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# Relationship between herkogamy, incompatibility and reciprocity with pollen–ovule ratios in *Melochia* (Malvaceae)

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## Abstract

Heterostyly is a floral polymorphism that increases pollination efficiency by promoting cross-pollination and reducing pollen wastage. Efficiency in pollination has been related to plant investment in gamete production and to the pollen to ovule ratio (P/O), which has been proposed as an indication of the likelihood of enough pollen grains reaching the stigmas to result in maximum reproductive success. In heterostylous species, cross-pollination is promoted by the reciprocal position of sexual organs between morphs and a heteromorphic incompatibility system, which precludes selfing and fertilizations among plants of the same morph. Morphological features like reciprocity (between morphs) and herkogamy (within morph) together with the breeding system are thought to influence pollination quality. Therefore, a close relationship between the pollination efficiency, morphological characteristics, and incompatibility would be expected. Pollination treatments and morphological measurements were carried out to describe the breeding system, herkogamy, and reciprocity of six *Melochia* species. Afterward, the relation between the P/O (as a surrogate of the efficiency in pollination), and reciprocity, herkogamy and incompatibility was evaluated. Monomorphic *M. nodiflora* and distylous *M. pyramidata* are self-compatible species, whereas the rest of the species are self- and morph-incompatible. There was a positive relationship between the P/O value and the degree of herkogamy and incompatibility. However, P/O values appear to increase when higher reciprocity is found in the populations. As expected, the lower values of P/O are associated with lower levels of herkogamy and compatibility in the *Melochia* species studied. The relationship between the factors is discussed under different scenarios of the pollinators' predictability.

**Keywords:** *herkogamy, heterostyly, incompatibility system, pollen limitation, pollen/ovule ratio, reciprocity*

## Introduction

The breeding system of a species indicates the extent to which individual plants rely on pollinators for seed production, and it also determines the species' ability to expand its distribution or colonize new habitats (Baker 1955; Cruden 1977). The breeding system is the evolutionary strategy, at least partly under genetic control, which influences the number, type, and distribution of potential partners during sexual reproduction (Neal & Anderson 2005). Self-incompatibility may prevent the potentially deleterious consequences of inbreeding (Rea & Nasrallah 2008), whereas self-compatibility may be a strategy used to ensure reproduction when mate availability is low or pollinators are either unpredictable or inefficient (Baker 1955; Cruden 1977). Indeed, persistent pollen limitation caused by either low pollinator availability or inefficient pollinators may favor the evolution of self-compatibility when selfing offers reproductive assurance (Knight et al. 2005). However, boundaries between self-compatibility and incompatibility are not rigorous in nature and the species often display variation in the incompatibility system, which allows them to produce some fruits after selfing (Lloyd & Schoen 1992; Castro et al. 2013; Costa et al. 2014). For this reason, the breeding system is best considered a continuous rather than a discrete character of plant populations (Vogler & Kalisz 2001). To deal with these intermediate cases, the use of reproductive indices that express the degree of species compatibility by contrasting fruit production after selfing and outcrossing has been proposed (Lloyd & Schoen 1992).

Efficiency in pollination has traditionally been related to plant investment in gamete production since the publication of Cruden's work (Cruden 1977; Chouteau et al. 2006). In his study on the association of pollen to ovule ratio (P/O) with breeding system variation, Cruden (1977) suggested that P/Os reflect the likelihood of sufficient pollen grains

getting to the stigmas to produce the maximum reproductive output; a hypothesis which has been supported empirically (Schlindwein & Medeiros 2006; Gong & Huang 2014). According to Cruden (1977), the more efficient the transfer of compatible pollen in a population, the lower is the P/O value. In complex breeding systems such as heterostyly, there are two morphs that only produce fruits when cross-pollinations occur between them. Since pollen from the same morph is incompatible, it would be reasonable, in this case, to estimate the efficiency of a particular morph as the likelihood of sufficient pollen grains of the opposite morph reaching its stigma.

In heterostylous species, the efficiency of pollination would be determined by physiological mechanisms (incompatibility) and also by morphological characteristics, such as those explained in detail below. Heterostyly is a floral polymorphism in which populations present two (distyly) or three (tristyly) morphs that differ reciprocally in the placement of anthers and stigmas between them; In the case of distylous species (as is the case of taxa included in this study), one morph presents the stigma above the anthers (L-morph), whereas the other has the stigma below the anthers (S-morph) (Barrett 1992). This variation has been interpreted to promote cross-pollination between morphs (Darwin 1877) and reduce pollen wastage (Cesaro et al. 2004), hence increasing the efficiency of pollination. Reciprocal herkogamy is usually accompanied by a heteromorphic and diallelic incompatibility system, which precludes selfing and fertilizations among plants of the same morph (Barrett 1992). However, there are several cases where the self-incompatibility system is not present in heterostylous species for different reasons (e.g. Barrett & Harder 2005; Pérez-Barrales et al. 2006; Consolaro et al. 2011; Faria et al. 2012; Ferrero et al. 2012). For example, heteromorphic incompatibility is lost when populations of heterostylous species lack one morph and compatibility arises as a way of reproductive assurance (e.g. Schoen et al. 1997; Mast et al. 2006) in certain cases where morphs only differ in style length (as in stigma height polymorphic groups like species of *Narcissus* or *Lithodora*; Pérez et al. 2004; Ferrero et al. 2012), or due to shifts in pollinators (e.g. Hodgins & Barrett 2008; Pérez-Barrales & Arroyo 2010). This lack of association between heterostyly and the incompatibility system has been evidenced in an evolutionary context in several unrelated groups (Ferrero et al. 2012; Santos-Gally et al. 2013).

