



## Unravelling the stylar polymorphism in *Melochia* (Malvaceae): reciprocity and ancillary characters

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Heterostyly is a genetic polymorphism in which plant populations possess two (distyly) or three (tristyly) morphs with flowers differing reciprocally in stigma and anther height. Sex organ deployment has been described as being highly variable among and within species of several distylous taxa belonging to different taxonomic groups. However, the number of studies considering within-species disparities is still limited. For a better overview of the existing amount of variation that can occur within and between heterostylous species, we sampled 46 populations of six *Melochia* spp., a style-polymorphic genus in Cuba. We characterized the floral morphology in all populations and described a set of ancillary characters per species. All of these *Melochia* spp. are distylous, except for the monomorphic *M. nodiflora*. The S-morph produces fewer, larger pollen than L-morphs, and has verrucose ornamentation. The L-morph produces reticulate pollen and has larger stigmatic papillae than the S-morph. The monomorphic *M. nodiflora* shows ancillary characteristics that are similar to the L-morph individuals in the related species. As expected, there are differences in ancillary characters among species and also dissimilarities in reciprocity among and within species of *Melochia*. Our results highlight the importance of considering intraspecific variation in the morphometric characterization of heterostylous taxa. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, **176**, 147–158.

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### INTRODUCTION

Heterostyly is a genetic polymorphism in which plant populations are composed of two (distyly) or three (tristyly) morphs that differ reciprocally in the heights of the stigmas and anthers in flowers (Barrett, 1992). Darwin (1877) suggested that the reciprocal placement of stigmas and anthers (reciprocal herkogamy) is a mechanism that promotes disassortative mating. Reciprocal herkogamy favours segregation-based pollen transfer on the body of the pollinator, in keeping with the heights of the anthers and stigmas of each morph. In addition, disassortative mating is also promoted through a heteromorphic incompatibility system, which precludes self- and intra-morph fertilization and mostly accompanies

morphological differences in heterostylous species (Barrett, 1992).

Different models have been proposed to describe the evolutionary process towards heterostyly (e.g. Charlesworth & Charlesworth, 1979; Lloyd & Webb, 1992a; Richards, 1998). These models mostly differ in the morphological ancestral condition they propose and the succession in the intermediate stages leading to the establishment of reciprocal herkogamy. Studies aimed at testing the evolution of heterostyly are based on evidence from shifts in floral morphology among closely related species. They normally use phylogenetic reconstructions that have contributed to the understanding of the changes likely to occur in the evolutionary process (e.g. Pérez, Vargas & Arroyo, 2003; Graham & Barrett, 2004; Morris, 2007; Ferrero *et al.*, 2009). Thus, a detailed comprehension of floral morphology is needed for a better understanding

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of the nature, origin and evolution of heterostyly (Dulberger, 1992).

Heterostyly has been described in about 28 families of angiosperm plants (Barrett & Shore, 2008), and it originated independently on more than 20 separate occasions among these families (Barrett, 1992). Hence, we would expect to find differences in the evolutionary process towards heterostyly in the different lineages in which it has evolved. Nevertheless, the evolution of heterostyly has been tested in only a few groups to date (e.g. Kohn *et al.*, 1996; Schoen *et al.*, 1997; Church, 2003; Graham & Barrett, 2004; Mast *et al.*, 2004; Truyens, Arbo & Shore, 2005; Morris, 2007; Ferrero *et al.*, 2011b, 2012). In all cases, the first step towards the elucidation of the evolution of the reciprocal condition is to characterize the morphological traits linked to the expression of the heterostylous syndrome, as they may provide some insight into the underlying evolutionary steps. These include stigma–anther separation (i.e. herkogamy), reciprocity in stigma and anther height, and ancillary characters.

Evolutionary forces exhibit their full expression at the population level, driving microevolutionary transformations in species (Barrett & Kohn, 1991). Therefore, population-level analyses (i.e. those considering possible variations in morphological variables among populations of the same species) are critical to gain a better understanding of the meaningful variations in character expression associated with heterostylous syndrome.

*Melochia* L. includes heterostylous and non-heterostylous species. *Melochia nodiflora* Sw. has been described previously as a monomorphic and self-compatible species (Martin, 1967), whereas distylous species, such as *M. tomentosa* L., are known to have an incompatibility system (Machado & Sazima, 2008). For this reason, it is an ideal study system in which to analyse changes in morphological traits associated with heterostyly. Although this genus is widely distributed in tropical and subtropical regions, and shows an outstanding variation in floral morphology, it has not been studied in detail (Martin, 1967; Machado & Sazima, 2008; Ramírez & Navarro, 2010). Previous papers have focused on several aspects of particular *Melochia* spp., but none has used a population-level approach (i.e. including a number of populations to account for intraspecific variation in morphological aspects). In this study, we characterize the floral morphology of six *Melochia* spp. at the population level, which may contribute to a better overview of the existing amount of variation that can occur within and between heterostylous species of this group. In addition to the morphological variables (corolla length, length of styles, length of stigma and anther height), we analysed the variation in ancillary

characters (pollen production, pollen size, pollen and stigmatic papillae morphology). *Melochia* spp. may provide elements that could shed light on the evolution or breakdown of heterostyly, if the expected morphological variation in characters associated with the heterostylous syndrome is confirmed.

