

Breeding system and pollination by mimicry of the orchid *Tolumnia guibertiana* in Western Cuba

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Abstract

The mimicry of malpighiaceae oil-flowers appears to be a recurrent pollination strategy among many orchids of the subtribe Oncidiinae. These two plant groups are mainly pollinated by oil-gathering bees, which also specialize in pollen collection by buzzing. In the present study, the floral ecology of the rewardless orchid *Tolumnia guibertiana* (Oncidiinae) was studied for the first time. The orchid was self-incompatible and completely dependent on oil-gathering female bees (*Centris poecila*) for fruit production. This bee species was also the pollinator of two other yellow-flowered plants in the area: the pollen and oil producing *Stigmaphyllon diversifolium* (Malpighiaceae) and the polliniferous and buzzing-pollinated *Ouratea agrophylla* (Ochnaceae). To evaluate whether this system is a case of mimetism, we observed pollinator visits to flowers of the three plant species and compared the floral morphometrics of these flowers. The behavior, preferences and movement patterns of *Centris* bees among these plants, as well as the morphological data, suggest that, as previously thought, flowers of *T. guibertiana* mimic the Malpighiaceae *S. diversifolium*. However, orchid pollination in one of the studied populations appears to depend also on the presence of *O. agrophylla*. Moreover, at the two studied populations, male and female pollination successes of *T. guibertiana* were not affected by its own floral display, and did not differ between populations. The results are discussed in relation to the behavior and preferences of *Centris* bees, as well as the differential presence and influence of each of the two floral models.

Keywords: *Centris*, Oncidiinae, *Ouratea*, self-incompatibility, *Stigmaphyllon*.

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Introduction

Many rewardless orchids specialize in deceiving pollinators (Dafni 1987; Jersáková *et al.* 2006, 2009). Nevertheless, deceptive orchids are reported worldwide to have lower reproductive success than their rewarding relatives (Neiland & Wilcock 1998; Tremblay *et al.* 2005) and the local co-occurring plants with which they share pollinators (Johnson *et al.* 2003b). This situation probably derives from the ability of insects to distinguish and avoid deceptive flowers after a certain number of visits (Johnson *et al.* 2004). However, some orchids seem to reduce the disadvantage of being deceptive by resembling and exploiting the research image of other plants (model plants) associ-

ated by their pollinators as important sources of rewards (Dafni & Ivri 1981a; Bell 1986; Johnson *et al.* 2003a; Peter & Johnson 2008). These relationships have been considered as Batesian mimicry systems in which the pollinators will learn more slowly to distinguish deceptive orchids.

In general, orchid species of *Oncidium*, *Tolumnia* and other genera in the subtribe Oncidiinae are expected to be mimics of Malpighiaceae members (Van der Cingel 2001; Powell *et al.* 2003; Renner & Schaeffer 2010). Both groups of plants overlap in their distribution: Oncidiinae is restricted to the Neotropics (Williams *et al.* 2001) where approximately 85% of Malpighiaceae also occur (Davis *et al.* 2002). They inhabit most Neotropical ecosystems and share both temporal (flowering) and spatial subniches (Powell *et al.* 2003; Carmona-Díaz & García-Franco 2009). These geographic and ecological ranges are also occupied by oil-gathering bees of *Centris* and relative genera (tribes

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Centridini, Exomalopsini and Tetrapediini; *sensu* Michener 2007), the main pollinators reported for both plant groups (Van der Pijl & Dodson 1966; Sazima & Sazima 1989; Van der Cingel 2001; Sigrist & Sazima 2004; Carmona-Díaz & García-Franco 2009). These bees depend completely on fatty compounds produced mainly on the elaiophores of Malpighiaceae and a few other plant families, including some orchids (Stpiczyńska *et al.* 2007; Renner & Schaeffer 2010). This dependence is supported by structural, physiological and behavioral adaptations of these bees to collect/process the oils (Simpson & Neff 1981; Buchmann 1987).

However, in this ecological context, most of the orchids involved do not offer oils (or other rewards), but rather exploit the dependence of females of these oil-collecting bees on malpighiaceae oil-producing flowers (Powell *et al.* 2003; Pansarin *et al.* 2008; Carmona-Díaz & García-Franco 2009). In other cases, a few species of orchids of the genus *Oncidium* and *Tolumnia*, pollinated by males of *Centris* bees, have been suggested to exploit the territoriality (Dodson & Frymire 1961; Van der Pijl & Dodson 1966; Nierenberg 1971) and mate-seeking behaviors of these males bees (Dod 1976). In the latter cases, malpighiaceae plants do not seem to play any role.

