *M. caracasana* with fruit and seed production under hand self-pollination only in thrum flowers; and *M. parvifolia* with fruit and seed production under hand self-pollination only in pin flowers. The statistical analysis revealed that seed set was significantly higher in thrum than in pin morphs in *M. caracasana*, and pin morphs produced significantly more fruits and seeds than thrum morphs in *M. parvifolia* under hand self-pollination (Table 3). The homomorphic population of *M. pyramidata* exhibited high fruit and seed set under spontaneous and hand self-pollination (Table 3).

Fruit set and seed set did not differ between legitimate crosses (pin × thrum and thrum × pin) in the three distylous species (Table 4). The statistical comparisons of hand self- and cross-pollination revealed that legitimate cross-pollination produce significantly more fruits and seeds than hand self-pollination in distylous species, except for fruit set in pin morphs of *M. parvifolia* (Table 4).

## Pollen/ovule ratio

The numbers of stamens per flower were very similar in all *Melochia* species (Table 5). The numbers of stamens per

**Table 2** Flower sizes of monomorphic and dimorphic species of *Melochia* and statistical comparisons between morphs for each distylous species and between monomorphic and dimorphic species

| *Melochia* species | Number | Flower length (mm) | Flower width (mm) | Herkogamy (mm) |
|---|---|---|---|---|
| Monomorphic | | | | |
| *M. crenata* | 13 | 6.4 (2.4) | 14.6 (2.4) | 0.1 (0.1) |
| *M. nodiflora* | 16 | 4.4 (0.2) | 4.3 (0.8) | 0.0 (0.0) |
| *M. pyramidata* var. *pyramidata* | 5 | 6.5 (0.6) | 5.3 (0.8) | 0.4 (0.1) |
| Mean (SD) | | 5.8 (1.8) | 8.1 (5.7) | 0.2 (0.2) |
| Dimorphic | | | | |
| *M. caracasana* | | | | |
| Pin | 5 | 10.2 (0.3) | 11.7 (0.4) | 2.9 (0.4) |
| Thrum | 5 | 11.0 (0.7) | 10.6 (1.2) | 2.4 (0.5) |
| *t* test (df 8) | | 4.03** | 3.17* | 2.78* |
| *M. parvifolia* | | | | |
| Pin | 10 | 10.7 (0.5) | 12.3 (0.9) | 4.2 (0.7) |
| Thrum | 10 | 10.4 (0.7) | 12.7 (1.7) | 3.4 (0.3) |
| *t* test (df 8) | | 1.15 (n.s.) | 0.67 (n.s.) | 4.04** |
| *M. tomentosa* | | | | |
| Pin | 10 | 10.9 (1.4) | 13.2 (2.0) | 3.1 (1.0) |
| Thrum | 10 | 12.2 (1.5) | 13.7 (1.5) | 4.1 (0.9) |
| *t* test (df 8) | | 2.18 (n.s.) | 0.65 (n.s.) | 0.63 (n.s.) |
| *M. villosa* var. *villosa* | | | | |
| Pin | 5 | 8.3 (0.7) | 7.7 (0.8) | 2.1 (0.6) |
| Thrum | 10 | 6.5 (2.1) | 10.8 (2.5) | 2.4 (1.7) |
| *t* test (df 8) | | 2.50* | 1.22 (n.s.) | 1.01 (n.s.) |
| Mean (SD) | | 10.0 (1.8) | 11.6 (1.9) | 3.1 (0.8) |
| Monomorphic–dimorphic comparison $F_{(1,9)}$ | | 15.1** | 3.5 (n.s.) | 68.4*** |

The data are presented as means (SD)

*n.s* not significant

*$P < 0.05$, **$P < 0.005$, ***$P < 0.0001$

**Table 3** Results of spontaneous self-pollination and hand self-pollination and statistical comparison between morphs of each of the four *Melochia* species

| *Melochia* species | Spontaneous self-pollination | | | | | Hand self-pollination | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Flowers (*n*) | Fruit (*n*) | Fruit set (%) | Seed (*n*) | Seed set (%) | Flowers (*n*) | Fruit (*n*) | Fruit set (%) | Seed (*n*) | Seed set (%) |
| *M. caracasana* | | | | | | | | | | |
| Pin | 98 | 0 | 0.0 | 0 | 0.0 | 50 | 0 | 0.0 | 0 | 0.0 |
| Thrum | 132 | 0 | 0.0 | 0 | 0.0 | 44 | 4 | 9.1 | 9 | 2.2 |
| $\chi^2$ test | | | | | | | | 2.48 (n.s.) | | 6.88* |
| *M. parvifolia* | | | | | | | | | | |
| Pin | 85 | 0 | 0.0 | 0 | 0.0 | 26 | 9 | 34.6 | 25 | 9.6 |
| Thrum | 58 | 0 | 0.0 | 0 | 0.0 | 31 | 0 | 0.0 | 0 | 0.0 |
| $\chi^2$ test | | | | | | | | 7.18* | | 26.25** |
| *M. pyramidata* var. *pyramidata* | 67 | 42 | 62.7 | 341 | 51.4 | 11 | 8 | 72.7 | 54 | 49.6 |
| *M. tomentosa* | | | | | | | | | | |
| Pin | 47 | 0 | 0.0 | 0 | 0.0 | 34 | 2 | 5.9 | 2 | 0.6 |
| Thrum | 40 | 0 | 0.0 | 0 | 0.0 | 38 | 4 | 10.5 | 7 | 1.9 |
| $\chi^2$ test | | | | | | | | 0.05 (n.s.) | | 1.56 (n.s.) |

*n.s.* not significant

*$P < 0.006$, **$P < 0.0001$

flower, pollen grains per anther, pollen grains per flower, and pollen/ovule ratio did not differ significantly between morphs of each distylous species, nor between distylous and homomorphic species (Table 5). However, slight differences were observed between distylous species. The pollen/ovule ratio was larger in pin than in thrum morphs of *M. caracasana*, and *M. villosa*; the contrary was found for *M. parvifolia* and *M. tomentosa* (Table 5). Pin morphs of *M. tomentosa* exhibited the lowest values of pollen/ovule ratio. Monomorphic species exhibited the lowest (*M. pyramidata*) and the highest (*M. crenata*) values of pollen/ovule ratio (Table 5).