## MATERIAL AND METHODS

### STUDY SPECIES

We studied six *Melochia* spp. from the Cuban archipelago: *M. nodiflora*, *M. pyramidata* L., *M. savannarum* Britt., *M. tomentosa*, *M. villosa* (Mill.) Fawc. & Rendle and *M. parvifolia* Kunth. All of the species are distributed throughout the country, except for *M. savannarum*, which is endemic to the siliceous white-sands in Pinar del Rio and Isla de la Juventud. Another three *Melochia* spp. (*M. manducata* C. Wright, *M. arenosa* Benth. and *M. bissei* A. Rodr.) have been reported in Cuba, but their current presence is doubtful (Rodríguez, 2000).

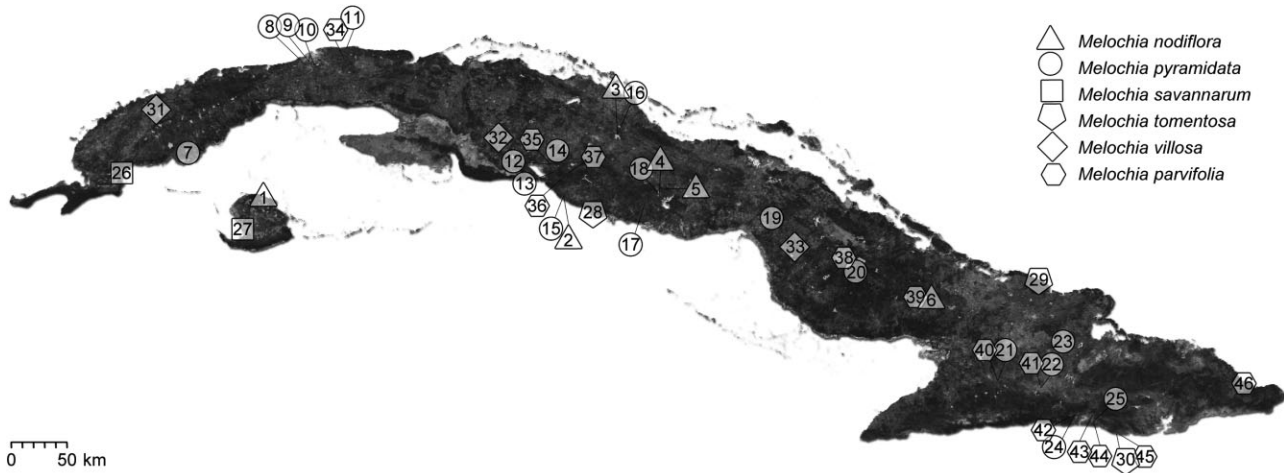
In Cuba, *Melochia* spp. are shrubs or subshrubs < 3 m in height, flowering and fruiting throughout the year. The most widespread species are ruderal and are common in crops as pioneer species and in disturbed areas (Goldberg, 1967).

### FLOWER MORPHOMETRICS

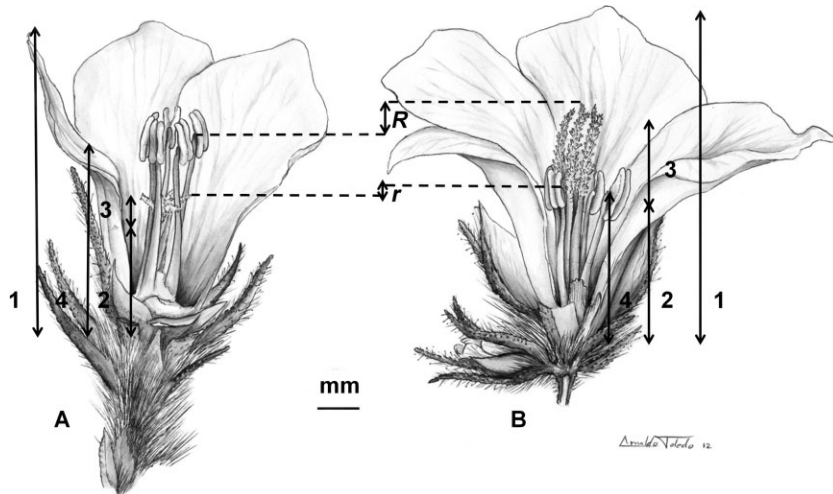
We sampled 46 populations of the six *Melochia* spp. over the whole archipelago: *M. nodiflora* (six populations), *M. pyramidata* (19), *M. savannarum* (two), *M. tomentosa* (three), *M. villosa* (three) and *M. parvifolia* (13) (Fig. 1; see Supporting Information, Table S1). The number of studied populations per species varied according to their natural abundance throughout the country. In each population, one flower per individual was collected, up to 100 flowers when possible. Flowers were preserved in 70% ethanol until processed in the laboratory. They were dissected and photographed under a magnifying glass. Traits on images were measured using ImageJ software (Rasband, 1997–2000). Voucher specimens were also collected and deposited at the ULV herbarium.

We characterized the flower morphology of *Melochia* spp. using four variables: (1) corolla length; (2) style length; (3) stigma length; and (4) anther height (Fig. 2). We measured the length of all five styles and the height of all five anthers in each flower to obtain average values. For each flower, we calculated the degree of herkogamy (i.e. stigma–anther separation within the flower).

