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Strength through unity: spatial affinity between morphs improves fitness in incompatible heterostylous *Melochia* (Malvaceae) species

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Abstract In heterostylous plants, both stilar polymorphism and incompatibility system favor legitimate pollination among individuals. Weak or partial expression of incompatibility may ensure progeny when mates or pollinators are scarce in unstable habitats, but under these conditions plants with heteromorphic incompatibility would be in disadvantage. In this work we determine how the spatial distribution of morphs and the effect of proximity to the nearest potential mates affect plants' reproductive output in four *Melochia* species. The general prediction of decreasing reproductive success with an increasing isolation of floral morphs in plants with heteromorphic incompatibility was corroborated only in one species (i.e. *M. tomentosa*). Meanwhile, the other species exhibit a spatial affinity between morphs (i.e. the number of individuals with the nearest neighbor of the opposite morph exceeds expectations upon a random distribution). For *M. savannarum* and *M. villosa* we could not detect any effect of proximity to potential mates on the seed-ovule ratio. This may be due to: (1) existence of pollinators with long flying distances, like butterflies, in the populations

and/or, (2) the possible occurrence of resource limitation. Spatial affinity between morphs in populations of heterostylous plants with heteromorphic incompatibility system increases reproductive success and may facilitate colonization of ephemeral habitats.

Keywords Heterostyly · Nearest-neighbor distance · Seed-ovule ratio · Segregation pattern · Spatial distribution

Introduction

Floral morphology in plants has evolved many times towards avoidance of self-interference and promotion of outcrossing (Ashman 2006; Barrett and Hodgins 2006; Darwin 1876; Willmer 2011). Stilar polymorphisms such as heterostyly are some of the best-known examples of such adaptations (Darwin 1877). Heterostylous species present populations with two (distyly) or three (tristyly) types of individuals or morphs that differ reciprocally in the height of the anthers and stigmas in their flowers; this is known as reciprocal herkogamy (Barrett 1992, 2002). For distylous species, individuals of the L-morph display the stigmas above the anthers whereas stigmas of the S-morph plants are placed below the anthers. Reciprocal herkogamy is often accompanied by an incompatibility system that prevents physiologically from selfing and from fertilization between individuals of the same morph (Barrett 1992). Both the reciprocal herkogamy and the incompatibility system have an important function favoring legitimate pollination, also termed disassortative mating (i.e. crosses between plants of different floral morph (Barrett 1992; Darwin 1877). However, the heteromorphic incompatibility system appears to have evolved independently reciprocal herkogamy (Ferrero et al. 2012), and in some cases

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it can be lost (reviewed in Barrett and Cruzan 1994; Ferrero et al. 2012; Santos-Gally et al. 2013) or modified to some extent (Barrett and Harder 2005; Brys et al. 2008; Faria et al. 2012; van Rossum et al. 2006).

The weak or partial expression of the morph-incompatibility may ensure progeny during mate or pollinator scarcity in ephemeral habitats (Baker 1955; Colautti et al. 2010) and the differences in the expression of incompatibility system between morphs can lead to differential levels of inbreeding depression (Weber et al. 2012, 2013) and also contrasting effects of isolation on plant reproductive success. For instance, in *Pulmonaria officinalis*, plants of the L-morph are capable of producing more seed after illegitimate crosses (both selfing and crosses among plants of the same morph) than those of the S-morph (Brys et al. 2008). In this species the spatial distribution and the degree of isolation of individuals of a morph with individuals of the opposing morph (hereinafter referred as “isolation”) affects differently both morphs in such a way that the S-morph plants depend more on nearby availability of potential mates of the opposite morph to produce fruits than the L-morph plants do (Brys and Jacquemyn 2010).