In particular, the pollination ecology of the genus *Tolumnia sensu* Chase (1986), with approximately 25 species mostly endemic to some Caribbean islands (Nir 2000), has been only descriptively addressed (Nierenberg 1971; Dod 1976; Van der Cingel 2001). As an exception, the most widely distributed species, *Tolumnia variegata* (Sw.) Braem, has been studied repeatedly in Puerto Rico (Ackerman & Montero-Oliver 1985; Ackerman & Galarza-Pérez 1991; Ackerman *et al.* 1997; Morales *et al.* 2010) and presented as a non-model form of food deception.

We examined for the first time the importance of oil-gathering bees in the reproductive fitness and breeding system of the Cuban endemic *Tolumnia guibertiana*. The co-occurrence of this orchid with malpighiaceae lianas and other plants frequented by oil-gathering bees has also been investigated to evaluate the effect of possible plant-plant interactions on the pollination scenario of this endemic orchid.

Materials and methods

Plant species

The twig epiphyte *Tolumnia guibertiana* (A. Richard) Braem occurs in Cuban lowland dry ecosystems (Llamacho & Larramendi 2005) (Fig. 1c,f,g). Plants are caespitose with 2–4 dark-green succulent leaves grouped in a fan-like shoot, usually including a basal pseudobulb. Each year, adult plants of *T. guibertiana* produce one (rarely two) inflorescence(s) from March to May (Díaz 1997). The

racemes are usually larger than the leaves with typical *Oncidium*-like flowers. This type of flowers have a labellum trilobed with a prominent callus; the column with a conspicuous tabula infrastigmatica and one column wing at each side of the stigma, with labellum and column joined in an angle $\geq 90^\circ$ [Chase *et al.* [2009]]. Each flower produces a single pollinarium with a viscidium frontally exposed. The pollinarium has a white stipe and two small caudicles, each of which supports a yellow pollinium.

Study sites

The study was carried out from March to May 2007, in two localities separated by 47.8 km: La Bajada (LB) (21°55'40"N, 84°29'83"W) and Cabo de San Antonio (CSA) (21°51'60"N, 84°57'00"W). Both localities are in the Biosphere Reserve 'Península de Guanahacabibes' in the western part of the Cuban archipelago.

At both sites, the orchid grows in the ecotone between evergreen microphyllous dry forests and mangrove swamps, on very rocky (karstic) terrains. Among the plants co-occurring with *T. guibertiana* in this area we found *Stigmaphyllon diversifolium* and *Ouratea agrophylla*. Both species have yellow flowers and bloom simultaneously with *T. guibertiana*. Moreover, the plants of *S. diversifolium* are the main phorophytes of *T. guibertiana* at the two localities (76% in LB and 87% in CSA; A. Vale 2007). In contrast, plants of *O. agrophylla* do not occur at CSA. Thus, *T. guibertiana* at CSA grows only in the presence of *S. diversifolium*.

Floral traits of *Tolumnia guibertiana*

In both populations the number of flowers per inflorescence of *T. guibertiana* and the initiation of fruits were recorded three times every 15 days. This monitoring was conducted to coincide with the flowering peak to its end. Collaterally, to quantify floral lifespan, one single flower on each of 12 individual orchids was randomly selected and the date of its opening and wilting annotated.

To characterize the morphological similarity between *T. guibertiana* and the putative model plants *S. diversifolium* and *O. agrophylla*, one flower per plant on 17 individuals of each species was photographed *in situ*. We took digital photographs using a standardized procedure (frontal view and planar position). Flowers were photographed at complete anthesis to avoid ontogenetic effects. Images were processed with the program ImageJ (National Institutes of Health, Maryland, USA) to obtain the following variables: (i) area of the flower (*fla*); (ii) area of the central element of the flower (*ace*); and (iii) the dimension of the grabbing structures (*wgs*). With regard to the grabbing structures we refer to the tabula infrastigmatica in *T. guibertiana* and the analogous structures of visitor's grabbing