Besides, in heterostylous species, morphological features like reciprocity in the heights of stigmas and anthers between morphs and herkogamy may also influence the quality of pollination in a population. Reciprocity defines the level of accuracy in pollen transference between plants promoting inter-morph pollination (Lloyd & Webb 1992b), whereas herkogamy (i.e. the spatial separation between anthers and stigmas within flowers) determines the extent of sexual interference between male and female functions (Webb & Lloyd 1986). Both characteristics vary among the species and populations established in different habitats (Faife-Cabrera et al. 2014; Brys & Jacquemyn 2015) and would thus be expected to affect the levels of compatible pollen reaching the stigmas [i.e. pollination efficiency *sensu* Cruden (1977)].

The morphological variation in heterostylous species could be used to analyze how a particular sex organ arrangement influences the efficiency in pollination (calculated as the pollen of the opposite morph over the number of available ovules). If Cruden's prediction (1977) was right, heterostylous systems would be expected to have lower values of P/O when the efficiency in pollination is higher as a result of (1) increased self-compatibility; (2) decreased herkogamy, since it would facilitate autonomous selfing; and (3) increased reciprocity, because the reciprocal position of the sexual organs results in an accurate deposition of compatible pollen on stigmas through pollen segregation over the length of the pollinators' body.

However, additional explanations for variation in P/O values have been proposed in the literature. Charnov (1979, 1982) argued that the opportunity for reproductive success would determine the allocation ratio (see also Queller 1984). Thus, among-species variation in P/O could be visualized as an expression of different allocation optima depending on the breeding system and specific ecological conditions. Neither theory is mutually exclusive since, to increase male fitness, a species with an inefficient pollination system requires a greater investment in pollen than those species with more effective pollination systems (Mione & Anderson 1992; Götzenberger et al. 2006, 2008).

*Melochia* is a cosmopolitan genus from tropical and subtropical regions (Dorr & Barnett 1989), with many species occurring in degraded habitats, or defined as early successional species (Goldberg 1967). In Cuba, there are currently five distylous species and one monomorphic species, *M. nodiflora*. All heterostylous species differ in their degree of polymorphism. *Melochia pyramidata* has both dimorphic and monomorphic populations in Cuba. The highest reciprocity is found in populations of *M. parvifolia*, whereas some populations of *M. villosa* and *M. tomentosa* exhibit the lowest values. Populations of *M. pyramidata*, *M. tomentosa*, and *M. villosa* are the most variable in terms of reciprocity. All the species show differences in pollen size between morphs [i.e. pollen from the short styled morph (S-morph) is larger than pollen from the long-styled morph (L-morph)], but pollen production differs

Table I. Studied *Melochia* species, localities, floral size, herkogamy distance, reciprocity between morphs and results for the type of compatibility in populations surveyed in Cuba.

| Species | Locality | $N_m$ (L/S) | Floral size (mm) (L/S) | Herkogamy (L/S) | Reciprocity degree | $N_{pt}$ (L/S) | Compatibility |
|---|---|---|---|---|---|---|---|
| *Melochia nodiflora* | Presa Minerva, Villa Clara (22°26′ N, 79°48′ W) | 50 | 4.8 ± 0.13 | 1.1 ± 0.03 | Monomorphic | 12 | Self-compatible |
| *M. pyramidata* | Santa Clara, Villa Clara (22°26′ N, 79°48′ W) | 14/15 | 7.1 ± 0.09/7.5 ± 0.09 | 2.7 ± 0.02/0.9 ± 0.02 | 0.85 | 12/12 | Self-compatible |
| *M. savannarum* | Siguanea, Isla de la Juventud (21°37′ N, 82°57′ W) | 30/32 | 7.4 ± 0.14/7.8 ± 0.17 | 2.3 ± 0.04/0.9 (0.04) | 0.71 | 15/15 | Self- and morph-incompatible |
| *M. tomentosa* | Verraco, Santiago de Cuba (19°53′ N, 75°34′ W) | 39/23 | 9.4 ± 0.15/8.3 ± 0.11 | 3.2 ± 0.01/2.3 ± 0.02 | 0.72 | 14/14 | Self- and morph-incompatible |
| *M. villosa* | Aguada de Pasajeros, Cienfuegos (22°25′ N, 80°49′ W) | 18/24 | 10.8 ± 0.06/11.0 ± 0.11 | 3.3 ± 0.01/1.1 ± 0.02 | 0.85 | 15/15 | Self- and morph-incompatible |
| *M. parvifolia* | Cumanayagua, Cienfuegos (22° 07′ N, 80°12′ W) | 43/41 | 12.4 ± 0.10/12.5 ± 0.09 | 4.3 ± 0.02/2.9 ± 0.01 | 0.87 | 8/10 | Self- and morph-incompatible |

Notes: Values for floral size and herkogamy distance are given for each morph as mean ± standard deviation (data from Faife-Cabrera et al. 2014). Reciprocity degree values were calculated following Sánchez et al. (2013), and range from 0 to 1, with 0 being the lowest reciprocity and 1 the highest (meaning perfect reciprocity). $N_m$: sample size for morphological measurements per morph. $N_{pt}$: sample size in pollination experiments for each morph. Pollen supplementation and controls were performed in 14 plants of each population.

significantly between morphs only in *M. tomentosa* and *M. villosa* and corollas are significantly larger only in S-morph flowers of *M. savannarum* (Faife-Cabrera et al. 2014). These variations, together with empirical evidence of differences in the breeding systems, would suggest that *Melochia* is an ideal group to use when testing questions related to the influence of the degree of herkogamy, reciprocity, and self-compatibility on pollination efficiency. Therefore, the main objective of this study is to characterize the breeding system of the six species of genus *Melochia* present in Cuba, evaluating the relationship between the incompatibility system, morphological characteristics and P/O ratios (modified from Cruden's (1977) proposal as a proxy of pollination efficiency). In particular, our aim is to answer the following questions: (i) Do heterostylous *Melochia* species, differing in type of polymorphism, present a heteromorphic incompatibility system? We expect species showing less reciprocity to be more compatible (at least within morph) than those showing perfect reciprocity between morphs. (ii) To what extent are the study populations (of different species and morphotypes) suffering from pollen limitation? We predict that species showing greater levels of pollen limitation would be more compatible since selfing offers reproductive assurance. (iii) Is there a relationship between the P/O and reciprocity, herkogamy, and the expression of self-incompatibility? We expect to find species with lower values of P/O

when the efficiency in pollination is higher as a result of increases in self-compatibility, decreases in herkogamy, and/or increases in reciprocity.