Biomass allocation to floral parts

Biomass allocation to floral parts was different across *Melochia* species. The biomass of the calyx and receptacle was significantly higher in pin morphs than thrum morphs of *M. caracasana*, and in thrum morphs than pin morphs of *M. villosa* (Table 6). Corolla biomass was significantly higher in pin morphs than thrum morphs of *M. caracasana* and *M. villosa*. Biomass allocation the to androecium was similar between pin and thrum morphs of *M. caracasana*, and significantly higher in thrum than in pin morphs of *M. parvifolia*, *M. tomentosa* and *M. villosa* (Table 6). Biomass allocation to the gynoecium was only significantly higher in pin morphs than in thrum morphs of *M. parvifolia* (Table 6). The dry weight of flowers was significantly higher in pin morphs than in thrum morphs of *M. caracasana* and *M. villosa*, and the contrary was found for *M.*

*tomentosa* (Table 6). The dry biomass of the androecium was the only floral part that differed significantly between homomorphic and distylous species, being higher in distylous species (Table 6).

The androecium/gynoecium biomass ratio tended to be larger in thrum morphs than in pin morphs of distylous species (Table 6). Biomass allocated to the androecium was higher than that to the gynoecium, except for the monomorphic species in which the androecium/gynoecium ratios were less than one (Table 6). The androecium/gynoecium ratio was significantly higher in distylous than in homomorphic species (Table 6). The reproductive/vegetative biomass ratio or reproductive effort of the flowers was less than one, which indicates that biomass allocation to the flowers of *Melochia* species is biased toward nonsexual structures, attraction and support (Table 6).

Reproductive efficiency

Natural fruit set varied among plant species and in some cases between morphs of distylous species (Table 7). The numbers of flowers per inflorescence and fruits per infrutescence were higher in distylous species than in monomorphic species, but only fruits per infrutescence was significantly higher in monomorphic species (Table 7). The numbers of flowers per inflorescence and fruits per infrutescence were similar between morphs of M. *parvifolia* and *M. villosa* In contrast, the numbers of flowers per inflorescence was higher in thrum than in pin morphs in *M. caracasana* and *M. tomentosa* (Table 7). The numbers of

**Table 4** Results of crosses between morphs and statistical comparisons of three *Melochia* species

| *Melochia* species | Cross-pollination | | | | | Hand self- vs. cross-pollination | |
|---|---|---|---|---|---|---|---|
| | Flowers (n) | Fruit (n) | Fruit/flower ratio | Seeds (n) | Seed set (%) | Pin[a] | Thrum[b] |
| *M. caracasana* | | | | | | | |
| Pin × pin | 33 | 0 | 0.0 | 0 | 0.0 | | |
| Thrum × thrum | 68 | 0 | 0.0 | 0 | 0.0 | | |
| Pin × thrum | 54 | 27 | 50.0 | 238 | 44.7 | | |
| Thrum × pin | 59 | 36 | 61.0 | 297 | 54.1 | | |
| $\chi^2$ test | | | 0.22 (n.s.) | | 2.54 (n.s.) | | |
| Fruit | | | | | | 19.0*** | 12.4** |
| Seed | | | | | | 153.4*** | 160.9*** |
| *M. parvifolia* | | | | | | | |
| Pin × pin | 21 | 6 | 28.6 | 28 | 13.3 | | |
| Thrum × thrum | 18 | 0 | 0.0 | 0 | 0.0 | | |
| Pin × thrum | 44 | 31 | 70.4 | 141 | 32.0 | | |
| Thrum × pin | 33 | 22 | 66.7 | 99 | 30.0 | | |
| $\chi^2$ test | | | 0.01 (n.s.) | | 0.13 (n.s.) | | |
| Fruit | | | | | | 1.9 (n.s.) | 14.6*** |
| Seed | | | | | | 28.6*** | 80.6*** |
| *M. tomentosa* | | | | | | | |
| Pin × pin | 41 | 1 | 2.4 | 1 | 0.2 | | |
| Thrum × thrum | 38 | 3 | 7.9 | 3 | 0.8 | | |
| Pin × thrum | 32 | 19 | 59.4 | 123 | 44.2 | | |
| Thrum × pin | 43 | 28 | 65.1 | 163 | 42.6 | | |
| $\chi^2$ test | | | 0.01 (n.s.) | | 0.08 (n.s.) | | |
| Fruit | | | | | | 9.9* | 10.2* |
| Seed | | | | | | 104.4*** | 105.1*** |

*n.s.* not significant

*$P < 0.002$, **$P < 0.0005$, ***$P < 0.0001$

[a] Hand self-pollination pin versus cross-pollination pin × thrum

[b] Hand self-pollination thrum versus cross-pollination thrum × pin

fruits per infrutescence only differed between morphs of *M. caracasana,* where infrutescence pin produced more fruit than thrum, which was opposite to the numbers of flowers per inflorescence. Fruit set ranged from 30.1% to 88.8% in all *Melochia* species, and ranged from 30.1% to 67.7% in distylous species, where fruit set was higher in pin than in thrum morphs of *M. caracasana*, *M. parvifolia* and *M. tomentosa;* but the opposite was found in *M. villosa* (Table 7).

The number of seeds per fruit ranged from 2.9 to 9.8 (Table 7). The number of seeds per fruit was significantly higher in monomorphic than in distylous species (Table 7). Fruit of pin morphs had significantly more seed than that of thrum morphs of *M. caracasana, M. parvifolia* and *M. tomentosa*, and the numbers were similar for both morphs of *M. villosa* (Table 7). The seed/ovule ratio or seed set was significantly higher in pin than in thrum morphs of *M. caracasana* and *M. parvifolia*. In contrast,

the seed/ovule ratios were similar in *M. tomentosa* and *M. villosa* (Table 7). The value of seed set was higher in monomorphic species than in distylous species (Table 7). Seed abortion was low in the species examined, with the highest values in *M. nodiflora*, and only differed between morphs of *M. villosa*. Relative fecundity ranged from 12.8% to 87.8% (Table 7). Fecundity was significantly higher in pin than in thrum morphs of *M. caracasana* and *M. parvifolia*, and was similar between morphs of each of the two distylous species (Table 7).

