

Chapter V

A new floral mimicry system between Turneraceae and Malvaceae is Müllerian but not mutualistic

Abstract

Known cases of floral mimicry, i.e. the sharing of a similar flower colour and display pattern by different plant species, involve a rewarding model and a rewardless mimic. Almost unknown in flowering plants is the perhaps much more common Müllerian type of mimicry where both the model and mimic offer rewards and for which it is assumed that both profit from their mutual resemblance. Here the adaptiveness of Müllerian similarity between flowers is explored for the first time, demonstrating that *Turnera sidoides* ssp. *pinnatifida* (Turneraceae) obtains a higher pollination service by sharing pollinators with *Sphaeralcea cordobensis* (Malvaceae). These two species resemble each other in their uncommon colour to the pollinators' eyes and their reward properties. Another geographical colour race of *T. sidoides* also mimics the displays of local Malvaceae. Instead of the expected mutualistic Müllerian mimicry, we have here evidence of a one-sided mimicry in which the mimic hitches a ride along with its successful rewarding model.

Introduction

To attract animal pollinators, plant species may either exhibit unique flower displays or imitate models that are available in their environment. In particular, the imitation of the flowers of one species by another is known as floral mimicry. Contrasting with antagonistic or Batesian flower mimicry, mutualistic or Müllerian mimicry involves two or more species with similar flowers that reward pollinators. It is believed that they benefit from their common advertising display by reaching a higher combined flower density (Dafni 1984, 1986, Roy and Widmer 1999). The idea that this kind of floral mimicry actually occurs, has been challenged for a number of reasons. Most important among them is the belief that improper pollen transference constrains its evolutionary emergence (Rathcke 1983, Waser 1983) by reducing male and female reproductive success through incongruent pollen deposition and stigma clogging. Further, the mere superficial similarity between the flowers to the human eye is insufficient for recognizing a mimicry system, i.e. mimics must resemble each other in flower display from the pollinator's perceptual point of view. In addition, mimics have to depend on the same individual pollen vectors for pollination, and the pollinators must be able to move freely between them. Finally, mimicry must have a positive effect on pollination and reproductive success of at least one of the mimics (Roy and Widmer 1999, Johnson 1994). This aspect, namely the adaptiveness of Müllerian flower mimicry, though a crucial one, has not been explored before (Roy and Widmer 1999).

Even if the above objections are resolved one could expect a mimic to have evolved to a greater similarity to its model or mimics to have coevolved to mutual similarity. This consideration is important in view of the possibility that convergent similarity might also be the result of two or more species meeting the sensorial preferences of shared pollinators. Thus, we expect that populations of a mimic growing with different models will deviate in flower colour from each other to resemble the respective co-occurring model.

To demonstrate the adaptiveness of flower similarity between species we studied the system formed by two plants that belong to families in which flower mimicry had not been suspected before: *Sphaeralcea cordobensis* Krapov. (Malvaceae) and *Turnera sidoides* L. ssp. *pinnatifida* (Poiret) Arbo (Turneraceae). We also provide evidence from geographical colour variations, that the latter member of the system imitates different co-occurring models.

Material and Methods

Sphaeralcea cordobensis and *Turnera sidoides* ssp. *pinnatifida* are self-incompatible and stress-tolerant perennial herbs that co-occur in the Córdoba Mountain Range (Argentina) and are pollinated by solitary bees. *S. cordobensis* has a restricted distribution range (Krapovickas 1949), while *T. sidoides* is widespread from Southern Bolivia to Central Argentina, including several geographical colour races (Solís Neffa 2000).