## Materials and methods

### Study system

In this study, we focused on six *Melochia* species currently present in Cuba. We selected one population of each species where pollination treatments and morphological measurements were carried out. The populations surveyed were randomly selected. Population characteristics such as location, mean floral size, mean herkogamy distance, and degree of reciprocity (see *Morphological features* section) are shown in Table I.

### Pollination treatments

To determine the breeding system of *Melochia* species, we carried out experimental crosses in one population per species from June 2011 to May 2012 (Table I). For distylous species (*Melochia pyramidata* L., *M. tomentosa* L., *M. parvifolia* H.B.K., *M. villosa* (Mill.) Fawc. and Rendle, *M. savannarum* Britt.), the following pollination treatments were conducted: (1) compatible inter-morph pollinations: flowers were emasculated, bagged, and pollinated with

pollen from flowers of the opposite morph [L × S – pollen from the short-styled morph (S-morph) on stigmas of the long -tyled morph (L-morph), S × L – pollen from L-morph on stigmas of S-morph]; (2) incompatible intra-morph pollinations: flowers were emasculated, bagged and pollinated with pollen from flowers of the same morph (L × L and S × S); (3) hand self-pollinations: bagged flowers of each of the two floral morphs were pollinated with their own pollen; (4) spontaneous self-pollinations: flowers of each of the two floral morphs were bagged without manipulation; (5) pollen supplementation: flowers were left opened and pollinated with pollen from the opposite morph, and (6) control treatment: flowers were exposed to natural pollination. For inter and intra-morph pollinations, we used a mixture of pollen from flowers of several plants that had recently opened. For the monomorphic species, *M. nodiflora*, the following treatments were carried out (1) xenogamy: emasculated flowers were bagged and pollinated with pollen from other individuals (as an equivalent to inter-morph pollination in distylous taxa); (2) hand self-pollinations: bagged flowers were pollinated with their own pollen; (3) spontaneous self-pollinations: flowers were bagged without manipulation; (4) pollen supplementation: flowers were left opened and additionally hand pollinated with pollen from other individuals, and (5) control treatment. In order to avoid the intrinsic differences associated with the genetic and ecological features of a particular plant, we used a block design to carry out all the treatments on each individual. A total of 8–15 plants of each morph per population was used (see Table I for details). There was a minimum distance of 5–7 m between pollen donors and hand-pollinated flowers to prevent mating between close relatives. After 4–5 weeks, fruit and seed production were recorded and seed to ovule ratio (S/O) was calculated as a reproductive response. We calculated S/O as an indication of reproductive success because it reveals the proportion of ovules that are fertilized after pollination and allow comparisons among taxa.

Statistical comparisons between pollination treatments were achieved through generalized estimating equation (GEE) models using the S/O obtained from pollination treatments as an intra-subject variable and an "independent" correlation matrix structure. GEE is useful for illustrating comparisons of groups where the units are independent between groups (Quinn & Keough 2002). Two independent analyses were carried out for each population, one to determine the breeding system and the other to assess pollen limitation in the populations. In both cases, we considered morph and treatments as fixed factors. For the analysis of breeding systems, we used the following treatments: inter-morph, intra-morph, hand self-pollinations,

and spontaneous self-pollinations; pollen limitation was evaluated with the other two (i.e. control and pollen supplementation). For all distylous species, we considered the fixed factor morph with two levels (L-morph and S-morph). For *M. nodiflora*, only the comparisons among treatments were carried out. The S/O ratio was modeled as a binomial distribution, with "logit" used as the link function. Statistical analyses were performed with the SPSS software (IBM 2010).

### Reproductive indices

Several reproductive indices were estimated using the values of S/O obtained from pollination treatments (see above).

Self and morph compatibility quantification for each species was assessed through the self-compatibility index (SCI) of Lloyd and Schoen (1992) and the morph compatibility index (MCI) of Ferrero et al. (2012). SCI was calculated as the ratio between S/O from self-pollination and inter-morph crosses and MCI as the ratio between S/O from intra and inter-morph crosses. MCI was not calculated for the monomorphic species *M. nodiflora*. Lloyd and Schoen (1992) defined a threshold of 0.75 for SCI. Index values above 0.75 correspond to self-compatible species, while species with values between zero and 0.75 are considered self-incompatible. In this study the same criteria was applied to MCI. The percentage of pollination limitation (PPL) was calculated as: PPL = [100 (PS − C)]/PS, where PS is the S/O of pollen-supplemented flowers and C is the S/O of the control flowers (Jules & Rathcke 1999).

### Characterization of reciprocity, herkogamy and P/O

For the morphological characterization of each population, we first calculated the reciprocity index following Sánchez et al. (2008, modified in Sánchez et al. 2013). This index is based on comparisons of the position of every single stigma height in the population with each stamen height measurement calculated for each whorl. The index has proven to be useful in the quantitative characterization of sexual reciprocity between morphs in heterostylous populations (Sánchez et al. 2010; Ferrero et al. 2011a). Next, we calculated the mean value of herkogamy per morph as the mean distance between the stigma and anther heights in each flower. Thus, the herkogamy in the L-morph was calculated by subtracting the anther height from the height of the stigma and vice versa for the S-morph.