### Significant variation in morphological and functional traits

Most of the traits differing significantly between morphs of each distylous species tended to be higher in pin than in thrum morphs of *M. caracasana* and *M. parvifolia*. Of 14 traits, 11 (78.6%) were higher in pin than in thrum morphs of

**Table 5** Pollen production and pollen/ovule ratio in seven *Melochia* species and statistical comparisons between morphs of each distylous species, and between monomorphic and dimorphic species

| *Melochia* species | Stamens per flower | | Pollen grains per anther | Pollen grains per flower | Pollen/ovule ratio |
|---|---|---|---|---|---|
| | Mean (SD) | Sample size | | | |
| Monomorphic | | | | | |
| *M. crenata* | 5.0 (0.0) | 40 | 2,898.3 | 14,941.6 | 1,494.2 |
| *M. nodiflora* | 5.0 (0.0) | 106 | 1,120.0 | 5,600.0 | 635.6 |
| *M. pyramidata* var. *pyramidata* | 5.1 (0.4) | 105 | 1,180.4 | 6,020.1 | 608.1 |
| Mean (SD) | 5.00 (0.0) | | 1,732.8 (1,009.8) | 8,853.9 (5,276.3) | 912.6 (503.8) |
| Dimorphic | | | | | |
| *M. caracasana* | | | | | |
| Pin | 5.0 (0.0) | 100 | 1,880.0 | 9,400.0 | 969.1 |
| Thrum | 5.0 (0.0) | 100 | 1,666.7 | 8.333.4 | 896.1 |
| Statistical test | 0.0[a] (n.s.) | | 0.02[b] (n.s.) | 0.10[b] (n.s.) | 0.01[b] (n.s) |
| *M. parvifolia* | | | | | |
| Pin | 5.0 (0.0) | 102 | 1,323.3 | 6,616.6 | 661.7 |
| Thrum | 5.0 (0.0) | 100 | 1,771.7 | 8,858.4 | 885.8 |
| Statistical test | 0.0[a] (n.s.) | | 0.23[b] (n.s.) | 0.48[b] (n.s.) | 0.14[b] (n.s) |
| *M. tomentosa* | | | | | |
| Pin | 4.9 (0.1) | 89 | 1,320.0 | 5,784.1 | 584.3 |
| Thrum | 4.9 (0.3) | 84 | 1,195.0 | 5,855.5 | 629.6 |
| Statistical test | 0.0[a] (n.s.) | | 0.01[b] (n.s.) | 0.00[b] (n.s.) | 0.01[b] (n.s) |
| *M. villosa* var. *villosa* | | | | | |
| Pin | 5.0 (0.0) | 78 | 1,564.6 | 7,822.9 | 899.2 |
| Thrum | 5.0 (0.0) | 89 | 1,231.6 | 6,158.2 | 691.9 |
| Statistical test | 0.0[a] (n.s.) | | 0.11[b] (n.s.) | 0.30[b] (n.s.) | 0.14[b] (n.s) |
| Mean (SD) | 5.0 (0.0) | | 1,494.1 (261.5) | 7,353.6 (1,429.9) | 777.2 (149.9) |
| Monomorphic–dimorphic comparison $F_{(1,9)}$ | 0.00 (n.s.) | | 0.23 (n.s.) | 0.39 (n.s.) | 0.34 (n.s.) |

*n.s.* not significant

[a]  *t* test

[b]  Chi square test

M. caracasana, and of 9 traits, 8 (88.9%) were higher in pin than in thrum morphs of *M. parvifolia* (Tables 2, 3, 6, 7). In contrast, the proportion of traits significantly different between pin and thrum morphs were in a similar proportion in *M. tomentosa* (three of six, 50.0%) and *M. villosa* (five of nine, 55.5%).

The classification of four distylous species based on the pin/thrum ratio of 25 characters examined (Tables 2, 5, 6 and 7, excluding ratios, fruit/flower by hand self-pollination, seed/ovule by hand self-pollination, fruit/flower by legitimate cross-pollination, seed/ovule by legitimate cross-pollination, and pin/thrum individual) show *M. parvifolia* to be the most different species followed by *M. caracasana*. *M. tomentosa* and *M. villosa* were close because most of the reproductive and functional characters occurred in a similar or complementary expression in pin and thrum morphs (Fig. 1a).

Pollination

*Melochia* species are pollinated by a great diversity of insects, which allows their classification as generalist pollination systems (Table 8). However, the relative abundance of each group or class of insect varies according plant species. Dipterans as pollinators were scarce or absent, and wasps and butterflies were relatively important groups of pollinators for *M. caracasana* and *M. parvifolia*. In contrast, bees represented the most abundant group of pollinators for *M. tomentosa*, and less abundant for the other two distylous species (Table 8). Cluster analysis of plant species according to the number of pollinator species of bees, wasps, flies, butterflies and sphingids indicated that *M. caracasana* and *M. parvifolia* had very similar pollination systems (Fig. 1b). *M. villosa* was related to these species because its pollination system is represented by a

**Table 6** Biomass allocation to flower and floral parts of seven *Melochia* species and statistical comparisons between morphs of each distylous species, and between monomorphic and dimorphic species *Melochia* species