In those heterostylous plants with a heteromorphic incompatibility system self- and within-morph fertilizations are prevented and no differences in inbreeding depression between morphs are expected a priori (Weber et al. 2013). Moreover, the proximity of potential mates in these cases (hereinafter referred as “spatial affinity”) is assumed to have a similar effect in the reproductive success of both morphs (Brys and Jacquemyn 2010; Ishihama et al. 2006; Levin 1974). Thus, in this case isolation of individuals should be particularly disadvantageous for mating opportunities (Brys et al. 2007). Besides, the role of pollinators must be considered irrespective of the distribution patterns of morphs in the populations. A more aggregated distribution of potential mates (i.e. each individual is usually nearby another of the opposite morph) is expected to improve fruit set when pollinators move between close plants (see Levin 1974). However, in populations where many individuals of the same morph are grouped together, pollinators flying short distances would promote illegitimate pollinations among individuals causing pollen clogging (Shore and Barrett 1984). In both cases, those effects could be diluted when pollinators' foraging strategy consist in longer flying distances.

Most *Melochia* species are heterostylous from tropical and subtropical regions (in America, from approximately 33°N to 33°S latitude on the east coast, and from 29°N to 12°S latitude on the west coast; in the Old World from 37°N in eastern Asia to 24°S in the Pacific Islands, and from 18°N to 16°S in western Africa and 2°S to 20°S in eastern Africa) (Goldberg 1967). Despite current information about floral morphology (Faife-Cabrera 2014; Faife-Cabrera et al. 2014; Martin 1967) and breeding system of

Table 1 *Melochia* species and populations studied in Cuba

Species	Population	Coordinates	Elevation
<i>M. savannarum</i>	Siguanea, Isla de la Juventud	21°37'N, 82°57'W	7
<i>M. tomentosa</i>	Caletones, Holguín	21°12'N, 76°14'W	4
<i>M. tomentosa</i>	Cayo Coco, Ciego de Ávila	22°31'N, 78°21'W	6
<i>M. villosa</i>	Aguada de Pasajeros, Cienfuegos	22°25'N, 80°49'W	40
<i>M. villosa</i>	Viñales, Pinar del Río	22°37'N, 83°44'W	143
<i>M. parvifolia</i>	El Salao, Santiago de Cuba	20°00'N, 75°45'W	45

Elevation is provided as meters above sea level

some species (Faife-Cabrera 2014; Machado and Sazima 2008; Ramírez and Navarro 2010) much work is needed to better understand its ecology.

Several *Melochia* species colonize disturbed environments (Goldberg 1967) and most of them are usually self- and intramorph-incompatible (Faife-Cabrera 2014; Machado and Lopes 2004; Machado et al. 2006; Machado and Sazima 2008; Ramírez and Navarro 2010). Most of these *Melochia* species have generalist pollinator assemblages composed of *Apis mellifera*, *Centris* sp., *Xylocopa* sp. and *Ascia monuste* (Faife-Cabrera 2014; Machado and Sazima 2008; Ramírez and Navarro 2010). In this study, we determine how the spatial closeness of compatible morphs (spatial affinity) affects the reproductive output of several *Melochia* species that have a heteromorphic incompatibility system. For this, we firstly determined the affinity levels between morphs in several populations. The affinity level refers to whether the number of individuals with the nearest neighbor of the opposite morph exceeds expectations based upon an assumption of random distribution (Levin 1974). Secondly, we examined the effect of distance to the nearest potential mates (i.e., the nearest individual of the opposite morph) on the seed-ovule ratio of plants in these populations and species.

Materials and methods

Sites and studied species

We studied six populations of four distylous species of the genus *Melochia* in Cuba (Table 1). All of them are perennial herbaceous plants flowering and fruiting throughout the year (Goldberg 1967). They show a dish-shaped corolla and dehiscent capsules that develop a maximum of 10 seeds (Rodríguez 2000). Two of these species are typically found at disturbed habitats: *M. villosa* (Mill.) Fawc. & Rendle and *M. parvifolia* Kunth; whereas *Melochia savannarum* Britt.

is restricted to siliceous white-sands in Pinar del Río and Isla de la Juventud, and *M. tomentosa* L. inhabits coastal xeromorphic thickets (Goldberg 1967; Rodríguez 2000). The presence of a heteromorphic incompatibility system has been recently confirmed for the four species in these studied populations (Faife-Cabrera 2014). The heteromorphic incompatibility system prevents from selfing and from pollinations among individuals of the same morph (see also Machado and Sazima 2008; Ramírez and Navarro 2010).