For each species, we determined the P/O per morph following a modification of Cruden (1977): the P/O of the S-morph was calculated as the number of pollen grains produced by the L-morph divided by the number of ovules produced by the S-morph. To

Table II. Results for the simplest GEE analysis of comparisons of S/O ratios obtained from pollination experiments in six *Melochia* species from Cuba.

| | Breeding system | | | | | | Pollen limitation | | | | | |
| | Morph | | | Treatment | | | Morph | | | Treatment | | |
| Species | df | Wald χ² | P | df | Wald χ² | P | df | Wald χ² | P | df | Wald χ² | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Melochia nodiflora* | | | | 3 | 2.763 | 0.430 | | | | 1 | 1.649 | 0.199 |
| *M. pyramidata* | 1 | 5.483 | **0.019** | 3 | 6.050 | 0.109 | 1 | 0.018 | 0.892 | 1 | 0.011 | 0.917 |
| *M. savannarum* | 1 | 0.161 | 0.688 | 3 | 69.185 | **<0.001** | 1 | 3.058 | 0.080 | 1 | 5.784 | **0.016** |
| *M. tomentosa* | 1 | 0.652 | 0.420 | | | | 1 | 0.105 | 0.746 | 1 | 10.022 | **0.002** |
| *M. villosa* | 1 | 7.343 | **0.007** | | | | 1 | 4.088 | **0.043** | 1 | 1.225 | 0.268 |
| *M. parvifolia* | 1 | 0.222 | 0.637 | | | | 1 | 0.328 | 0.567 | 1 | 0.282 | 0.595 |

Notes: Treatments to test the type of breeding system were analyzed separately from those used to test pollen limitation. No interactions between morph and treatment were significant so they were excluded from the models. For *M. nodiflora*, the monomorphic species, only the comparison among treatments was carried out. In *M. parvifolia*, *M. tomentosa*, and *M. villosa*, comparisons were not carried out because of the zero values of some treatments (see Figure 1). For each analysis, the degrees of freedom (df), value for the Wald chi-square statistic (Wald χ²), and probability value (P) are shown. Significant differences at α = 0.05 are highlighted in bold print.

do this, for each population, we counted the pollen grains in one anther per developed bud in 10 individuals of each morph. Each anther was set in a glycerine drop on a microscope slide. Pollen grains were counted under a light microscope following Castro et al. (2008) (see also Ferrero et al. 2011b). We estimated the pollen production per flower by multiplying the count values by the number of anthers per flower (i.e. five anthers in all species). The number of ovules was determined in all species using 10 floral buds of each morph per population. For this purpose, the ovaries were dissected and the ovules inside were counted visually under a stereoscopic microscope.

*Relationship between P/O, self-compatibility and herkogamy*

To test whether P/O values were related to self-incompatibility and herkogamy, we performed a generalized linear model (GLZ) with herkogamy and SCI (of each morph and species) as fixed factors using a "Poisson" distribution with a "log" link function. Since the L-morph and S-morph might produce a different number of pollen grains (and thus generate differences in the P/O ratios, given that the number of ovules is constant), we carried out one independent analysis for each morph. Then, we analyzed the reciprocity independently because the reciprocity index results in a single value for each population. For this reason, we considered the mean values of P/O in the populations and a similar GLZ (like the one described previously) was carried out using reciprocity as a fixed factor.

**Results**

*Pollination treatments*

The results of the comparisons between pollination treatments are shown in Table II (see also additional

data in Appendix 1). Significant differences among treatments in the seed/ovule (S/O) were found for all dimorphic species except *M. pyramidata*. In this species, seeds were also found after spontaneous self-pollination treatment. In all of them, S/O values from pollinations between morphs were greater than for selfing and intra-morph crosses (Figure 1), which means that all of them showed some kind of heteromorphic incompatibility. In *M. parvifolia*, *M. tomentosa*, and *M. villosa*, statistical comparisons were not possible because of zero values in some treatments; nevertheless, differences between treatments are also given in Figure 1. No significant differences were found between treatments in *M. nodiflora*, the monomorphic species, either. We found significant differences between morphs in two species, *M. pyramidata* and *M. villosa* (Table II). Both species present higher S/O ratios in S-morph flowers.

Comparisons between supplemented pollinations and control treatments differed significantly for *M. savannarum* and *M. tomentosa* revealing pollen limitation in those populations (Table II). For *M. villosa*, no significant differences were found between the control and the supplemented pollination; however, there were differences in the S/O ratio between morphs. S/O ratios were, like in the other pollination treatments, greater in S-morph flowers of this species.

*Reproductive indices*

Values for the self and morph compatibility indices are shown in Table III. The values of the SCI were greater than 0.75 for *M. nodiflora* and *M. pyramidata*, which suggests that these two species are self-compatible. In *M. pyramidata*, the SCI values were 0.54 and 0.77 in the L- and S-morph respectively. According to these results, S-morph appears to be capable of producing more seeds than the L-morph
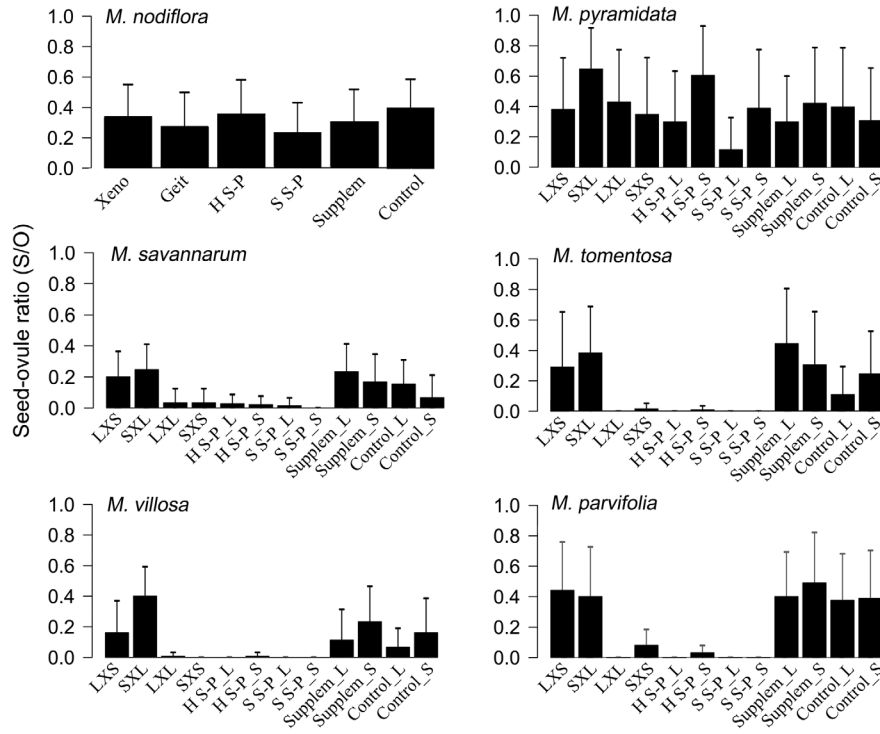
Figure 1. Mean ± standard deviation of S/O after hand-pollination treatments in *Melochia* species. Treatments: Inter-morph pollinations (L × S, S × L) – L × S – pollen from S-morph on L-morph stigmas, S × L – pollen from L-morph on S-morph stigmas; Intra-morph pollinations (L × L, S × S); Hand self-pollination (H S-P); Spontaneous self-pollination (S S-P); Supplementation (Supplem); Control (natural pollination). When differences between morphs were found, treatments were separated in the figure. L-morph (L) and S-morph (S) are indicated at the end of the treatment nomenclature. Additional treatment for monomorphic *M. nodiflora* was xenogamy (Xeno).