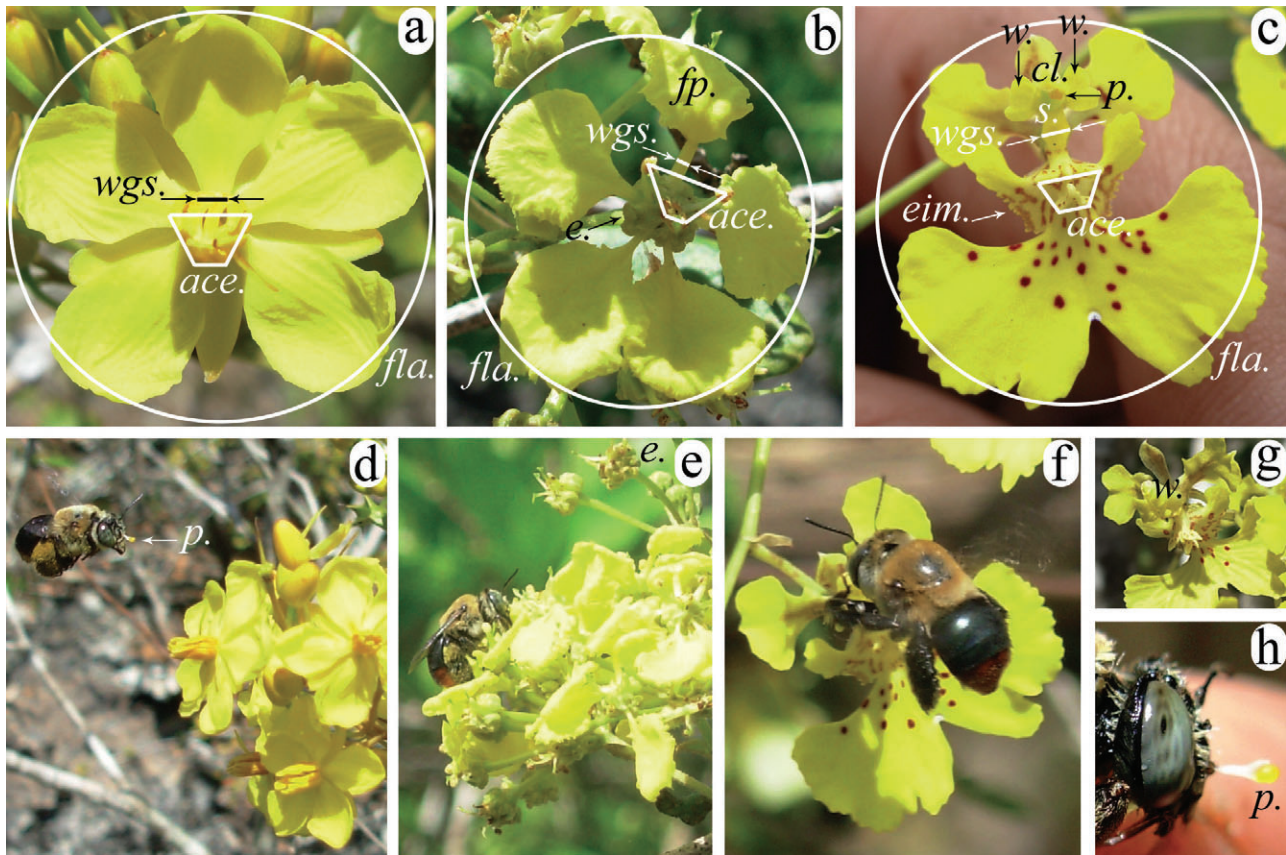


Fig. 1 Guild of yellow-flowered plants pollinated by females of *Centris poecila* at La Bajada (Cuba), and measurements conducted for comparing its morphology and the activity of their main pollinator. (a) *Ouratea agrophylla*, (b) *Stigmaphyllon diversifolium*, (c) *Tolumnia guibertiana* (note the pollinia on the stigma), (d) a female of *C. poecila* hovering for transferring the pollen obtained from *O. agrophylla* to its hind legs (note the pollinarium of *T. guibertiana* on the bee frons), (e) a female of *C. poecila* biting the base of the flag petal while gathering oils with all its legs from the elaiophores of a *S. diversifolium* flower, (f) a female of *C. poecila* biting the tabula infrastigmatica of *T. guibertiana* while grasping the labellum central margins for non-existent oils, (g) a flower of *T. guibertiana* in the post-pollination phase showing the plugging of both column wings and (h) details of the head of a *C. poecila* female with a pollinarium at its frons. *wgs*, wideness of the grabbing structures; *ace*, area of the central element; *fla*, floral area; *e*, elaiophores; *fp*, flag petal; *w*, wings of the column; *cl*, clinandrium; *p*, pollinium or pollinia; *s*, stigma.

in the other two species (see Fig. 1a–c and the explanation below). Flower area was obtained considering the mean value of the broadest horizontal axis and the broadest vertical axis as the diameter of the flower circumference. As the central element we considered the orchid callus and the conjuncts of stamens and pistils in the flowers of the other two plants (Fig. 1a–c). The area of the central element of the flowers was calculated as the area of the trapezium formed among the four extremes of the central element of these flowers.