We classified flowers as L-morphs (when stigmas were positioned above the anthers) and S-morphs (when the opposite pattern was found). Next, corolla



**Figure 1.** Geographical location of the 46 studied populations belonging to six species of the genus *Melochia*.



**Figure 2.** Variables measured for floral morphometric characterization of *Melochia* species and populations: 1, corolla length; 2, style length; 3, stigma length; 4, anther height (to the point of insertion of the anther). A, S-morph flower; B, L-morph flower. *R* and *r* refer to the reciprocity (i.e. separation between stigmas of one morph and anthers of the opposite morph) calculated for each level of the sexual organs (high and low, respectively). Illustration: Arnaldo Toledo; using *Melochia savannarum* flowers.

length and stigma–anther separation were tested for differences between morphs and populations with two-way mixed analyses of variance (ANOVAs). We defined morph as a fixed factor and population as random. For *M. nodiflora*, a monomorphic species, we used a one-way ANOVA model II to assess differences between populations. Statistical analyses were performed with SPSS software (version 19, SPSS, Chicago, IL, USA).

Two different methods were used to analyse the reciprocity between floral morphs in each population. First, we calculated the reciprocity of the stigma–anther position for both levels of the sexual organs,

i.e. for the high level, the average separation between the stigmas of the L-morph and the anthers of the S-morph and, for the low level, the average separation between the stigmas of the S-morph and the anthers of the L-morph. Measures are represented as *R* and *r*, respectively, in Figure 2 (for similar calculation of the index, see Faivre & McDade, 2001). We tested for differences in reciprocity between both levels (high and low) with a Monte Carlo Student’s *t*-test (9999 randomizations; PAST version 2.14; Hammer & Harper, 2006). Then, we calculated the reciprocity index following Sánchez, Ferrero & Navarro (2008) (modified in Sánchez, Ferrero &

Navarro, 2013). This index compares the stigma–anther height of all flowers in the population and is useful for the examination of differences in reciprocity between populations.

#### ANCILLARY CHARACTERISTICS

##### *Pollen production and pollen size*

Ancillary characters were measured in only one population per species. To evaluate the pollen produced by each morph, we followed Castro, Silveira & Navarro (2008). We selected seven to ten floral buds of each morph from different plants in one population per species (84 floral buds were analysed; populations surveyed are shown in Supporting Information, Table S2). In each bud, we selected an anther. Under a magnifying glass, we carefully dissected the anther on a microscope slide, extracting all pollen grains and placing them in a drop of glycerin. The pollen grains were then quantified by direct observation under a light microscope. The total production of pollen grains was estimated by multiplying the counts by the number of anthers (i.e. five) of each flower. Differences in pollen production between morphs were tested with a Student's *t*-test. In addition, we calculated the ratio of pollen grains between morphs (*L/S*) to examine relative differences in pollen production between each one.

Differences in pollen size between morphs were assessed by measuring the diameter of 20 pollen grains in seven or eight individuals per morph for the same populations as were used in the pollen counts (71 individuals, see also Supporting Information, Table S2). As *Melochia* pollen grains are spherical (Fig. 5), we measured the diameter of each pollen grain. Pollen size dimorphism between morphs was determined with a Student's *t*-test. We also calculated the ratio between S/L morph diameters.

##### *Pollen and stigmatic papillae morphology*

Pollen samples from ten floral buds (per morph and species) were acetolysed, mounted on stubs and coated with gold in a sputtering Emitech K550X. We used the samples from the same populations as surveyed for pollen production and pollen size. Pollen images were taken with a scanning electronic microscope (SEM) FEI Quanta 200, under vacuum conditions. For each species and morph, we characterized the shape, exine sculpturing type, and number and type of apertures.

Stigmatic papillae were dehydrated with a critical point drier (CPD) and the uncoated samples were mounted on stubs for SEM observation. Images of stigmatic papillae were obtained as described for pollen samples. The flowers were the same as those used to obtain pollen samples for SEM observation

(ten flowers from ten individuals of each morph and species).