We studied the resemblance in visual display from the pollinator's perceptual point of view. To this purpose, we measured the petal reflectance of *T. sidoides* and its possible mimicry partner flowers' spectra by means of a SD 2000 spectrometer (Ocean Optics, Florida, USA). We recorded reflectance spectra from Córdoba Province, Argentina (31°15'S; 64°18'W) where a pink race of *T. sidoides* grows together with *S. cordobensis* and in Salta Province (24°39'S; 65°22'W) where a yellow race co-occurs with two additional malvaceous plant species: *Sida rhombifolia* and *Modiolastrum malvifolium*. To explore the extent of variation of flower colour within Turneraceae, we obtained reflectance spectra of eight subspecies and species related to *T. sidoides* ssp. *pinnatifida* from the living collection of IBONE (Instituto de Botánica del Nordeste, Corrientes, Argentina). Additionally, to investigate whether the colour of mimicry partners is widespread in the community, we recorded the reflectance spectra of coflowering species at the Córdoba site. The perceptual similarity of the floral colours was estimated using the Receptor Noise Limited model of honeybee colour vision (Vorobyev *et al.* 2001). The obtained colour loci were represented in the bee's perceptual colour space, in which a critical distance of 2.3 units represents the threshold for colour discrimination (Vorobyev *et al.* 2001), and one unit corresponds to one standard deviation of bee photoreceptor noise. To estimate the dispersion of colour loci between flower species, the mean chromatic distances between all individual loci pairings were calculated. In addition, single floral displays were visualised as they are seen by the bees (Vorobyev *et al.* 1997). To this purpose, flowers were imaged through a set of chromatic filters (Vorobyev *et al.* 1997). The estimated bee photoreceptor excitations in the short (S), medium (M) and long (L) wavelength ranges were coded with the primary monitor colours, and the resolution of a bee compound eye was simulated for an angular subtense of the floral display of 16° (6-9 cm distance) which lies within the perceptual range of chromatic pattern cues (Hempel de Ibarra *et al.* 2001).

To compare rewarding properties we covered newly opened flowers for five hours and quantified with 5 μ l microcapillaries nectar amounts of fifty flowers of *S. cordobensis* and twenty eight flowers of *T. sidoides*. Nectar concentrations were measured in fifteen flowers of *T. sidoides* and fifteen flowers of *S. cordobensis* using a hand refractometer (Atago) in Brix % scale. Mann-Whitney *U* tests were used to compare nectar volume and concentration.

To determine whether pollen vectors were shared among mimics, we analysed pollinator assemblage, pollen loads on pollinators and flowering phenologies. The pollen loads of 63 bees captured when visiting the flowers of either species were examined under the microscope. Pollen of mimics and other co-flowering plants was identified by comparison with reference pollen samples from the same plant communities. We determined flowering phenologies by checking the number of flowers produced in six periods of fourteen days each, covering a year's flowering season (September – December 2002). The match between the phenology of both species was examined by means of Pearson's correlation index.

We studied the possible benefit of both species flowering together in terms of pollination services by comparing female reproductive success in plants from mixed and single species patches. For *T. sidoides* the number of conspecific pollen grains on the stigma was used as measure of the effectiveness of pollinator success. Since this species is heterostylous, stigmas and anthers are at different levels making spontaneous self-pollination almost impossible, thus pollen found on the stigmas is a good measure of pollen transference by pollen vectors. Pollen loads on the stigmas of 142 plants (61 from mixed and 81 from single species patches) were analyzed with epifluorescent microscopy. The number of conspecific pollen grains/flower was compared between plants in mixed and single species patches with Mann Whitney *U* test. For *S. cordobensis* the number conspecific pollen grains on the stigmas was not an adequate measure of pollinator services since the stigmas are intermingled with numerous anthers making spontaneous pollen transfer within a flower possible. For this reason we preferred to use fruit set (fruits/flowers) as measure of female fitness in this species; though it is not as precise as pollen deposition on the stigmas to show pollinator effectiveness. Arcsine-transformed fruit set was compared between 140 and 76 plants growing respectively in mixed and single species patches, using a *t*-test.

The effect of patch density on female reproductive success (fruit set) was analysed for both species in nine mixed patches of increasing combined density. A regression analysis was performed to test whether patch density increases reproductive success. The consequence of

improper pollen transfer on reproductive success of *T. sidoides* (only conspecific pollen were observed in all *S. cordobensis* stigmas inspected) was examined by comparing the arcsine transformed percentage of heterospecific pollen on fruiting and non-fruiting stigmas, using a *t*-test. This was possible because stigmas remain undamaged on initiate fruits and wilted flowers of *T. sidoides*.