| *Melochia* species | Number | Calyx and receptacle | Corolla | Androecium | Gynoecium | Flower | Androecium/ gynoecium ratio | Reproductive/ vegetative ratio |
|---|---|---|---|---|---|---|---|---|
| Monomorphic | | | | | | | | |
| *M. crenata* | 8 | 1.73 (0.32) | 1.22 (0.08) | 0.28 (0.06) | 0.39 (0.06) | 3.62 (0.40) | 0.72 | 0.23 |
| *M. nodiflora* | 22 | 0.39 (0.13) | 0.21 (0.07) | 0.09 (0.03) | 0.33 (0.14) | 1.01 (0.26) | 0.27 | 0.70 |
| *M. pyramidata* var. *pyramidata* | 10 | 0.46 (0.30) | 0.35 (0.06) | 0.12 (0.03) | 0.18 (0.03) | 1.11 (0.32) | 0.67 | 0.37 |
| Mean (SD) | | 0.86 (0.75) | 0.59 (0.55) | 0.16 (0.10) | 0.30 (0.11) | 1.91 (1.48) | 0.55 (0.25) | 0.43 (0.24) |
| Dimorphic | | | | | | | | |
| *M. caracasana* | | | | | | | | |
| Pin | 10 | 2.24 (0.20) | 1.55 (0.09) | 0.60 (0.07) | 0.49 (0.60) | 4.47 (0.33) | 1.22 | 0.28 |
| Thrum | 10 | 1.66 (0.19) | 1.05 (0.22) | 0.62 (0.05) | 0.34 (0.06) | 3.67 (0.40) | 1.82 | 0.35 |
| Statistical test (*t* test, df 18) | | 6.87*** | 5.72*** | 0.07 (n.s.) | 0.15 (n.s.) | 4.65*** | | |
| *M. parvifolia* | | | | | | | | |
| Pin | 10 | 1.00 (0.12) | 0.87 (0.19) | 0.40 (0.06) | 0.29 (0.04) | 2.58 (0.41) | 1.38 | 0.36 |
| Thrum | 10 | 0.93 (0.09) | 0.74 (0.07) | 0.71 (0.07) | 0.21 (0.02) | 2.59 (0.25) | 3.38 | 0.55 |
| Statistical test (*t* test, df 18) | | 1.18 (n.s.) | 2.03 (n.s.) | 7.23*** | 6.86*** | 0.07 (n.s.) | | |
| *M. tomentosa* | | | | | | | | |
| Pin | 10 | 1.12 (0.16) | 1.19 (0.29) | 0.33 (0.07) | 0.33 (0.05) | 2.97 (0.55) | 1.00 | 0.28 |
| Thrum | 10 | 1.16 (0.12) | 1.29 (0.02) | 0.72 (0.09) | 0.31 (0.03) | 3.49 (0.35) | 2.32 | 0.42 |
| Statistical test | | 0.63 (n.s.) | 1.03 (n.s.) | 8.31*** | 0.83 (n.s.) | 2.52* | | |
| *M. villosa* var. *villosa* | | | | | | | | |
| Pin | 10 | 0.52 (0.05) | 0.74 (0.13) | 0.23 (0.04) | 0.17 (0.05) | 1.67 (0.21) | 1.35 | 0.32 |
| Thrum | 10 | 0.57 (0.12) | 0.43 (0.12) | 0.30 (0.07) | 0.15 (0.03) | 1.45 (0.25) | 2.00 | 0.45 |
| Statistical test (*t* test, df 18) | | 2.88** | 2.19* | 2.75* | 1.08 (n.s.) | 2.13* | | |
| Mean (SD) | | 1.15 (0.57) | 0.98 (0.36) | 0.49 (0.19) | 0.28 (0.11) | 2.86 (1.01) | 1.81 (0.77) | 0.38 (0.09) |
| Monomorphic–dimorphic comparison $F_{(1,9)}$ | | 0.65 (n.s.) | 2.37 (n.s.) | 8.06* | 0.04 (n.s.) | 1.89 (n.s.) | 10.37** | 0.27 (n.s.) |

The data are presented as mean (SD) milligrams

*n.s.* no significant

$*P < 0.05$, $**P < 0.01$, $***P < 0.0001$

**Table 7** Flower, fruit and seed production, and fecundity in seven *Melochia* species and statistical comparisons between morphs of distylous species and between monomorphic and dimorphic species

| *Melochia* species | Flower/inflorescence | | Fruit/infrutescence | | Fruit set (%) | Seed/fruit | | Ovule/flower | | Seed set (%) | Aborted seed/fruit | | Fecundity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Sample size | Mean (SD) | Sample size | | Mean (SD) | Sample size | Mean (SD) | Sample size | | Mean (SD) | Sample size | |
| **Monomorphic** | | | | | | | | | | | | | |
| *M. crenata* | 4.9 (1.6) | 32 | 2.3 (1.3) | 31 | 46.9 | 8.7 (1.0) | 26 | 10.0 (0.0) | 30 | 87.0 | 0.11 (0.3) | 26 | 40.3 |
| *M. nodiflora* | 9.4 (3.4) | 47 | 5.2 (2.2) | 103 | 55.3 | 5.5 (4.9) | 98 | 8.8 (2.1) | 107 | 62.5 | 1.37 (1.2) | 98 | 25.9 |
| *M. pyramidata* var. *pyramidata* | 3.6 (0.9) | 143 | 3.2 (1.0) | 157 | 88.8 | 9.8 (1.4) | 108 | 9.9 (0.2) | 105 | 98.9 | 0.02 (0.1) | 108 | 87.8 |
| Mean (SD) | 5.9 (3.0) | | 3.6 (1.5) | | 63.7 (22.2) | 8.0 (2.2) | | 9.6 (0.7) | | 82.8 (18.6) | 0.5 (0.7) | | 51.3 (32.4) |
| **Dimorphic** | | | | | | | | | | | | | |
| *M. caracasana* | | | | | | | | | | | | | |
| Pin | 17.0 (6.9) | 100 | 10.2 (4.2) | 100 | 60.0 | 5.9 (2.5) | 104 | 9.7 (0.6) | 100 | 60.8 | 0.97 (1.2) | 104 | 30.5 |
| Thrum | 21.5 (8.3) | 79 | 8.7 (4.6) | 103 | 40.5 | 4.6 (2.1) | 101 | 9.3 (0.6) | 100 | 49.4 | 0.89 (1.1) | 101 | 16.1 |
| Statistical tests | 3.98***[a] | | 2.42*[a] | | 41.9**[b] | 3.97**[a] | | 4.53**[a] | | 6.40*[b] | 0.49 (n.s.)[a] | | 6.05*[b] |
| *M. parvifolia* | | | | | | | | | | | | | |
| Pin | 12.7 (5.4) | 95 | 8.6 (7.9) | 105 | 67.7 | 4.9 (0.3) | 61 | 10.0 (0.0) | 102 | 49.0 | 0.00 (0.0) | 61 | 33.2 |
| Thrum | 13.3 (3.8) | 80 | 7.0 (4.6) | 109 | 52.6 | 2.9 (1.5) | 134 | 10.0 (0.0) | 100 | 29.0 | 0.05 (0.2) | 134 | 15.0 |
| Statistical tests | 0.83 (n.s.)[a] | | 1.82 (n.s.)[a] | | 10.0***[b] | 4.20****[a] | | 0.00 (n.s.)[a] | | 28.95****[b] | 1.39 (n.s.)[a] | | 6.29*[b] |
| *M. tomentosa* | | | | | | | | | | | | | |
| Pin | 6.7 (2.9) | 52 | 4.2 (2.2) | 98 | 62.7 | 7.3 (1.8) | 93 | 9.9 (0.5) | 91 | 73.7 | 0.00 (0.0) | 93 | 46.2 |
| Thrum | 10.3 (6.4) | 50 | 4.2 (2.2) | 154 | 40.8 | 6.7 (1.5) | 89 | 9.3 (0.8) | 102 | 72.0 | 0.00 (0.0) | 89 | 40.8 |
| Statistical tests | 4.30****[a] | | 0.00 (n.s.)[a] | | 2.65***[b] | 2.44*[a] | | 4.54****[a] | | 0.34 (n.s.)[b] | 0.00 (n.s.)[a] | | 1.99 (n.s.)[b] |
| *M. villosa* var. *villosa* | | | | | | | | | | | | | |
| Pin | 54.2 (29.9) | 79 | 16.3 (9.9) | 79 | 30.1 | 3.9 (1.3) | 57 | 8.7 (0.9) | 103 | 44.8 | 0.21 (0.5) | 57 | 12.8 |
| Thrum | 52.1 (35.9) | 23 | 18.9 (12.8) | 23 | 36.3 | 4.2 (0.9) | 81 | 8.9 (1.1) | 93 | 47.2 | 0.23 (0.6) | 81 | 16.2 |
| Statistical tests | 0.28 (n.s.)[a] | | 1.03 (n.s.)[a] | | 8.38***[b] | 1.16 (n.s.)[a] | | 1.40 (n.s.)[a] | | 0.20 (n.s.)[b] | 2.54*[b] | | 1.43 (n.s.)[b] |
| Mean (SD) | 23.5 (18.8) | | 9.8 (5.3) | | 48.8 (13.7) | 5.0 (1.5) | | 9.5 (0.5) | | 53.2 (14.9) | 29.4 (40.4) | | 26.3 (13.3) |
| Monomorphic–dimorphic comparison $F_{(1,9)}$ | 3.89 (n.s.) | | 5.15* | | 2.06 (n.s.) | 6.73* | | 0.06 (n.s.) | | 8.58** | 0.27 (n.s.) | | 3.84 (n.s) |