## RESULTS

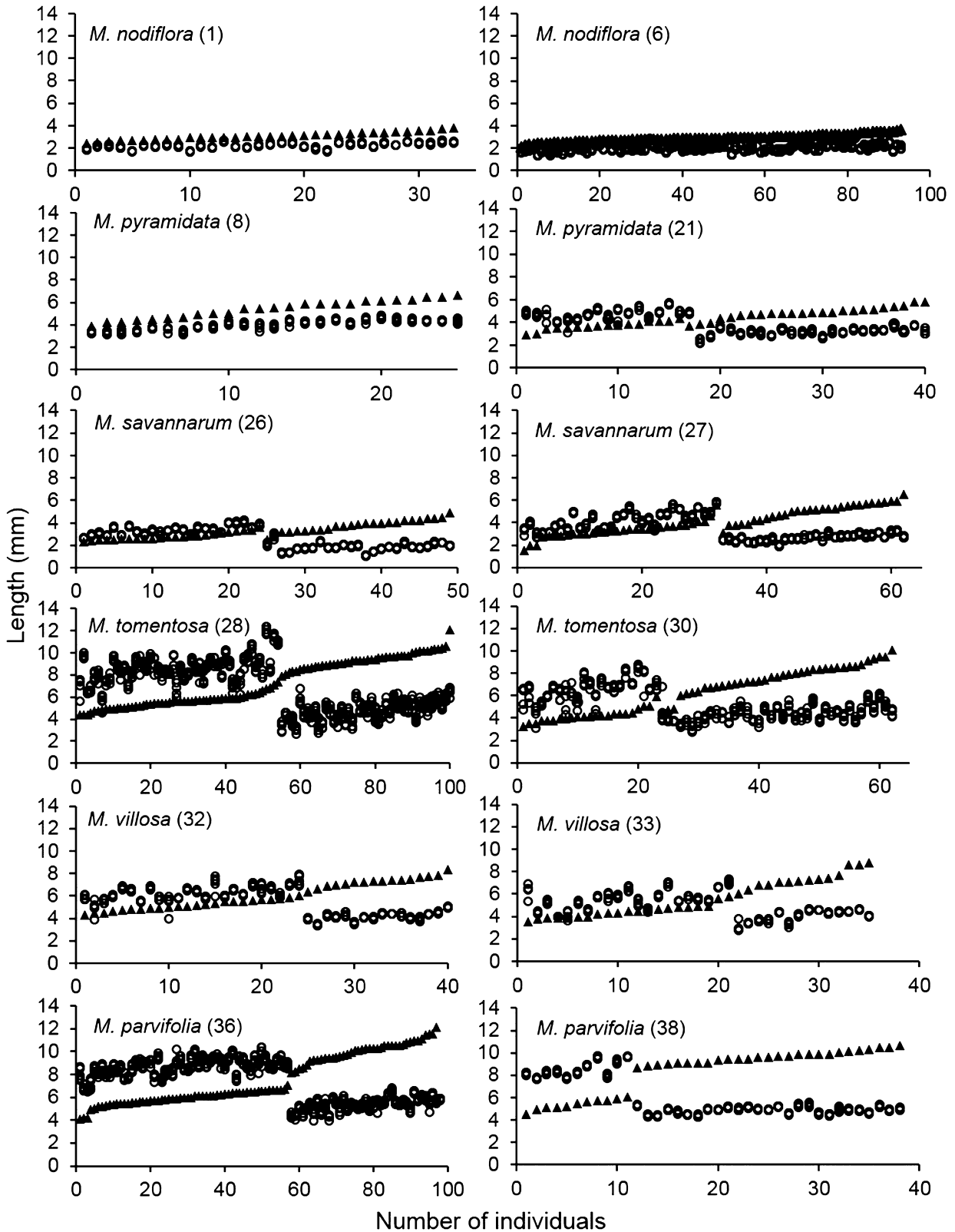
### FLOWER MORPHOMETRICS

Five of the six *Melochia* spp. had dimorphic populations (see Supporting Information, Table S1). All populations of *M. nodiflora* were monomorphic, with flowers showing approach to herkogamy (i.e. style length exceeded anther height in all individuals in the populations). In the case of *M. pyramidata*, despite the fact that most of its populations presented both S-morph and L-morph plants, in one population only L-morph individuals were found (Supporting Information, Table S1). In addition, there was a wide variation in the length of the style and the anther height of individuals within and between populations. Herkogamy was more conspicuous in L-morph flowers in the majority of the populations (Fig. 3; Supporting Information, Table S1). Stigma–anther separation differed significantly between morphs and between populations, except for populations of *M. pyramidata* and *M. tomentosa* (Table 1). Interactions between morph and population were significant when comparing herkogamy in all species, except *M. savannarum* and *M. villosa*. Monomorphic *M. nodiflora* had lower herkogamy than any of the morphs in the rest of the species (Fig. 3).

Comparisons of corolla length between populations and morphs showed significant differences among populations in the six *Melochia* spp., but not between morphs (Table 2). A significant interaction between these factors was only found in *M. parvifolia*. For the monomorphic *M. nodiflora*, significant differences between populations were also found (Table 2).

Reciprocity between sexual organs at both levels (high and low), measured as stigma–anther separation between stigmas of one morph and anthers of the opposite morph, was significantly greater at the low level in populations of *M. parvifolia* ( $t = 8.88$ ,  $P = 0.0001$ ), *M. villosa* ( $t = 2.24$ ,  $P = 0.0001$ ) and *M. tomentosa* ( $t = -2.64$ ,  $P = 0.0134$ ), but not for *M. savannarum* ( $t = -0.99$ ,  $P = 0.3295$ ). Conversely, *M. pyramidata* showed a significantly higher reciprocity at the high organ sex level ( $t = -2.64$ ,  $P = 0.0134$ ) (see Supporting Information, Table S1).

Values of the reciprocity index showed broad variation within and between species (Fig. 4). Higher reciprocity (i.e. higher reciprocity index value) was found in populations of *M. parvifolia* (0.82–0.92), whereas some populations of *M. villosa* and *M. tomentosa* exhibited the lowest values (0.62 and 0.67, respectively). Populations of *M. pyramidata* (0.74–0.96), *M. tomentosa* (0.67–0.86) and *M. villosa* (0.62–0.85) were the most variable (Fig. 4).



**Figure 3.** Relation between style length (▲) and anther height (○) in several (at least two) contrasting populations of six *Melochia* spp. from Cuba. The numbers in parentheses identify some of the populations represented in Figure 1 and Table S1 (Supporting Information).