Results

Floral colour and display pattern

The petal reflectance spectra of mimicry partners, showed a very close match. According to the model of bee vision used, *T. sidoides* was indistinguishable in petal colour from supposed mimicry partners: *S. cordobensis* in Córdoba and *M. malvifolium* – *S. rhombifolia* in Salta. The respective loci of the floral reflectances lay close together in the bee's perceptual colour space within the threshold of discrimination (mean chromatic distance: 1.08 ± 0.58 S.D. Fig. 1a). Both mimicry partners showed a similar degree of colour variability (*T. sidoides*: 1.10 ± 0.50 S.D.; *S. cordobensis*: 1.02 ± 0.62 S.D.). The geographical race of *T. sidoides* spp. *pinnatifida*, from Salta province (Argentina), was different in colour for bees from the former race (mean chromatic distance 2.94 ± 0.84 S.D., Fig. 1a). It resembled local malvaceae in its yellowish colour for the bee eye (mean chromatic distance between colour loci of *T. sidoides* and the putative mimicry partners of 1.77 ± 0.76 S.D. and 1.37 ± 0.5 S.D. for *S. rhombifolia* and *M. malvifolium*, respectively, Fig. 1a). Also bee-views of the floral displays of the mimicry partners were similar, as shown in Fig. 1b. Other co-flowering species of the Córdoba community were dissimilar in colour (mean chromatic distances to *T. sidoides*: 11.1 ± 3.84 S.D; range 4.9 to 17.5 units; $n = 15$ species, Fig. 2) except for *Abutilon pauciflorum*, a malvaceous plant scarce in the area and similar to *S. cordobensis*.

Inspecting phylogenetic relatives, we found that flower colouration of geographical races of *T. sidoides* ssp. *pinnatifida*, other subspecies of *T. sidoides*, and species of Turneraceae resulted in a wide range of bee-subjective colours (Fig. 3).

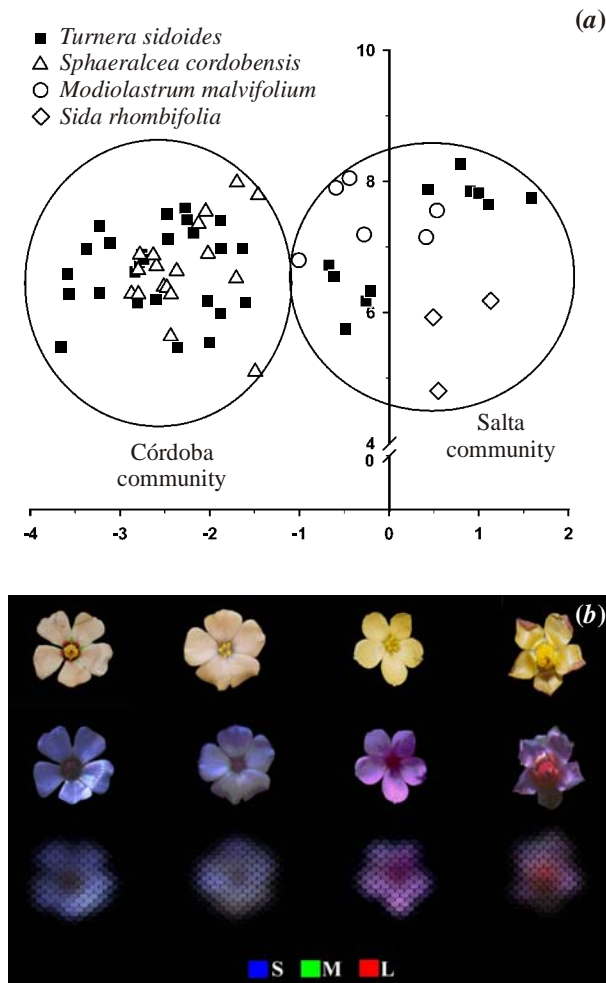


Fig. 1 Two colour races of *Turnera sidoides* ssp. *pinnatifida* resemble their models.

(a) The enclosed left group, flowers of *T. sidoides* ssp. *pinnatifida* from Córdoba populations resembled those of the local Malvaceae *Sphaeralcea cordobensis*. In the right group, flowers of *T. sidoides* ssp. *pinnatifida* from Salta populations resembled those of two Malvaceae: *Modiolastrum malvifolium* and *Sida rhombifolia*. The colour loci of their petals in the perceptual colour space of bees (Receptor Noise Limited model) are intermingled and within a radius of 2.3 units which correspond to the threshold distance between loci, thus, being indistinguishable to bees.

(b) Floral displays as seen by the human and the bee eye: Floral displays of *T. sidoides* ssp. *pinnatifida* (central columns) and their respective models, *Sphaeralcea cordobensis* (left-most column) and *Modiolastrum malvifolium* (right-most column), in two distant populations, Córdoba (first and second columns) and Salta (third and fourth columns). Appearance to human eye (first row), and bee-views of floral displays (second and third rows) where primary colours (blue, green, red) label the bee photoreceptor excitations (S, M, L). The third row simulates the low spatial resolution of a bee eye corresponding to a distance to the flower (16° angular subtense or 6-9 cm distance) where bees start exploiting chromaticity of floral colouration as visual cue.