*n.s.* no significant

[a] *t* test

[b] Chi square test

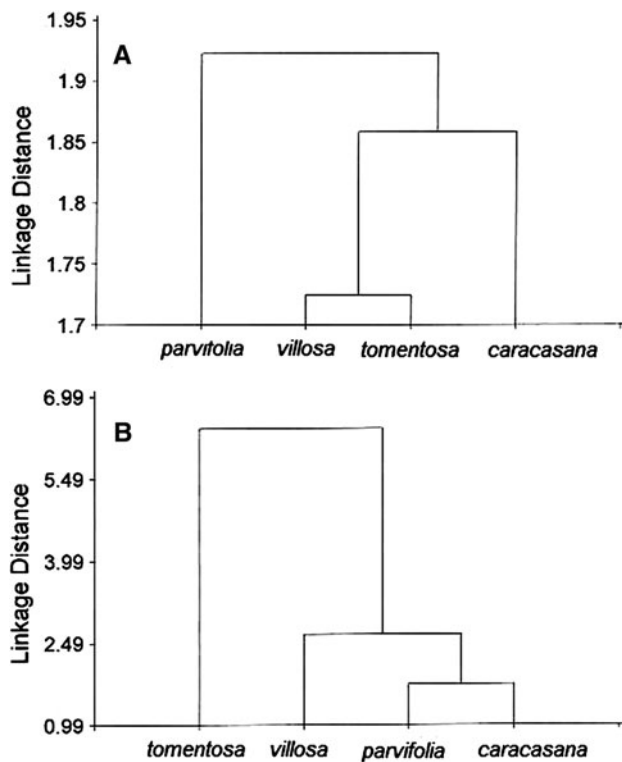$*P < 0.05$, $**P < 0.002$, $***P < 0.005$, $****P < 0.0001$

Fig. 1 Cluster analyses of distylous species of *Melochia* using as grouping variables (**a**) the pin/thrum ratios of all characters evaluated and (**b**) the number of pollinator groups (bees, wasps, flies, butterflies, and sphingids) pollinating each plant species

few species of butterflies (Ramírez and Brito, 1992). In contrast, *M. tomentosa* is predominantly pollinated by bees, and is therefore classified as a different species (Fig. 1b).

## Discussion

### Levels of self-incompatibility

More fruits and seeds were produced after legitimate cross-pollination than after hand self-pollination of *Melochia* species, which indicates that the three distylous species are self-incompatible, as recorded previously for *M. villosa* and *M. tomentosa* (Martin, 1967; Ramírez and Brito 1990; Machado and Sazima 2008). Moreover, fruit and seed set from pin × thrum and thrum × pin crosses did not differ significantly in any of the distylous *Melochia* species, indicating the complementarity of the two morphs for reproductive success. Under the self-incompatibility system, populations of distylous species are at equilibrium (Teixeira and Machado 2004a; Arroyo et al. 2002; Coelho and Barbosa 2004; Rossi et al. 2005; and many others). The nonsignificant variation between pin:thrum plant frequency in *Melochia* species seem to be correlated with the level of

self-incompatibility. However, hand self-pollination and illegitimate cross-pollination among pin individuals of *M. parvifolia* and among thrum individuals of *M. caracasana* produced some fruits and seeds, suggesting incipient steps toward self-compatibility, which could be associated with deviation from the expected frequency at equilibrium in *M. caracasana, M. parvifolia* and *M. villosa*.

The population of *M. pyramidata* studied was homomorphic. However, Martin (1967) reported homomorphic and distylous populations of *M. pyramidata* in Puerto Rico. On the basis of such evidence, homostyly and self-compatibility in *M. pyramidata* and *M. nodiflora* may originate in a distylous self-incompatible ancestor, as shown in some studies of homostylous self-compatible species (Coelho and Barbosa 2003). Breakdown in heterostylous taxa may results in the production of homostylous plants (Sobrevila et al. 1983), which frequently occur by a crossover that combines the style length and self-compatibility group of one form, such as pin, with the stamen length and pollen compatibility group of the opposite form (Baker 1966).