Table III. Results of the self-compatibility index (SCI, Lloyd & Schoen 1992), morph-compatibility index (MCI, Ferrero et al. 2012), and percentage of pollination limitation (PPL, Jules & Rathcke 1999) based on the values of S/O obtained from pollination treatments (see methods).

| Species | SCI | | MCI | | PPL | |
|---|---|---|---|---|---|---|
| | L | S | L | S | L | S |
| *Melochia nodiflora* | **0.87** | | | | −29.55 | |
| *M. pyramidata* | 0.54 | **0.77** | **1.13** | 0.54 | −33.33 | 27.45 |
| *M. savannarum* | 0.01 | 0.04 | 0.17 | 0.14 | 44.46 | 55.57 |
| *M. tomentosa* | 0.00 | 0.01 | 0.00 | 0.04 | 76.56 | 19.05 |
| *M. villosa* | 0.00 | 0.01 | 0.04 | 0.00 | 41.18 | 31.43 |
| *M. parvifolia* | 0.00 | 0.04 | 0.00 | 0.20 | 20.45 | 6.25 |

Notes: Negative values of PPL mean that the S/O in the control treatment is higher than in the supplemented one. For SCI and MCI, bold numbers represent values above 0.75 and correspond to self-compatible species. For *Melochia nodiflora*, MCI was not calculated. SCI = S/O from self-pollination/S/O from inter-morph crosses. MCI = S/O from intramorph/S/O from inter-morph crosses. PPI = [100 (PS − C)]/PS; where PS: S/O of pollen-supplemented flowers; C: S/O of control flowers.

after self-pollination. Specifically for *M. villosa*, the SCI value is close to zero since L-morph flowers produced no fruits after self-pollination, and S-morph

flowers produced just a few seeds (Table III). The low values of the SCI in *M. savannarum, M tomentosa, M. villosa*, and *M. parviflora* are due to flowers that produced seeds after cross-pollination, but not after self-pollination (see Figure 1).

We found similar patterns for the MCI values. *M. pyramidata* presented values of MCI above 0.75. For *M. villosa*, which showed statistical differences between morphs, few fruits were produced after the intra-morph pollination of L-morph flowers, but no fruits and seeds were produced for S-morphs flowers. The low values of MCI in *M. savannarum, M tomentosa, M. villosa*, and *M. parviflora* indicate that they are morph-incompatible species.

Values of PPL are also summarized in Table III. We found the highest values (≥44%) for *M. savannarum* and *M. tomentosa*. For *M. villosa* (the only species for which differences were found between morphs), L-morph flowers presented greater pollen limitation (higher PPL values) than S-morph flowers (Table III). *M. nodiflora, M. pyramidata*, and *M. parvifolia* exhibited no pollen limitation in the populations studied (the PPL values of these species are even negative, which means that the S/O in the control treatment is higher than in the supplemented one. However, they are not statistically different; see the "Pollination treatments" section).

Table IV. P/O ± standard deviation in populations of six *Melochia* species.

| Species | P/O ratio | |
|---|---|---|
| | L | S |
| *M. nodiflora* | 154.3 ± 42.2 | |
| *M. pyramidata* | 359.1 ± 54.4 | 363.1 ± 46.9 |
| *M. savannarum* | 497.5 ± 121.9 | 541.4 ± 111.1 |
| *M. tomentosa* | 459.3 ± 76.2 | 550.6 ± 98.9 |
| *M. villosa* | 622.5 ± 97.7 | 827.2 ± 103.3 |
| *M. parvifolia* | 752.4 ± 92.7 | 867.6 ± 108.9 |

Notes: P/O ratios for a particular morph (e.g. S-morph) were calculated as the number of pollen grains of the opposite one, e.g. L-morph) over its number of ovules. L: long-styled morph; S: short-styled morph. For *M. nodiflora* just one value of P/O is shown because it is a monomorphic species.

### *Characterization of reciprocity, herkogamy and P/O*

Values of reciprocity and herkogamy for all the species studied (and for each morph in heterostylous species) are shown in Table I. Reciprocity was lower in *M. savannarum* and *M. tomentosa* and similar in the rest of distylous species. The lowest values of herkogamy were found in *M. nodiflora*, *M. pyramidata*, and *M. savannarum*. The number of ovules per flower is the same for all the species (10), but the number of pollen grains is variable among the species. The results of P/O calculation for each morph are shown in Table IV.

### *Relationship between P/O, self-compatibility, and herkogamy*

We found a significant relationship between P/O and both herkogamy in the two morphs (S-morph: $\beta = 0.24 \pm 0.03$, $z = 9.58$, $p < 0.001$, $N = 6$; L-morph: $\beta = 0.11 \pm 0.02$, $z = 4.40$, $p < 0.001$, $N = 5$) and the incompatibility systems characterized by SCI (S-morph: $\beta = -1.01 \pm 0.09$, $z = -11.22$, $p < 0.001$, $N = 6$; L-morph: $\beta = -0.50 \pm 0.08$, $z = -6.10$, $p < 0.001$, $N = 5$).