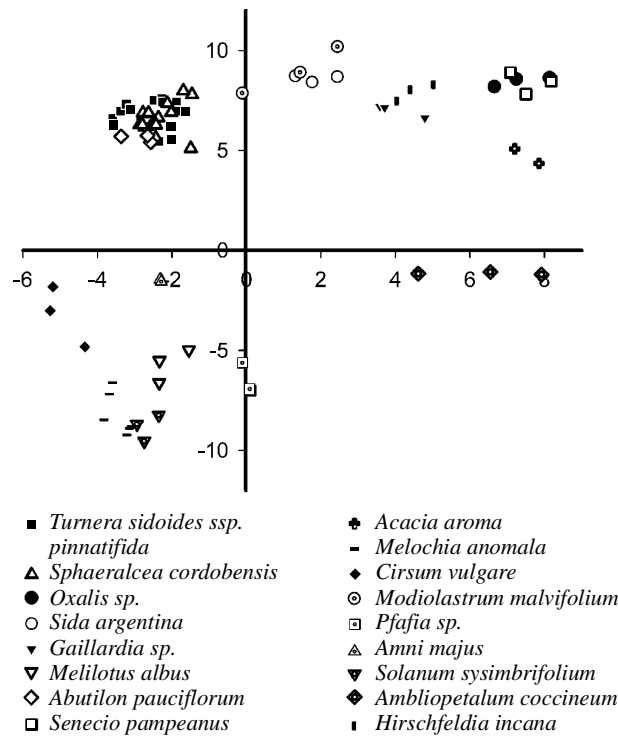


Fig. 2 Colours of mimetic species and co-flowering plants of the Córdoba community. *Sphaeralcea cordobensis* and *Turnera sidoides* share their sector of perceptual colour space of bees (Receptor Noise limited model) only with *Abutilon pauciflorum*, another Malvaceae rarely present in the site. Colours of all the other species are clearly distinguishable for bees.

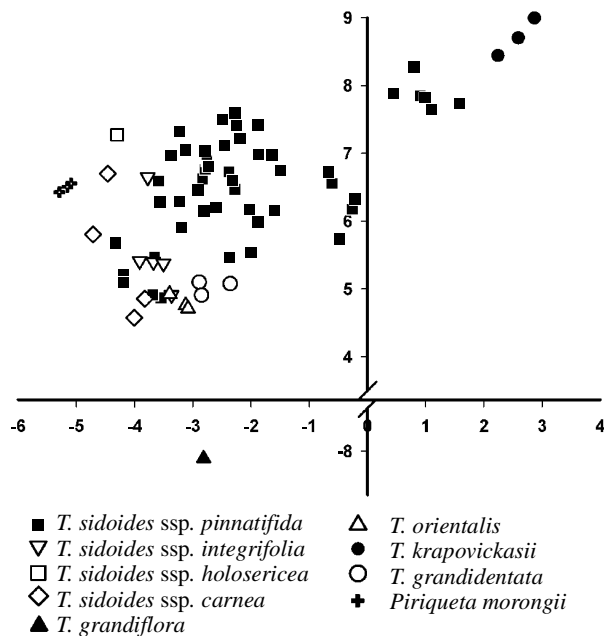


Fig. 3 Colour variation in Turneraceae. Colour variation among the whole geographical range of *T. sidoides* ssp. *pinnatifida*, including pink and yellow races. Other *T. sidoides* sub-species and closely related turneraceous species which display different colour variants are presented. Most of them are different from *T. sidoides* ssp. *pinnatifida* to bee eyes. The colour loci of their petal reflections are represented in the perceptual colour space of bees (Receptor Noise Limited Model).

Nectar

The sugar concentration of the nectar differed significantly between mimicry partners in the Cordoba site (Mann-Whitney U test: $U = 32$; $p < 0.001$). The concentration of *S. cordobensis* (36.79 ± 8.25 Brix %) was approximately twice as high as that of *T. sidoides* (20.97 ± 10.62 Brix %). Nectar volume also differed significantly between species (Mann-Whitney U test: $U = 312.5$; $p < 0.001$), but this time *T. sidoides* (2.82 ± 1.43 μ l) had about twice as much nectar as *S. cordobensis* flowers (1.47 ± 0.99 μ l). Hence, reward in terms of sugar amount was similar between species.