Spatial affinity between morphs

In each of the populations studied, we randomly chose 10 plants per morph and identify the morph of the nearest neighbor. We assessed the spatial affinity between morphs (i.e. the frequency of individuals with the nearest neighbor of the opposite morph) in the populations using the segregation index (S) proposed by Levin (1974). The S index reflects the degree to which individuals of one morph are related to each other and with the opposite morph, following the concept of segregation elaborated by Pielou (1961). The S index was thus calculated as follows: $S = 1$ (observed number of LS and SL pairs/expected number of LS and SL pairs), where LS indicates the L-morph is the focal plant and the S-morph is the nearest neighbor and SL denotes combinations of S-morph focal plant and L-morph one as the nearest neighbor. The index could take the following values: zero for an unsegregated population; 1 for a completely segregated population, in which plants of the same morph are closer than expected by chance; negative values in the index S point to a spatial affinity between the floral morphs which means it will be more frequent to find in the population individuals of different morph together than with a random pattern (Levin 1974). Significance of spatial pattern (S index) was tested through Chi square 2×2 contingency tables. Contingency tables were built with the observed and expected proportions of each of the four types of nearest-neighbor relationships between morphs, at each population (i.e. the combinations LL, SS, LS, SL).

Effect of distance to potential mates

In order to test the effect of proximity of compatible neighborhood on sexual reproductive success, we used the same 10 flowering individuals of each floral morph in each population. For each selected plant we collected the fruits in a branch to quantify seed to ovule ratio, as an indicative of legitimate pollination. The branches in the plants were chosen seeking for the same size and exposition in all of them. In plants with a heteromorphic incompatibility system the number of seeds per fruit should depend on the quality of the pollen deposited in their stigmas, since only the legitimate pollen would develop in viable seeds. We

Table 2 Values of segregation index (S), following Levin (1974) and results of the Chi square (χ^2) (and their *P*-values) between the observed and expected frequency of the four types of nearest-neighbor relationships between morphs, at each population (i.e. the combinations LL, SS, LS, SL)

Species (population)	S	χ^2	<i>P</i> -value
<i>M. savannarum</i> (Siguanea)	−0.60	7.20	0.01
<i>M. tomentosa</i> (Caletones)	−0.42	2.57	0.11
<i>M. tomentosa</i> (Cayo Coco)	−0.20	0.95	0.33
<i>M. villosa</i> (Aguada de Pasajeros)	−0.60	7.20	0.01
<i>M. villosa</i> (Viñales)	−0.60	4.67	0.03
<i>M. parvifolia</i> (El Salao)	−0.60	7.50	0.01

Ten individuals per morph at each population were used for S calculation. Significant differences are highlighted in bold

also counted the number of opened flowers and fruits present in the branch at this time to account for possible effects of plant vigor on the reproductive output.

Besides, for each of the individuals analyzed, we calculated both the distance to the nearest individual of the opposite morph and also the harmonic mean of the five measurements to the closer potential mates. We used the harmonic mean rather than other measures of central tendency because it is not so affected by extreme values, thus being more conservative for the remoteness of the neighborhood (Ferber 1931). With this information we tested the effect of proximity of the neighborhood on seed to ovule ratio of each morph.

For the statistical analysis we used Generalized Lineal Models for each species. The distance to the nearest individual of the opposite morph and floral morph were set as fixed factors, while flower production and fruit production were incorporated as covariates. Additional models were performed with the same specification but using instead as factor the harmonic mean of the distance to the five nearest individuals of the opposite morph. These latter models were employed to assess the possible effects of the neighborhood composed by individuals of the opposite morph. Analyses were performed in R (R Core Team 2013) using the ‘quasibinomial’ as family type with a ‘logit’ link function. To perform the models, we applied a log-transformation of both distances and we transformed flower production and fruit production using $\log(X + 1)$. We chose a significant level of $\alpha = 0.05$.