### Floral size and herkogamy

All self-incompatible distylous *Melochia* have flowers larger than their counterpart self-compatible monomorphic species. Self-compatibility recorded in *M. pyramidata* and in *M. nodiflora* (Martin 1967) is associated with the smallest flower lengths. In contrast, outcrossers may favour a larger attractive structure for individual flowers to attract more pollinators (Tomimatsu and Ohara 2006), and consequently enhance pollination success in self-incompatible distylous and homomorphic partial self-compatible flowers of *Melochia*.

Morphometric differences have been frequently recorded between floral morphs of distylous species (Hernández and Ornelas 2003; Teixeira and Machado 2004a, 2004b), including *Melochia* species (Martin 1967; Machado and Sazima 2008). In our study, flower sizes were similar between morphs of each distylous species, except in *M. caracasana* and *M. villosa*. Differences between pin and thrum flower sizes have been considered as complementary to floral dimorphism (Hernández and Ornelas 2003), and contribute to the functionality of the distylous system (Ganders 1979). The longest thrum flowers could be related to a shorter separation between anthers and stigmas of *M. caracasana*. Similarly, the longest pin flowers exhibited the shortest separation between anthers and stigmas in *M. villosa*. Both cases suggest that the longest flowers in distylous species may have shorter separation between anthers and stigmas, which could have certain consequences on the levels of self-compatibility. The longest morphs may increase pollen donation (thrum) or pollen receipt (pin).

**Table 8** *Melochia* species, visitor species, pollen load, and characterization of pollination system

| *Melochia* species | Visitors | | | Pollen load | Abundance | Visitor activity | Pollination system (specialization) |
|---|---|---|---|---|---|---|---|
| | Order | Family | Species | Position | | | |
| *M. caracasana* | Diptera | Syrphidae | *Ornidia obesa* | Head, proboscis and/or buccal parts | Scarce | Pollinator | Fly, bee, wasp, butterfly (generalist) |
| | Hymenoptera | Apidae | *Apis mellifera* | Head, proboscis and/or buccal parts, legs, ventral (thorax, abdomen) | Abundant | Pollinator | |
| | | Halictidae sp. | *Trigona fascipennis* | Legs | Scarce | Visitor | |
| | | Megachilidae | *Megachile* sp. | Legs | Scarce | Visitor | |
| | | Pompilidae | *Pepsis vitripennis* | Legs | Abundant | Pollinator | |
| | | | *Pepsis* sp. | Head, ventral (thorax) | Abundant | Pollinator | |
| | | | | Head, ventral (thorax) | Scarce | Pollinator | |
| | Lepidoptera | Hesperiidae | *Antigonus erosus* | —a | | Visitor | |
| | | | *Chiomara asychis* | —a | | Visitor | |
| | | | *Typhedanus undulatus* | —a | | Visitor | |
| | | | *Urbanus pronus* | —a | | Visitor | |
| | | | *Zopyrion satyrina* | —a | | Visitor | |
| | | Nymphalidae | *Nestra cana* | Proboscis and/or buccal parts | Abundant | Pollinator | |
| | | Papilionidae | *Papilio praeon thrason* | Proboscis and/or buccal parts | Scarce | Visitor | |
| | | Pieridae | *Ascia monuste* | Proboscis and/or buccal parts | Scarce | Visitor | |
| | | Pyralidae | *Syngamia florella* | Proboscis and/or buccal parts | Scarce | Visitor | |
| *M. parvifolia* | Hymenoptera | Apidae | *Apis mellifera* | Proboscis and/or buccal parts, legs, ventral (thorax, abdomen) | Abundant | Pollinator | Bee, wasp, sphingid (generalist) |
| | | Sphecidae | *Ammophilla gracilis* | Legs, ventral (abdomen) | Scarce | Pollinator | |
| | | | *Prionix thomae* | Head, legs, ventral (thorax) | Abundant | Pollinator | |
| | | Vespidae | *Polybia sericea* | Ventral (thorax, abdomen) | Scarce | Pollinator | |
| | Lepidoptera | Hesperiidae | *Cogia calchas* | —a | | Visitor | |
| | | | *Pyrgus oileus orcus* | —a | | Visitor | |
| | | Sphingidae | *Aellopus clavipes* | Proboscis and/or buccal parts | Abundant | Pollinator | |
| | | | *Aellopus titan* | Proboscis and/or buccal parts | Abundant | Pollinator | |
| *M. tomentosa* | Diptera | Bombyliidae | *Heterostylum hirsutum* | Proboscis and/or buccal parts | Abundant | Pollinator | Fly, bee (generalist) |
| | | | | Ventral (thorax, abdomen) | Scarce | Pollinator | |
| | Hymenoptera | Anthophoridae | *Centris (Centris) niveofasciata* | Ventral (thorax) | Scarce | Pollinator | |
| | | | *Centris (Centris) poecila* | Proboscis and/or buccal parts, ventral (thorax) | Abundant | Pollinator | |
| | | | *Centris (Hemisiella) lanipes* | Legs, ventral (thorax) | Scarce | Pollinator | |
| | | | *Centris (Hemisiella) trigonoides* | Legs, ventral (thorax, abdomen) | Scarce | Pollinator | |
| | | | *Xylocopa fimbriata* | Head, ventral (thorax) | Abundant | Pollinator | |
| | | | *Xylocopa* sp. | Legs, proboscis and/or buccal parts, ventral (thorax) | Abundant | Pollinator | |
| | | Megachilidae | *Megachile* sp. | Legs, ventral (thorax, abdomen) | Abundant | Pollinator | |
| | Lepidoptera | Pieridae | *Ascia monuste* | —a | | Visitor | |

a Without pollen load, visitor

The fact that there is no significant difference in the distance between stigmas and anthers between floral morphs seems to indicate a reciprocal herkogamy (Vuilleumier 1967; Ganders 1979; Barrett 1992). The largest herkogamy in pin morphs of *M. parvifolia* and *M. caracasana* may also indicate high variation in assortative pollen flow, and consequently could affect morph frequency (Arroyo et al. 2002; Pérez et al. 2003; Coelho and Barbosa 2004). Furthermore, nonherkogamous species of *Melochia* indicates a relationship with the levels of self-compatibility: *M. pyramidata* is autogamous self-compatible, *M. crenata* is partially self-compatible (Lemus-Jiménez and Ramírez 2005), and *M. nodiflora* is self-compatible (Martin 1967).