Pollinators and flowering phenology

The major pollinators captured at the flowers of both plant species were solitary Euphorini bees of the genera *Diadasia* (two species) and *Leptometriella* (one species) and Colletidae bees of the genus *Leioproctus* (three species). These six species represent 85.29% of the captured bees. We found pollen from either plant species on all of them and of both species on the majority of the captured bees (45 of 68 bees). The bees' pollen loads on ventral parts of the thorax and abdomen and on the scopae consisted mainly of *S. cordobensis* (75.58 %), to a lesser extent *T. sidoides* (16.98 %) and other pollen types (7.44 %). Flowering phenologies were significantly correlated between both plant species ($r = 0.83$; $p = 0.04$).

Pollination service and reproductive success

Loads of conspecific pollen on stigmas of *T. sidoides* were significantly higher in mixed-species patches than in single-species patches (Fig. 4a), indicating higher pollinator services in the former. In contrast, fruit set of *S. cordobensis* did not differ between mixed and single species patches (t -test, $t = -1.65$, $p = 0.10$; mixed patches mean fruit set: 51.5 %; single species patches mean fruit set: 56.8 %). Fruit set of *S. cordobensis* was strongly related to the overall flower density ($n = 9$; $R^2 = 0.51$; $p < 0.01$). Likewise, *T. sidoides* fruit set was significantly correlated with the overall flower density in mixed patches, which was dominated by *S. cordobensis* being 54 times more dense (Fig. 4b).

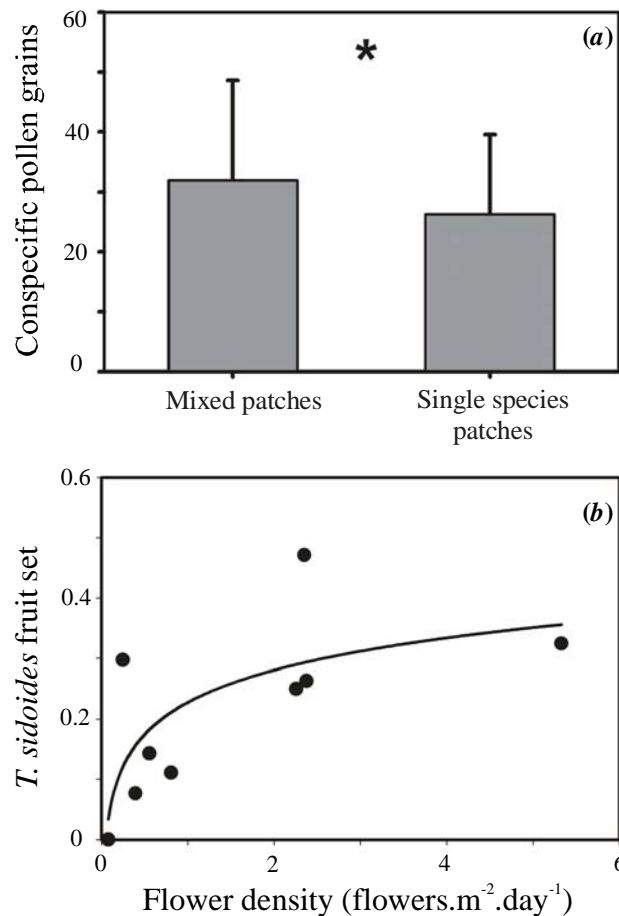


Fig. 4 Impact of patch composition and flower density on *Turnera sidoides* reproductive success.

(a) Growing together enhanced pollination services: more conspecific pollen was found on *T. sidoides* stigmas in the mixed- than in the single-species patches (Mann-Whitney *U* test: $n = 142$ plants; $U = 1964$; $P = 0.04$). Error bars represent standard errors.

(b) Growing together with the malvaceous model is of benefit to *T. sidoides*, since its fruit set is positively correlated with the combined flower density of *T. sidoides* and *S. cordobensis* in mixed patches (logarithmic regression: fruit set = $0.54 \pm 0.13 \ln$ flower density; $n = 9$; $R^2 = 0.57$; $P = 0.02$).