**Table 1.** Comparisons of stigma–anther separation between morphs and populations in six *Melochia* spp. from Cuba. Results correspond to a one-way analysis of variance (ANOVA) for the monomorphic *M. nodiflora* and two-way mixed effects ANOVAs for distylous species. Significant differences for  $\alpha = 0.05$  are highlighted in bold

Species	Factor	df	<i>F</i>	<i>P</i>
<i>M. nodiflora</i>	Population	5,325	10.86	< <b>0.001</b>
<i>M. pyramidata</i>	Morph	1,18	116.99	< <b>0.001</b>
	Population	18,18	1.61	0.170
	Morph × population	18,1115	7.49	< <b>0.001</b>
<i>M. savannarum</i>	Morph	1,1	126.56	<b>0.006</b>
	Population	1,1	948.55	<b>0.021</b>
	Morph × population	1,107	0.013	0.908
<i>M. tomentosa</i>	Morph	1,2	115.24	<b>0.009</b>
	Population	2,2	11.67	0.076
	Morph × population	2,239	19.31	< <b>0.001</b>
<i>M. villosa</i>	Morph	1,2	6921.92	< <b>0.001</b>
	Population	2,2	35.50	<b>0.027</b>
	Morph × population	2,152	0.03	0.976
<i>M. parvifolia</i>	Morph	1,12	86.03	< <b>0.001</b>
	Population	12,12	3.70	<b>0.016</b>
	Morph × population	12,871	10.87	< <b>0.001</b>

**Table 2.** Comparisons of corolla length between morphs and populations in six *Melochia* spp. from Cuba. Results correspond to a one-way analysis of variance (ANOVA) for the monomorphic *M. nodiflora* and two-way mixed effects ANOVAs for distylous species. Significant differences for  $\alpha = 0.05$  are highlighted in bold

Species	Factor(s)	df	<i>F</i>	<i>P</i>
<i>M. nodiflora</i>	Population	5,325	21.19	< <b>0.001</b>
<i>M. pyramidata</i>	Morph	1,18	0.01	0.940
	Population	18,18	10.64	< <b>0.001</b>
	Population × morph	18,1115	1.42	0.120
<i>M. savannarum</i>	Morph	1,1	48.44	0.091
	Population	1,1	1052.31	<b>0.020</b>
	Population × morph	1,107	0.07	0.791
<i>M. tomentosa</i>	Morph	1,2	12.35	0.068
	Population	2,2	71.09	<b>0.014</b>
	Population × morph	2,239	1.22	0.296
<i>M. villosa</i>	Morph	1,2	0.298	0.591
	Population	2,2	1515.79	<b>0.001</b>
	Population × morph	2,152	0.02	0.978
<i>M. parvifolia</i>	Morph	1,12	0.15	0.704
	Population	12,12	171.34	< <b>0.001</b>
	Population × morph	12,871	2.04	<b>0.019</b>

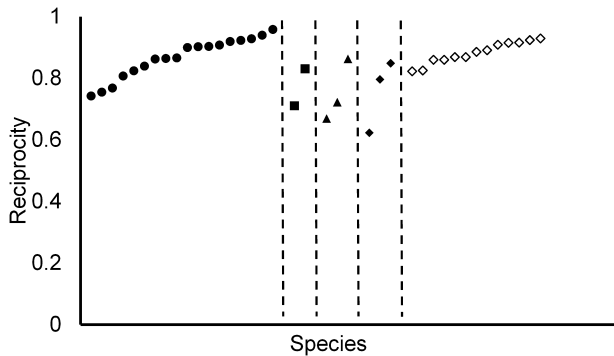
## ANCILLARY CHARACTERISTICS

*Pollen production and pollen size*

*Melochia* species produced from 2115 to 11 520 pollen grains per flower. The lowest pollen production rate was found in *M. nodiflora*, and the highest value was observed in *M. tomentosa*. Pollen production differed significantly between morphs in *M. tomentosa* and *M. villosa*. L-morph flowers produced more or equal numbers of pollen in comparison with S-morph

flowers (see Supporting Information, Table S2). The ratio calculated between the pollen production of L- and S-morph flowers ranged from 0.96 (*M. pyramidata*) to 1.65 (*M. tomentosa*).

Results for mean pollen size, measured as pollen diameter, ranged from 35 to 48  $\mu\text{m}$  (Supporting Information, Table S2). The S-morph pollen of all studied *Melochia* spp. was significantly larger than L-morph pollen (S/L ratios ranged between 1.13 and 1.21) and,



**Figure 4.** Population-level variability of reciprocity between stigma and anther height in the dimorphic populations of *Melochia*: ●, *M. pyramidata*; ■, *M. savannarum*; ▲, *M. tomentosa*; ◆, *M. villosa*; ◇, *M. parvifolia*.

in the monomorphic *M. nodiflora*, pollen showed a similar size to that of L-morph individuals in the distylous species *M. pyramidata* and *M. tomentosa* (Supporting Information, Table S2).

#### Pollen and stigmatic papillae morphology

Pollen grains of *Melochia* spp. are spherical or subspherical and tricolporate. There were differences between morphs in pollen exine ornamentation in all species. In all dimorphic species, pollen sculpturing was reticulate in the L-morph and verrucose in the S-morph (Fig. 5). The pollen of the monomorphic species *M. nodiflora* showed a similar exine ornamentation as the L-morph pollen of the other distylous species studied.

Stigmatic papillae also differed in morphology between morphs in all species. Generally, L-morphs had more elongated stigmatic papillae than S-morphs, although small differences were found for *M. pyramidata*. Again, stigmatic papillae of the monomorphic *M. nodiflora* were similar to those described for L-morph stigmas in the other species studied (Fig. 6).