Pollen/ovule ratio

Self-incompatible distylous and self-compatible autogamous species exhibited pollen/ovule ratios ranged from 608 to 1,494, where *M. crenata*, which is partially self-compatible, has the largest pollen/ovule ratio. Pollen/ovule ratios for self-incompatible and self-compatible *Melochia* species correspond to facultative xenogamous species according Cruden (1977). Although Cruden (2000) recognized that there is tremendous variation in pollen/ovule ratios among species with equivalent breeding systems, low variation in pollen/ovule ratios may occur in plant species with diverse breeding systems, such as genera predominantly distylous (Coelho and Barbosa, 2003). The similarity in pollen/ovule ratios across *Melochia* species could be determined primarily by their taxonomic affinity, together morphological and ecological attributes, such as floral morphology, and animal pollination; and may also suggest that homostylous species have their origin in distyly, the comparatively high values of pollen/ovule ratios in homostylous self-compatible species being an indicator of a retained ancestral trait.

There are some variations in pollen/ovule ratios, which could be associated with a certain level of self-compatibility in morphs of distylous species. The pollen/ovule ratio was lower in thrum than in pin individuals of *M. caracasana* and *M. villosa*; the opposite was found in *M. parvifolia* and *M. tomentosa*. In the first case, some seeds were produced via self-pollination in thrum morphs of *M. caracasana*. In the second case, there was significant fruit and seed set via self-pollination in pin morphs of *M. parvifolia*. These trends are primarily associated with variations in the number of pollen grains per anther because the number of stamens per flower and ovules per flower are homogeneously distributed across *Melochia* species. Furthermore, self-compatibility and the pollen/ovule ratio between morphs may indicate light changes synchronized between morphs of distylous species, where male investment decreases together with the occurrence of self-compatibility (Charlesworth and Charlesworth 1987; Lloyd 1987).

Biomass allocation to flowers

It is well known that resource investment per flower often decreases with increasing selfing (Ornduff 1969; Cruden and Lyon 1985; Sato and Yahara 1999). Dry weights of flowers, which are correlated with the biomass allocated to the corolla enhancing pollination attraction, were higher in pin than in thrum morphs of *M. caracasana* and *M. villosa* (Morgan 1992; Sakai 1993; Delph et al. 1996). In some cases the highest dry weight of flowers was associated with some level of selfing (i.e. the pin morph of *M. villosa*), but in the other cases the lowest dry weight of flowers was not associated with the level of selfing. The lowest biomass allocated to flowers of one of the two morphs tended to be related to the production of some fruits and seeds via hand self-pollination: the thrum morph of *M. caracasana* and *M. villosa*, and the pin morph of *M. parvifolia*. On the other hand, the highest biomass allocated to one of the two floral morphs was correlated with the absence of selfing and with biomass allocated to the perianthum, increasing attractiveness and therefore enhancing pollination.

Allocations to male and female functions will be unequal when the gain in the contribution to fitness differs for equal proportionate increases in allocation to the two different sex functions (Charnov 1979). Biomass allocation to the androecium tends to be significantly higher in thrum than in pin morphs, and for the gynoecium tends to be higher in pin than in thrum morphs across *Melochia* species, but being only statistically significant for *M. parvifolia*. These trends indicate maleness for thrum and femaleness for pin individuals. In this context, self-incompatible species are expected to allocate more resources to male than female function (Charlesworth and Charlesworth 1987; Lloyd 1987). The self-compatible pin morph of *M. parvifolia* allocates significantly more biomass to the gynoecium than the thrum morph; and the self-incompatible thrum morph allocates more biomass to the androecium than the pin morph. The large biomasses allocated to male function promote an androecium/gynoecium ratio higher than unity for distylous species. In contrast, in the monomorphic populations of *M. nodiflora*, *M. pyramidata* and *M. crenata*, androecium/gynoecium ratios were less than one, which is similar to autogamous species previously studied (e.g. Valerio and Ramírez 2003). Moreover, distylous self-compatible species exhibited significantly higher biomass allocated to the androecium than homomorphic self-compatible species, indicating that more biomass allocation to female than male function is associated with autogamy or some level of selfing, and confirms that selfing plants

allocate less resource to male than to female function (Charlesworth and Morgan 1991).

Allocation to attractive structure may be high in out-crossing species and is expected to be low in selfing populations (Charlesworth and Charlesworth 1987; Lloyd 1987; Tomimatsu and Ohara 2006). Furthermore, the reproductive/vegetative biomass ratio or reproductive effort of the flowers is less than one, which indicates that biomass allocation to flowers of *Melochia* species is biased towards nonsexual structures, attraction, support and protection of developing fruit. However, the reproductive/vegetative ratio does not seem to show any relationship with the level of selfing among *Melochia* species. Apparently, allocation to the calyx and corolla does not always respond similarly to an increase in selfing (Brunet 1992). Given that cross-pollination is necessary for reproductive success in both morphs, they should not differ in attributes that contribute to attracting visitors (Leege and Wolfe 2002). The highest reproductive effort of thrum flowers is primarily related to a significantly higher biomass allocated to the androecium of thrum than pin flowers. The highest sex allocation to thrum morphs, in addition, could contribute to an asymmetrical pollen transfer between floral morphs which could enhance the susceptibility of the pin morph to self-compatibility and the eventual transition toward a monomorphic population. Allocation to an attractive structure should also be high when pollinators are limiting and there is competition for animal visits (Charlesworth and Morgan 1991). Allocation to pollination attraction was found to be high in all *Melochia* species, irrespective of the level of self-compatibility, which indicates that pollination mediated by insects may be an important event for reproduction in *Melochia* species.