Interference

We examined the pollen loads on stigmas of both species. No *T. sidoides* pollen was found on *S. cordobensis* stigmas, whereas pollen of both species was observed on most *T. sidoides* stigmas (85.22% of 142 flowers). The proportion of improper pollen was low (21.36 ± 17.29 %, $n = 142$) considering the much higher proportion of *S. cordobensis* flowers in mixed patches and its higher number of anthers. Heterospecific pollen did not affect fecundity, since no significant difference was evident in the percentages of heterospecific pollen found on the stigmas of fruiting and non-fruiting flowers of *T. sidoides* (*t*-test; $t = -0.41$; $p = 0.68$; fruiting flowers: 17.08 ± 16.14 %, $n = 88$; non-fruiting flowers 16.37 ± 15.84 %, $n = 54$).

Discussion

We provide several lines of evidence to show that *Turnera sidoides* ssp. *pinnatifida* forms a mimicry system with *Sphaeralcea cordobensis* and other malvaceous species. First, flower colour and colour patterns of the mimics are indistinguishable to bees; second, geographical colour variants of *T. sidoides* match in colours with different local malvaceous plants species; fourth, pollinators may move freely between mimics because flowering occurs simultaneously in the same patches; fifth, specialized pollinators are shared between both species (see below); sixth, female reproductive success of both species increases with combined flower density.

The fact that mimics offer similar reward in terms of sugar content, which is the reward feature claimed to be evaluated by bees during foraging (Varju and Núñez 1991), indicates that the object of this study is a Müllerian mimicry system. Contrary to expectations (Roy and Widmer 1999), pollination services are better in mixed populations than in populations containing only one mimic species (*T. sidoides*). This is reasonable considering that *S. cordobensis* is a dominant species in the community with a much higher density than the other mimicry partner. *T. sidoides* does not contribute significantly to combined flower density but is benefited by the increased flower display of *S. cordobensis*. Thus, we propose that *S. cordobensis* and other malvaceous species are actually models while *T. sidoides* ssp. *pinnatifida* is the mimic. This also suggests that advergent evolution, *i.e.* to higher similarity of mimic to model, and not convergent evolution, *i.e.* to mutual similarity, took place in the formation of the present mimicry system (Johnson *et al.* 2003a).

We can also discern that the system in question arose through mimicry and not as a result of convergence toward traits attractive to common pollinators. We base our observation on the following evidence: First, intra-population spectral reflectances of the mimic and the model match very well, while inter-population differences in the model's spectral reflectance are associated with equivalent changes in the mimic; second, the flower colours of the mimic and the model are rare in the plant community studied in Córdoba. Other flowering plants occupy wide areas of the perceptual colour space of bee vision and are not superposed with *S. cordobensis* and *T. sidoides* (plus the rare *Abutilon pauciflorum*), which occupy alone one whole sector of this colour space.

Flower colour is not phylogenetically constrained – both in the Turneraceae and in *T. sidoides* ssp. *pinnatifida* – since a wide variation in flower colour is exhibited at both family and

species level, which suggests that flower colour has an adaptive value in these plants. Additionally, in *T. sidoides* this variation could be explained by the above mentioned colour superposition with two geographical colour variants between the mimic and differently-coloured malvaceous models.

The same pollinators were recorded on the plants of the mimicry pair. They were carrying pollen of both species. Among these bees, it is interesting that *Diadasia* and *Leptometriella* are known as specialists for *Sphaeralcea* among other Malvaceae in North and South America (Sipes and Tepedino 2005). These findings, together with the phenological superposition, and the presence of *S. cordobensis* pollen on *T. sidoides* stigmas, indicate that putative mimics share individual pollinators and that these can always move freely between them.

The strong prevalence of *S. cordobensis* in the mixed species patches carries the problem of improper pollen transfer onto the stigmas of *T. sidoides* which would impair fruit set and entails a selective factor impeding mimicry (Roy and Widmer 1999). We postulate the existence of a strong mechanism that prevents heterospecific pollen from clogging stigma in *T. sidoides*, because of low amounts of improper pollen found. Also, heterospecific pollen did not seem to impair fecundity of *T. sidoides*, so apparently there are no inhibitory properties of improper pollen.

We suggest the following scenario for the evolution of the present mimicry system. The *S. cordobensis* behaves as a “magnet species” (Johnson *et al.* 2003 b), attracting pollinators conditioned to its dominant floral display. Only plants with flowers resembling such display would be able to profit from the specialized pollinators attracted by the magnet species. These results suggest that selection for flower colour acts on the less abundant mimic, favouring resemblance with the more abundant flower model, and not compensating mutual resemblance of both species in the system.

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