## DISCUSSION

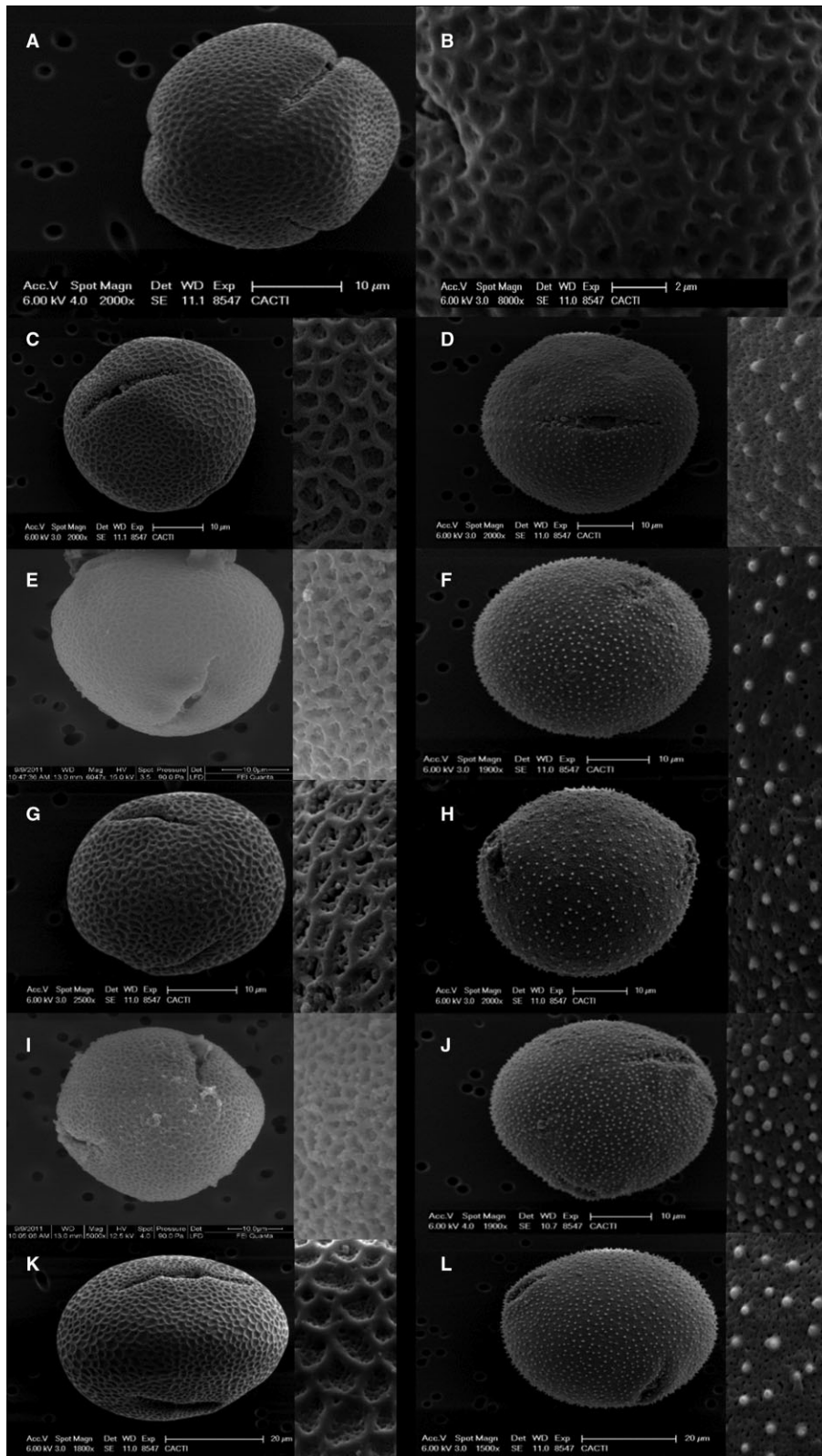
According to our results, most *Melochia* spp. in the Cuban archipelago can be described as dimorphic and distylous, with two floral morphs (see Fig. 3 and Supporting Information, Table S1) and a wide variation in reciprocity within and between species. The monomorphism of *M. nodiflora* has already been described on other Caribbean islands and on the mainland (Goldberg, 1967; Martin, 1967; Rondón, 2009; Ramírez & Navarro, 2010). This species has the smallest flowers, a feature which, among others, has been associated with shifts from heterostyly to homo-

styly, similar to the situation in *Amsinckia* Lehm. and *Eichhornia* Kunth (Schoen *et al.*, 1997; Barrett & Shore, 2008).

Our data reveal that *M. pyramidata* has both dimorphic and monomorphic populations in Cuba, in accordance with previous observations in this species in other study areas (Martin, 1967; Ramírez & Navarro, 2010). *Melochia pyramidata* is widely distributed in tropical and subtropical areas (Goldberg, 1967). According to Baker's law, self-compatibility has been found to facilitate the establishment of sexually reproducing populations following long-distance dispersal (Baker, 1955; see also Pannell & Barrett, 1998; Costa *et al.*, 2014). Thus, self-compatibility in *M. pyramidata* (Martin, 1967; Ramírez & Navarro, 2010) could be related to the wide distribution of dimorphic populations of this species and of monomorphic ones. Especially in heterostylous species, a breakdown in the self-incompatibility system can allow reproduction if only one morph is available (Faria *et al.*, 2012; Castro *et al.*, 2013).