As expected, lower values of P/O were associated with a lower herkogamy and a higher degree of compatibility (i.e. higher values of SCI). We also found a significant relationship between P/O and reciprocity ($\beta = 1.72 \pm 0.27$, $z = 6.31$, $p < 0.001$, $N = 6$) where greater values of P/O were related to higher reciprocity.

### Discussion

#### *Breeding system*

*Melochia* species in Cuba present a wide variety of breeding systems. Our results reveal that not all species present the heteromorphic incompatibility system that is normally associated with reciprocal herkogamy in heterostylous plants. The distylous *M. pyramidata* and the monomorphic *M. nodiflora* are confirmed to be self-compatible species (Martin

1967; Ramírez & Navarro 2010) and crosses between individuals of the same morph are also successful in the former. Populations of *M. pyramidata* are known to present both monomorphic and dimorphic populations [see Faife-Cabrera et al. (2014) for further details]. These two species, moreover, spontaneously produce fruits and seeds (see Figure 1). In Cuba, *M. pyramidata* and *M. nodiflora* are primary colonizer species, with a wide distribution (Rodríguez 2000), and the self-compatibility condition may facilitate the colonization process or their establishment in degraded environments like roadsides, abandoned crops, and pasture fields (see Baker 1955, 1967; Cheptou 2012). In these areas, pollinators may be scarce or unpredictable (Baker 1955; Cruden 1977; Cheptou 2012). Therefore, self-compatibility together with partially spontaneous selfing (see Figure 1 and Appendix 1) could be a way to ensure sexual reproduction (Jürgens et al. 2002). Moreover, the ability to carry out spontaneous selfing could explain the fact that they exhibited the lowest PPL values as compared with the other species surveyed and these negative PPL values could point to the possible effect of stigma clogging or differences in pollen load quality between treatments.

When looking for differences between morphs, in the case of *M. pyramidata*, we found differences between L-morph and S-morph fecundity (see Figure 1) as well as in their degree of compatibility (see Table III). S-morph plants exhibit higher fecundity, possibly due to their greater self-compatibility (SCI). For this self-compatible species, these results may also reflect differences between L and S stigmas in pollen competition and/or inbreeding depression. The same results were found for *M. villosa*, which also showed a lower value of pollen limitation in the S-morph flowers. Dissimilarities in fecundity and compatibility could be related to differences in herkogamy distances between morphs, since the S-morph has a smaller herkogamy than the L-morph in *M. pyramidata* and *M villosa* (Table I). However, such differences in herkogamy between morphs also appear in other species in which compatibility and fecundity are equal for both morphs.

Asymmetries in degree of self-compatibility between morphs have been found in other genera (e.g. Richards 2003; Brys et al. 2008; Faria et al. 2012; Simón-Porcar et al. 2015) and usually confirm the criterion of Lloyd and Schoen (1992) regarding the quantitative nature of this feature. In addition, the few fruits produced after hand self-pollination, only for the S-morph in *M. villosa*, could reflect the variability in breeding expression in the population and a kind of flexibility in the self-incompatibility of these S-morph flowers. Perhaps this could be associated with the existence of a lower herkogamy between sexual whorls in this morph (see Table I),

which is also found in other genera and can bias the efficiency of pollen transfer between morphs (Baena-Díaz et al. 2012; Faria et al. 2012).

Both habitat quality and floral display could influence the costs of plant reproduction, a key issue in plant colonization (Cheptou 2012; Euler et al. 2012). *Melochia parvifolia* and *M. villosa* usually inhabit disturbed environments. These two species are self- and morph-incompatible (see Figure 1) and present large flowers (Table I). Larger flowers facilitate their success as colonizers since they are more attractive for pollinators ensuring reproduction. On the other hand, *M. pyramidata* or *M. nodiflora* are self-compatible and present smaller flowers, but they also co-exist in disturbed habitats. It would therefore appear that *Melochia* species present different colonization strategies.

The capacity for self-fertilization has been proposed as a way to reduce pollen limitation in self-compatible species (Larson & Barrett 2000). Thus, the lack of pollen limitation in *M. nodiflora* and *M. pyramidata* is probably a result of their capacity for spontaneous selfing. However, we found pollen limitation in the populations of two species with a heteromorphic incompatibility system: *M. tomentosa* and *M. savannarum*. The populations of these two species are periodically affected by fires (Borhidi 1991). Such environmental perturbations decreases the community of local pollinators and hence increases pollen limitation (Ashman et al. 2004). Unreliable visitations of pollinators lead to differences in pollen limitation even among populations of heterostylous species (Baker et al. 2000).

### Efficiency of pollination (P/O)

The results of the analysis of the P/O values revealed a close relationship with herkogamy and the degree of incompatibility in the species studied. Although we did not test whether variations in P/O could be the result of different resource allocations (see Charnov 1979, 1982), there seems to be a relation between the efficiency in pollen transfer and these two features. As expected, more compatible species with lower values of herkogamy present lower P/O values. These findings support Cruden's conclusion that P/O ratios decrease with increase in the degree of self-fertilization. Since compatible species depend less on pollinators and selfing ensures reproductive success, plants do not need to invest many resources in gamete production (Cruden 1977). On the other hand, a small separation between sexual organs also favors a lower pollen investment, since it enhances the chances of pollen grains reaching the stigmas (de Vos et al. 2014). Indeed, the *Melochia* species with the highest herkogamy are the most incompatible (see Tables I and III).