Results

Spatial affinity between morphs

According to the values of segregation index (S), all the studied populations showed spatial affinity between floral

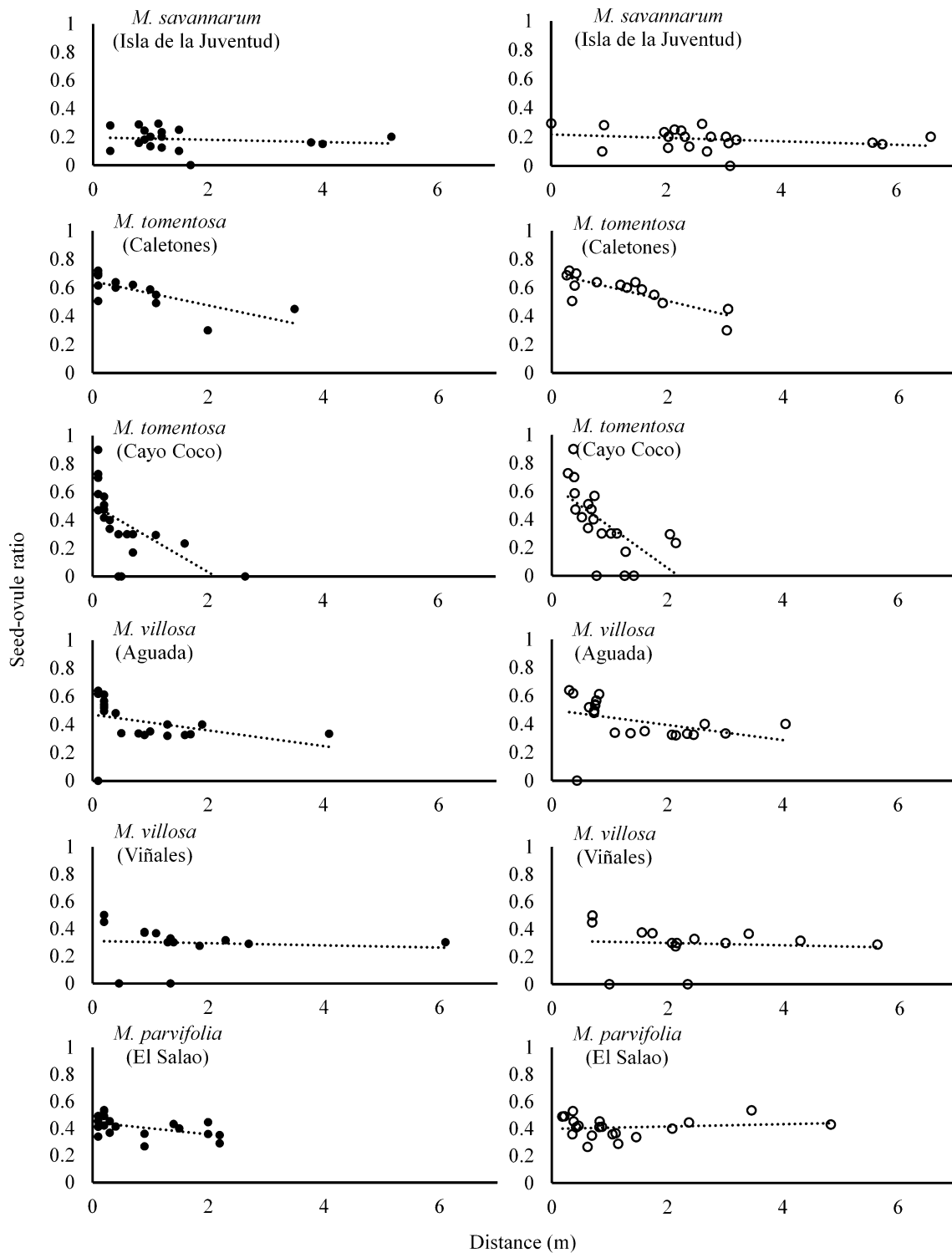


Fig. 1 Relation between seed-ovule ratio (S/O) and (1) distance to the nearest potential mate (*filled circle*) and (2) the harmonic mean distance to the five nearest potential mates (*open circle*), in

populations of four self-incompatible species of the genus *Melochia*. Population names are given in parenthesis

morphs (Table 2). Populations of *M. tomentosa* presented the highest values of S. However, Chi square tests were not significant for any populations of this species,

indicating instead a random spatial association of plants. For the populations of the other three species there were statistically significant departures from random spatial