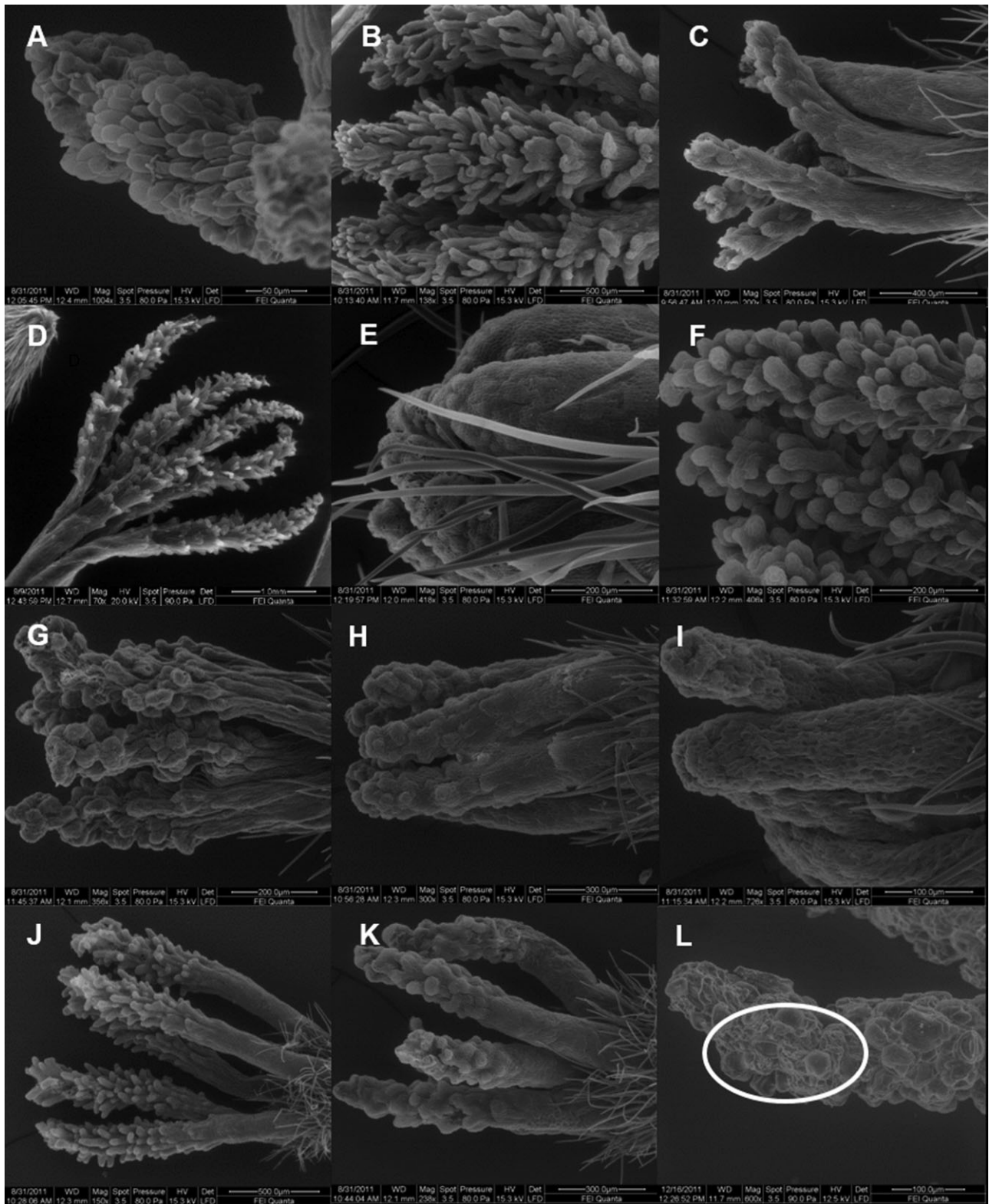
As corolla length may be an indicator of resource investment to floral display (Herrera, 2009), differences in this trait could be considered as a measurement of pollinator attraction at the population level. Larger flowers may increase the number of pollinator visits (Thompson, 2001) and improve the reproductive success of plants (Karron & Mitchell, 2012). Corollas are significantly larger in S-morph flowers of *M. savannarum*. Female fitness in flowers of the S-morph could be at a disadvantage when compared with the L-morph because of the under-exposure of the stigmas in the corolla (Ganders, 1974; Dulberger, 1992; Pérez-Barrales & Arroyo, 2010). In this case, a higher floral size could increase female fitness in S-morph flowers by attracting more pollinators to compensate for this weakness (Dulberger, 1992).

The analysis of ancillary characters exhibited significant differences between morphs in dimorphic populations. All species showed differences in pollen size as expected (i.e. S-morph pollen is larger than L-morph pollen), whereas pollen production differed significantly between morphs only in *M. tomentosa* and *M. villosa*. Higher pollen production rates are generally associated with smaller pollen sizes (e.g. Dulberger, 1992; Chen & Zang, 2010), a relationship that can be attributed to a trade-off between number and size (Vonhof & Harder, 1995; Cruden, 2000). Other ancillary characters, such as the sculpture of pollen exine and stigma, could be involved in the physiology of the incompatibility mechanism of heterostylous species (Dulberger, 1975, 1992). Pollen grains of L-morph flowers of *Melochia* spp. are reticulate, whereas the S-morph shows verrucose pollen ornamentation. These differences could have a key role in the capture, adhesion (Luu, Heizmann &



**Figure 5.** Scanning electron micrographs of pollen grains of L- and S-morph flowers of the studied *Melochia* spp. Patterns of exine sculpturing are also shown at the right side of each pollen grain (8000 $\times$ ). A, B, *Melochia nodiflora*; C, D, *M. pyramidata*; E, F, *M. savannarum*; G, H, *M. tomentosa*; I, J, *M. villosa*; K, L, *M. parvifolia*.