However, we did not find the expected relationship between reciprocity and P/O. Despite the fact that more reciprocal systems are likely to result in the higher efficiency of pollinators (Lloyd & Webb 1992a), we could not corroborate our predictions of a lower P/O. Instead of reciprocity, the plant's dependence on pollen vectors appears to demand a higher investment in gamete production, as proposed by Cruden (1977). However, it must be noted that in this approximation, a single value of P/O was used for each population even though differences in P/O values have been described (due to unequal pollen production by morphs). For this reason, we would like to point out that future approximations using independent indices per morph might result in different trends.

### Melochia as a case study

In the *Melochia* species studied, the lower values of P/O (a possible indicator of the efficiency in pollen transfer) are associated with lower levels of herkogamy and compatibility. The species studied here are mostly incompatible and, in general, inhabit "disturbed" habitats where pollinators are erratic. For this reason, we would expect herkogamy to have a strong effect on the success of pollination in these heterostylous species because it influences the rates of self-pollination and self-interference. However, in cases where pollinators are more predictable, we would expect reciprocity to have an even greater effect. Studies in other groups of heterostylous species occupying more stable environments may shed light on this question.

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## Supplemental data

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## References

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, et al. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85: 2408–2421.

Baena-Díaz F, Fornoni J, Sosenski P, Molina-Freaner FE, Weller SG, Pérez-Ishiwara R, Domínguez CA. 2012. Changes in reciprocal herkogamy during the tristyly–distyly transition in *Oxalis alpina* increase efficiency in pollen transfer. J Evol Biol 25: 574–583.

Baker HG. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9: 347–348.

Baker HG. 1967. Support for Baker's law-as a rule. Evolution 21: 853–856.

Baker AM, Barrett SCH, Thompson JD. 2000. Variation of pollen limitation in the early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae). Oecologia 124: 529–535.

Barrett SCH. 1992. Heterostylous genetic polymorphisms: Model systems for evolutionary analysis. In: Barrett SCH, editor. Evolution and function of heterostyly. Berlin: Springer-Verlag. pp. 1–29.

Barrett SCH, Harder LD. 2005. The evolution of polymorphic sexual systems in daffodils (*Narcissus*). New Phytol 165: 45–53.

Borhidi A. 1991. Phytogeography and vegetation ecology of Cuba. Budapest: Akadémiai Kiado.

Brys R, Jacquemyn H. 2015. Disruption of the distylous syndrome in *Primula veris*. Ann Bot-London 115: 27–39.

Brys R, Jacquemyn H, Hermy M, Beeckman T. 2008. Pollen deposition rates and the functioning of distyly in the perennial *Pulmonaria officinalis* (Boraginaceae). Plant Syst Evol 273: 1–12.

Castro S, Ferrero V, Costa J, Sousa AJ, Castro M, Navarro L, et al. 2013. Reproductive strategy of the invasive *Oxalis pes-caprae*: Distribution patterns of floral morphs, ploidy levels and sexual reproduction. Biol Invasions 15: 1863–1875.

Castro S, Silveira P, Navarro L. 2008. How flower biology and breeding system affect the reproductive success of the narrow endemic *Polygala vayredae* Costa (Polygalaceae)? Bot J Linn Soc 157: 67–81.

Cesaro AC, Barrett SCH, Maurice S, Vaissiere BE, Thompson JD. 2004. An experimental evaluation of self-interference in *Narcissus assoanus*: Functional and evolutionary implications. J Evol Biol 17: 1367–1376.

Charnov EL. 1979. Simultaneous hermaphroditism and sexual selection. Proc Natl Acad Sci-Biol 76: 2480–2484.

Charnov EL. 1982. The theory of sex allocation. Princeton: Princeton University Press.

Cheptou PO. 2012. Clarifying Baker's law. Ann Bot-London 109: 633–641.

Chouteau M, Barabé D, Gibernau M. 2006. A comparative study of inflorescence characters and pollen-ovule ratios among the genera *Philodendron* and *Anthurium* (Araceae). Int J Plant Sci 167: 817–829.

Consolaro H, Silva SCS, Oliveira PE. 2011. Breakdown of distyly and pin-monomorphism in *Psychotria carthagenensis* Jacq. (Rubiaceae). Plant Spec Biol 26: 24–32.

Costa J, Ferrero V, Loureiro J, Castro M, Navarro L, Castro S. 2014. Sexual reproduction of the pentaploid short-styled *Oxalis pes-caprae* allows the production of viable offspring. Plant Biol 16: 208–214.

Cruden RW. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Darwin C. 1877. The different forms of flowers on plants of the same species. London: John Murray.

Dorr LJ, Barnett LC. 1989. A revision of *Melochia* section Physodium (Sterculiaceae) from Mexico. Brittonia 41: 404–423.

Euler TV, Ågren J, Ehrlén J. 2012. Floral display and habitat quality affect cost of reproduction in *Primula farinosa*. Oikos 121: 1400–1407.

Faife-Cabrera M, Ferrero V, Navarro L. 2014. Unravelling the stylar polymorphism in *Melochia* species (Malvaceae): Reciprocity and ancillary characters. Bot J Linn Soc 176: 147–158.

Faria R, Ferrero V, Navarro L, Araujo AC. 2012. Flexible mating system in distylous populations of *Psychotria carthagenensis* Jacq. (Rubiaceae) in Brazilian Cerrado. Plant Syst Evol 298: 619–627.

Ferrero V, Arroyo J, Castro S, Navarro L. 2012. Unusual heterostyly: Style dimorphism and self-incompatibility are not tightly associated in *Lithodora* and *Glandora* (Boraginaceae). Ann Bot 109: 655–665.

Ferrero V, Castro S, Sánchez JM, Navarro L. 2011a. Stigma-anther reciprocity, pollinators, and pollen transfer efficiency in populations of heterostylous species of *Lithodora* and *Glandora* (Boraginaceae). Plant Syst Evol 291: 267–276.

Ferrero V, Chapela I, Arroyo J, Navarro L. 2011b. Reciprocal style polymorphisms are not easily categorized: The case of heterostyly in *Lithodora* and *Glandora* (Boraginaceae). Plant Biol 13: 7–18.