## Reproductive efficiency

Fruit set is frequently similar between pin and thrum individuals of distylous self-incompatible species at equilibrium (Coelho and Barbosa 2004; Sobrevila et al. 1983), which have been associated with symmetrical pollen flow (Coelho and Barbosa 2004). Fruit and seed set variation may be partially explained by pollinator attraction together the self-incompatibility hypothesis (Sutherland and Delph 1984). Fruit set variation between morphs of distylous species could be in part determined by the number of reproductive structures. Fruit set tends to increase when the number of flowers per inflorescence decreases (Ramírez and Berry 1995). The number of flowers per inflorescence was higher in thrum than in pin morphs in *M. caracasana* and *M. tomentosa*, which indicates more attraction to pollination (Sutherland and Delph 1984; Sutherland 1986). Furthermore, the pin morphs of *M. caracasana*, *M. parvifolia* and *M. tomentosa* have higher fruit set than thrum morphs,

probably related to the fact that self-pollination of out-crossed flowers can result in a significant reduction in seed set (Barrett et al. 1996; Arroyo et al. 2002), and indicates that pin morphs are biased toward female function. In addition, the largest values of seed/ovule ratio or seed set and seeds per fruit of monomorphic populations compared with distylous species are also probably associated with loss of the adverse effect of the self-incompatibility reaction in self-compatible species (Sutherland and Delph 1984; Sutherland 1986). The presence of self-compatibility and femaleness in biomass allocation to pin flowers could enhance high fruit set. Monteiro et al. (1991) found a larger fruit set in pin than in thrum morphs of *Psychotria barbiflora*, which was associated with a tentative female function.

## Pollination

*Melochia* species are pollinated by a great diversity of insects, which allow them to be classified as generalist pollination systems. The levels of generalization seem to be higher in *M. caracasana* and *M. parvifolia* (bee, wasp, butterfly and sphingid pollination) than in *M. tomentosa* (bee pollination) and *M. villosa* (butterfly pollination). Stone (1996) has shown that bees and long-tongued lepidopterans are comparatively more proficient for disassortative pollination, a condition for proper functioning of reciprocal herkogamy. Therefore, the pollination system seems to play an important role in the reciprocity of reproductive traits, and consequently on the isoplethic condition of the populations, operating as a selective force enhancing a self-compatible breeding system, as suggested previously by Barrett (1992). The most generalized pollination system in *M. caracasana* and *M. parvifolia* is correlated with morphology and the colour of the flowers. Open and short-tube flowers allow unspecialized visits of insects with short mouth parts, which is enhanced because the white flowers of *Melochia* may attract a large number of specialized and unspecialized visitor species. White flowers reflect all wavelengths in equal proportions and therefore may be seen by a large spectrum of visitors (Kevan 1978).

## Evolutionary trends

The relative variation between morphs in relation to plant frequency, herkogamy and fecundity, together the pollination system, suggest that *M. parvifolia* and *M. caracasana* are under selective forces as shown by an incipient variation in breeding system strategy. In this analysis, *M. caracasana* and *M. parvifolia* are biased towards the pin morph, representing a different evolutionary condition compared to *M. tomentosa* and *M. villosa*. In addition,

*M. caracasana* and *M. parvifolia* are similar according their pollination systems. However, trends in morphological and functional characters of pin and thrum morphs are different in these species, which suggests that particular traits of each species, may predispose to reciprocity between morphs, and consequently may enhance or constrain different evolutionary trends in natural populations of *Melochia*.

Fecundity and seed set were significantly higher in pin than in thrum morphs of *M. caracasana* and *M. parvifolia*. The largest values of fecundity for pin individuals of these species suggest an asymmetrical fitness between pin and thrum morphs. High fecundity in pin individuals indicated a higher female contribution to the population and could increase deviation from the equilibrium. Although both plant species exhibited an incipient anisoplethy, *M. parvifolia* was biased towards the pin morph and *M. caracasana* was biased towards the thrum morph. Self-compatibility of the pin morph of *M. parvifolia* could enhance a monomorphic population as in other species previously recorded (Sobrevila et al. 1983; but see Consolaro et al. 2005). This trend is supported by the following attributes: (1) short-lived species tend to be self-compatible (i.e. Ramírez and Brito 1990; Jaimes and Ramírez 1999) therefore allowing self-compatibility in distylous species; (2) high levels of fruit set in pin individuals may enhance female fitness; and (3) high levels of herkogamy in pin morphs avoids self-pollination and consequently inbreeding depression (Thomson and Brunet 1990; Barrett 1992; Dulbergers 1992).

*M. caracasana* exhibited different trends in morphological and functional traits, together with ecological features, which could be interpreted as an incipient step in the evolution to unisexuality from distyly. This is arguing as follows:

1. Generalist pollination system. Switch from distyly to dioecism is most likely the result of a form of pollinator-mediated selection for femaleness in the pin morph and maleness in the thrum morph (Beach and Bawa 1980; Bawa and Beach 1981). In addition, pollination processes may be disrupted in fragmented habitats (van Rossum et al. 2006) such as the *M. caracasana* habitat.

2. Self-pollination in thrum individuals enhanced by the position of the stigma below the stamens may produce inbreeding depression in female fitness, and separation of the sexes has evolved because it reduces inbreeding (Charlesworth and Charlesworth 1979; Bawa 1980; Freeman et al. 1997).

3. Thrum individuals occur in a higher numbers than pin individuals, as so male individuals frequently outnumber female in dioecious species (Ibarra-Manríquez and Oyama 1992; Oliveira 1996).

4. The higher number of flowers per inflorescence in thrum individuals increases male fitness as a result of flower excess and pollen donation (Sutherland and Delph 1984; Sutherland 1986). On the other hand, an increase in maternal reproductive investment or the maturation of more fruits should result in a disproportionate gain in female fitness (Givnish 1980). Pin morphs exhibited higher fecundity than thrum morphs, which could be considered as potentially female, or female fitness is higher in this morph. By contrast, thrum morphs seemed to be biased toward maleness.

5. Dioecy is frequently associated with woody species (Beach and Bawa 1980; Bawa and Beach 1981). The perennial habit of *M. caracasana* could be an important attribute predisposing to changes toward dioecy.

6. Small population size in fragmented habitats such as the remnant of secondary deciduous forest seems to create the opportunity for morph-specific fitness (van Rossum et al. 2006).

Finally, distyly in *Melochia* is characterized by instability in morph frequency, self-incompatibility, biomass allocation to floral parts and reproductive efficiency. Unspecialized and generalist pollination systems seem to be an important selective force enhancing deviation from the equilibrium in distylous species. Indirect evidence suggests that homomorphic species are derived from distylous ancestors. Deviation from the equilibrium of distylous species and the evolution of homostylous from distylous is also influenced by life history traits predisposing to variations in reproductive biology.

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