**Figure 6.** Stigmatic papillae of six *Melochia* spp. using scanning electron microscopy. A, *M. nodiflora* (monomorphic species); B, C, *M. savannarum*; D, E, *M. villosa*; F, G, *M. parvifolia*; H, I, *M. pyramidata*; J, K, *M. tomentosa*; L, pollen grain on stigmatic papillae in *Melochia pyramidata*. For each species, stigmatic papillae of L- and S-morphs, respectively, are shown.

Dumas, 1997), recognition and hydration of pollen (Zinkl & Preuss, 2000), when they interact with stigmatic papillae. Future work on the reproductive systems of these species will help to clarify this.

Moreover, pollen of the monomorphic *M. nodiflora* has ornamentation similar to that of the L-morph in dimorphic populations. This could shed light on the putative evolutionary process towards heterostyly evolution or breakdown in the genus. Other monomorphic *Melochia* spp. have the same pollen ornamentation, as is the case for *M. corchorifolia* L. (M. Faife-Cabrera, V. Ferrero & L. Navarro, unpubl. data). However, there is no dimorphism in the pollen sculpturing of distylous species from *Melochia* section *Physodium* (Dorr & Barnett, 1989). Species of *Physodium* have reticulate pollen ornamentation, like *M. nodiflora* and L-morph flowers of distylous species.

However, *M. nodiflora* is self-compatible (M. Faife-Cabrera *et al.*, unpubl. data) and has the lowest herkogamy among all the studied species. Next lowest value of anther–stigma separation appears in *M. pyramidata*, which is also self-compatible (Ramírez & Navarro, 2010). These two features (low herkogamy and capacity of selfing) could facilitate independent fertilization in cases in which there is a scarcity of pollinators or low pollination efficiency. Distylous *Melochia* spp. exhibit higher herkogamy in L-morph flowers. Differences in herkogamy between morphs were found between populations, as reported previously in other heterostylous plants (Faivre & McDade, 2001). The differences in herkogamy in some *Melochia* spp., reported in previous papers (Ramírez & Navarro, 2010), are confirmed here, even when comparisons are made between populations of the same species. We could not identify any geographical patterns in these differences, which could be explained by founder events and/or local differences in pollinator communities, together with occasional propagation as a result of human activity.

Considering the high and low sexual organ levels, reciprocity between anthers and stigmas appears to be greater at the low level. This has already been described for other distylous genera, such as *Lithodora* Griseb. and *Glandora* D. C. Thomas, Weigend & Hilger (Boraginaceae) (Ferrero *et al.*, 2011b). However, in other stylar dimorphic cases, such as *Narcissus* L., opposite results have been found (Baker, Thompson & Barrett, 2000; Cesaro *et al.*, 2004). The model proposed by Lloyd & Webb (1992b) for the evolution of heterostyly argues that the segregated deposition of pollen from the two morphs on different parts of the body of the pollinator would select the most reciprocal phenotypes in a population. However, selection may operate on one sexual level, but not on the other, if the behaviour and efficiency of the pollinator differs at each level (high

or low). According to the descriptions, in species with tubular flowers visited by specialized pollinators, selection operates in the same way on both levels. However, flowers of *Melochia* are open with only a small floral tube at the base, so that only the low level is inside the tube. Under these conditions, although the high level is exposed to the visit of any ineffective visitor, the low level is available for only more efficient pollinators specialized in disassortative pollen transfers (M. Faife-Cabrera *et al.*, unpubl. data). The higher reciprocity at this level could be a consequence of more accurate pollen transfer by pollinators in the lower parts of the flower.

Differences in the degree of herkogamy and reciprocity have been described previously in other genera as a result of variations in the assemblage of pollinators between populations (Ferrero *et al.*, 2011a). In the case of *Melochia* spp., they are frequently distributed in perturbed habitats (e.g. road borders, crops, grazing areas; Goldberg, 1967) with a preponderance of generalist pollinators (Ramírez & Navarro, 2010). These areas are under changing environmental pressures that could determine spatio-temporal variations in the assemblage of pollinators (see Herrera, 1988; Guitián, Guitián & Navarro, 1996; Navarro, 2000), which may also determine shifts in pollen transfer efficiency.

The analysis of whether the wide variation in terms of reciprocity between populations and ancillary characters in *Melochia* spp. is related to changes in the patterns of pollinators and/or shifts in the degree of incompatibility is a challenge. This type of analysis should be conducted in a phylogenetic context to elucidate the evolutionary mechanisms of heterostyly. The results of this descriptive study demonstrate that this group of plants is a good system for the study of evolutionary processes involving the gain or loss of heterostyly.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Sample size, corolla length, pistil length, anther height and reciprocity values for both levels of the sexual organs for the studied populations of *Melochia* species. Arithmetic median and coefficient of variation (CV) in millimetres are shown. For monomorphic populations (i.e. with no differentiation in L- and S-morphs), results are shown in the L-morph columns.

**Table S2.** Arithmetic mean and coefficient of variation (CV) of pollen production estimations, and pollen diameter ( $\mu\text{m}$ ), of six *Melochia* species from Cuba. Ratio L/S of pollen production (PPR) and ratio S/L of pollen diameter (PDR) are given. Values in bold differ significantly at  $P < 0.05$ . L, long-styled morph; S, short-styled morph; *N*, sample size.