Table 3 Results for the Generalized Lineal Models on the effect of distance to the nearest potential mate (and neighborhood), flower and fruit number, and morph type, on seed-ovule ratio in populations of four self-incompatible *Melochia* species

Species	Variables	Nearest potential mate		Neighborhood mate	
		<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
<i>Melochia savannarum</i> (Isla de la Juventud)	Distance	−0.187	0.854	−0.381	0.708
	Flowers	−0.733	0.475	−0.599	0.558
	Fruits	0.154	0.775	2.048	0.059
	Morph	0.762	0.458	0.722	0.481
<i>M. tomentosa</i> (Caletones)	Distance	−2.511	0.033	−2.525	0.033
	Flowers	0.208	0.840	0.376	0.716
	Fruits	0.357	0.729	0.249	0.809
<i>M. tomentosa</i> (Cayo Coco)	Distance	−4.432	>0.001	−3.420	0.004
	Flowers	−0.373	0.714	−0.307	0.763
	Fruits	0.287	0.778	0.750	0.465
<i>M. villosa</i> (Aguada de Pasajeros)	Distance	−1.305	0.211	−1.109	0.285
	Flowers	−0.765	0.456	−0.746	0.467
	Fruits	2.092	0.054	2.025	0.061
<i>M. villosa</i> (Viñales)	Distance	−1.436	0.185	−1.229	0.250
	Flowers	1.135	0.286	1.042	0.324
	Fruits	0.991	0.348	1.032	0.329
<i>M. parvifolia</i> (El Salao)	Distance	−1.608	0.129	−0.022	0.983
	Flowers	0.177	0.862	−0.514	0.615
	Fruits	−0.239	0.814	−0.442	0.665
	Morph	−0.967	0.349	−1.625	0.125

To test for the neighborhood effect, mean harmonic distance to the five nearest potential mates was used as independent variable. Significant differences are highlighted in bold

association (Table 2). In these populations is more likely to find different morphs together than expected by chance.

Effect of the nearest potential mate and neighborhood on seed-ovule ratio

The relation between the seed-ovule ratio (S/O) and the distance to the nearest potential mate and neighborhood is shown in Fig. 1. In all species, distances to the five nearest neighbors of the opposite morph were no further than six meters for most individuals in the studied populations (Fig. 1). Only for the two populations of *M. tomentosa* an increasing distance to the nearest potential mate and to the neighborhood of potential mates was significantly related to a decrease in the seed-ovule ratio (Table 3). Non-significant differences between floral morphs or no effect of

flower and fruit number on plant on seed-ovule ratio were found for any of the species and populations surveyed.

Discussion

Spatial distribution of potential mates varies in the *Melochia* species studied and has different effects on individuals' reproductive success. Despite all the surveyed *Melochia* species are self-incompatible and reproduction is not feasible between plants of the same morph (Faife-Cabrera 2014; Machado and Sazima 2008; Ramírez and Navarro 2010), only populations of *M. tomentosa* exhibited a significant effect of spatial distribution on seed-ovule ratio. In this species, the seed-ovule ratio was negatively associated with increases in the distance to potential mates, which was our general expectation for heterostylous species with heteromorphic incompatibility system.

Differential effects of distance to the nearest mates on seed-ovule ratio could be explained through the analysis of spatial distribution of morphs and their relation with the activity of pollinators. Most of the populations surveyed in this study have their five nearest-neighbor of the opposite morph in the surrounding six meters, showing, additionally, a spatial affinity between morphs (negative values of S index, see Table 2). A random spatial distribution of the morphs can be expected in distylous populations where disassortative mating is prevalent due to negative frequency-dependent selection, unless some causes determine a distinct pattern like vegetative growth, differential niche exploitation favoring selective survivorship of one morph over another or founder events (Levin 1974; Nicholls 1986; van Rossum and Triest 2007). There are many examples in literature describing spatial segregation of L and S morphs (e.g., Levin 1974; Hodgins and Barrett 2008; Weller 1981), random spatial distribution (e.g., Ornduff 1980; Schou 1983) or spatial affinity (e.g., Nicholls 1986; Ornduff 1980; Wolfe 2001). The aggregated disposition of morphs suggests that legitimate pollinations occur within a few meters from the pollen donor (Brys et al. 2007; Wolfe 2001). This appears to be the case of most studied *Melochia* species. However, in *M. tomentosa* morphs appear to be rather randomly distributed in the populations. In this species, a decrease in the distance to the nearest potential mate (and also to the neighborhood formed by individuals of the opposite morph) appears to boost the levels of legitimate pollination. In relation to the neighborhood proximity, the existence of many legitimate pollen sources could improve individual seed production (Schemske and Pautler 1984).