Goldberg A. 1967. The genus *Melochia* L. (Sterculiaceae). Contrib US Nat Herb 34: 191–363.

Gong YB, Huang SQ. 2014. Interspecific variation in pollen-ovule ratio is negatively correlated with pollen transfer efficiency in a natural community. Plant Biol 16: 843–847.

Götzenberger L, Durka W, Kühn I, Klotz S. 2006. The relationship of pollen-ovule ratio and seed size: A comparative test of a sex allocation hypothesis. Evol Ecol Res 8: 1101–1116.

Götzenberger L, Kühn I, Klotz S. 2008. Effects of habitat disturbance and pollination type on the interspecific variation in pollen-ovule ratios. Preslia 80: 423–437.

Hodgins KA, Barrett SCH. 2008. Geographic variation in floral morphology and style-morph ratios in a sexually polymorphic daffodil. Am J Bot 95: 185–195.

IBM Corp. 2010. IBM SPSS statistics for windows. Version 19.0. North Castle, New York.

Jules ES, Rathcke BJ. 1999. Mechanisms of reduced trillium recruitment along edges of old-growth forest fragments. Conserv Biol 13: 784–793.

Jürgens A, Witt T, Gottsberger G. 2002. Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: Correlation with breeding system, pollination, life form, style number, and sexual system. Sex Plant Reprod 14: 279–289.

Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, et al. 2005. Pollen limitation of plant reproduction: Pattern and process. Ann Rev Ecol Evol Syst 36: 467–497.

Larson BMH, Barrett SCH. 2000. A comparative analysis of pollen limitation in flowering plants. Biol J Linn Soc 69: 503–520.

Lloyd DG, Schoen DJ. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. Int J Plant Sci 153: 358–369.

Lloyd DG, Webb CJ. 1992a. The evolution of heterostyly. In: Barrett SCH, editor. Evolution and function of heterostyly. Berlin: Springer-Verlag. pp. 151–178.

Lloyd DG, Webb CJ. 1992b. The selection of heterostyly. In: Barrett SCH, editor. Evolution and function of heterostyly. Berlin: Springer-Verlag. pp. 179–207.

Martin FW. 1967. Distyly, self-incompatibility, and evolution in *Melochia*. Evolution 21: 493–499.

Mast AR, Kelso S, Conti E. 2006. Are any primroses (*Primula*) primitively monomorphic? New Phytol 171: 605–616.

Mione T, Anderson GJ. 1992. Pollen-ovule ratios and breeding system evolution in *Solanum* section Basarthrum (Solanaceae). Am J Bot 79: 279–287.

Neal PR, Anderson GJ. 2005. Are "mating systems" "breeding systems" of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? Plant Syst Evol 250: 173–185.

Pérez R, Vargas P, Arroyo J. 2004. Convergent evolution of flower polymorphism in *Narcissus* (Amaryllidaceae). New Phytol 161: 235–252.

Pérez-Barrales R, Arroyo J. 2010. Pollinator shifts and the loss of style polymorphism in *Narcissus papyraceus* (Amaryllidaceae). J Evol Biol 23: 1117–1128.

Pérez-Barrales R, Vargas P, Arroyo J. 2006. New evidence for the Darwinian hypothesis of heterostyly: Breeding systems and pollinators in *Narcissus* sect. Apodanthi. New Phytol 171: 553–567.

Queller DC. 1984. Pollen-ovule ratios and hermaphrodite sexual allocation strategies. Evolution 38: 1148–1151.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Ramírez N, Navarro L. 2010. Trends in the reproductive biology of Venezuelan *Melochia* (Malvaceae) species. Plant Syst Evol 289: 147–163.

Rea AC, Nasrallah JB. 2008. Self-incompatibility systems: Barriers to self-fertilization in flowering plants. Int J Develop Biol 52: 627–636.

Richards J. 2003. Primula. 2nd ed. Portland, OR: Timber Press.

Rodríguez A. 2000. Sterculiaceae. Flora de la República de Cuba, Fascículo 3(4) Serie A, Plantas Vasculares [Sterculiaceae. The Flora of the Cuban Republic, vol 3(4) Series A, Vascular plants]. Konigstein: Koeltz Scientific Books.

Sánchez JM, Ferrero V, Arroyo J, Navarro L. 2010. Patterns of style polymorphism in five species of the South African genus *Nivenia* (Iridaceae). Ann Bot 106: 321–331.

Sánchez JM, Ferrero V, Navarro L. 2008. A new approach to the quantification of degree of reciprocity in distylous (*sensu lato*) plant populations. Ann Bot-London 102: 463–472.

Sánchez JM, Ferrero V, Navarro L. 2013. Quantifying reciprocity in distylous and tristylous plant populations. Plant Biol 15: 616–620.

Santos-Gally R, Gonzalez-Voyer A, Arroyo J. 2013. Deconstructing heterostyly: The evolutionary role of incompatibility system, pollinators, and floral architecture. Evolution 67: 2072–2082.

Schlindwein C, Medeiros PCR. 2006. Pollination in *Turnera subulata* (Turneraceae): Unilateral reproductive dependence of the narrowly oligolectic bee *Protomeliturga turnerae* (Hymenoptera, Andrenidae). Flora 201: 178–188.

Schoen DJ, Johnston MO, L'Heureux AM, Marsolais JV. 1997. Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). Evolution 51: 1090–1099.

Simón-Porcar V, de Castro A, Herrero M, Arroyo J. 2015. Ovarian self-incompatibility in *Narcissus papyraceus* (Amaryllidaceae) is the result of a pre-zygotic response. Bot J Linn Soc 177: 629–643.

Vogler DW, Kalisz S. 2001. Sex among the flowers: The distribution of plant mating systems. Evolution 55: 202–204.

de Vos JM, Wüest RO, Conti E. 2014. Small and ugly? Phylogenetic analyses of the "selfing syndrome" reveal complex evolutionary fates of monomorphic primrose flowers. Evolution 68: 1042–1057.

Webb CJ, Lloyd DG. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. New Zeal J Bot 24: 163–178.