Moreover, pollinator foraging behavior may affect the quality of pollen loads (Herrera 1987). Some pollinators forage intensively, minimizing flying distances and selecting patches of flowers rather than solitary flowers.

This behavior has been described for generalist hymenoptera pollinators like *Apis mellifera* (Herrera 1987; Waddington 1980). Indeed, this is a very frequent floral visitor of both *M. tomentosa* and *M. parvifolia* (Machado and Sazima 2008; Ramírez and Navarro 2010). Since plants of *M. parvifolia* display a strong spatial affinity between morphs (values of $S = -0.60$) closer neighbors are expected to be of the opposite morph. The short flights of pollinators like *A. mellifera*, and other hymenopterans with a similar behavior, will then result in great amounts of legitimate pollen deposited on stigmas. However, in *M. tomentosa*, where there is not spatial affinity between morphs, the effect of proximity to potential mates is more intense—as the values of seed-ovule ratio indicate. The disposition of morphs in this species could favor an insect to visit successive plants of the same morph more frequently than in the rest of the species, and this may result in greater amounts of pollen wasted.

A similar reproductive success between morphs matches with the expression of the heteromorphic incompatibility system in *M. tomentosa* and its similar capacity to set fruits and seeds (Machado and Sazima 2008). Differences in reproductive success between morphs have been found rather for self-compatible species in which one of the morphs shows higher degree of compatibility (Brys and Jacquemyn 2010) or in populations with unequal morph frequencies as a result of the negative frequency-dependent selection (Brys et al. 2007; Eckert et al. 1996; Thompson et al. 2003). Additionally, the number of flowers and fruits has no effect on the seed-ovule ratio. This means that plant reproductive success does not depend heavily on the vigor of the plants in the species.

On the other hand, the results found for *M. savannarum* and *M. villosa* may have also two main causes. The first of these putative causes is the incidence of pollinators with longer flying distances, like butterflies. Butterflies are usual pollinators of several *Melochia* species (see Kato et al. 2008; Machado and Sazima 2008; Ramírez and Navarro 2010). They describe longer flying distances than other pollinators, like hymenopterans, when foraging on flowers (Herrera 1987). Additionally, although pollinators can transfer enough amounts of pollen to maximize seed set, plants could not reach that maximum if they suffer of resource limitation. In many cases, resource limitation rather than pollen limitation could affect fruit or seed set (Ashman et al. 2004; Guitián et al. 1996; Guitián and Navarro 1996; Knight et al. 2006; Navarro 1996, 1998; Yang et al. 2005), although both can act at the same time (Brookes et al. 2008). This effect of resource limitation on seed-ovule ratio could be especially critical for these two species of *Melochia* due to the low level of nutrients in the soils they occupy (Borhidi 1991; Rodríguez 2000). The fact that just *M. savannarum* and *M. villosa* are precisely the

two species that do not produce a maximum seed set even after pollen supplementation is an indicative of this prediction (Faife-Cabrera 2014). For the endemic *M. savannarum*, resource limitation could be particularly important since it inhabits at the strongly poor-nutrient siliceous white-sands savannahs of the western region of the Cuban archipelago, exposed, in addition, to recurrent fires (Borhidi 1991; Rodríguez 2000).

Spatial affinity between morphs is the pattern more common in the studied *Melochia* species. In heterostylous species with a heteromorphic incompatibility system the affinity between morphs appears to be advantageous when colonizing unpredictable habitats by ensuring a higher probability of pollinators achieving legitimate pollinations. However, the causes of the prevalent spatial affinity rather than the expected random distribution of morphs in the *Melochia* populations, as well as how this spatial pattern has arisen are still unclear, and remains here as an important question to be answered in the future.

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