The tabula infrastigmatica (flanked by arrows and labeled as *wgs* in Fig. 1c) is a key structure in the pollination ecology of *Oncidium*-like flowers (*sensu* Stpicyńska *et al.* 2007) because their pollinators bite this floral element to obtain the necessary stability for foraging on the flowers. We have observed the same bee behavior during

previous pollinator censuses on the other two yellow-flowered plant species; this has also been reported in the literature (e.g. Sigrist & Sazima 2004). Thus, the bitten structures from those flowers (the base of the upper petal of *S. diversifolium* flowers and of the upper anther in *O. agrophylla* flowers) were interpreted as analogous to the tabula infrastigmatica of *T. guibertiana* flowers. As a result we refer to these three structures as ‘grabbing structures’ and their wideness (*wgs*) was compared among species (Fig. 1a–c).

After conducting hand pollinations in 12 plants, all changes in the pollinarium and in the column wings were registered (Fig. 1c,g). For this, each pollinarium was removed with a toothpick and deposited onto a piece of paper for marking the position of the pollinia. After each minute (for 7 min) the new position of the pollinia was

also marked and the resulting angles were quantified. The highest angle swept by each pollinarium along the measurements and the time in which that angle was reached were annotated. On the next day we inspected the changes in color and consistency of the flowers artificially pollinated, and the position of the structures near to the stigma, particularly the position of the column wings. In addition, we assessed the occurrence of other changes in color, shape or turgidity of the column and the labellum capable of diminishing flower attractiveness for bees.

As we detected heterogeneous variances in the floral traits compared in the present study ($P < 0.05$ in all variables according to the Levene test), comparisons were carried out using a robust ANOVA (Welch) and pairwise comparisons were conducted using a Games–Howell test. We consider the populations to be independent groups, despite species identity. The level of significance of the hypothesis tests was 0.05.

Breeding system of Tolumnia guibertiana

At LB, 15 flowered plants were excluded from insect visits using silk bags. In these plants the fruit production was monitored periodically until the end of the flowering season. Collaterally, 192 flowers exposed to pollinators (from 99 inflorescences, one per plant) were used as a control. Fruit set was quantified as the response variable of this experiment.

Plants with four or six flowers each were artificially pollinated with an entire pollinarium: half of the flowers were pollinated with geitonogamous pollen and the other half with allogamous pollen (30 flowers in total per treatment in 12 plants). Pollen donor plants for cross-pollinations (allogamy) were at least 10 m from the receptors. The position of each treatment within the same inflorescence was randomized to avoid architectural effects.

Pollination ecology

To determine the visitor spectrum of *T. guibertiana*, *O. agrophylla* and *S. diversifolia* we conducted simultaneous observations of the three species, from sunrise to sunset, in periods of 15 min alternated with 2 min pauses to account for a total of 21 h. Observations were made in an area of 2.0 m × 2.0 m × 3.5 m that included at least five blooming individuals of *T. guibertiana* each day and flowers of the other two species. The site selected for the observations was LB because of the coexistence of these three plant species at this locality. Before starting observations, the number of flowers of each of the three species was registered to standardize the response variables.

Insect visits to the flowers were classified as: contact visits (when the insect landed on the flower) or effective

visits (when the insect removed/deposited some pollinia in the case of *T. guibertiana*, or when it touched the sexual structures in the case of the other plant species). Each visitor that arrived from a place further than 3 m away during the same census was considered to be a 'different' individual.

Behavioral aspects of the insects were registered when possible: the use of legs and mandibles during foraging, the displaying of buzzing behavior on flowers, and the type of floral reward collected/consumed at each visit. In addition, it was noted for each visitant which floral parts were used by the insects to land, which were bitten to hold their bodies while foraging, and finally what parts they manipulated to collect/consume each form of floral reward.

Whenever an insect landed on an orchid flower, it was annotated which flower species was visited before and after. A direct landing on orchid flowers (without the observation of previous flowers visitation) and departures from the orchid flowers to far places were recorded as 'visits from none plant' and 'departures to none plant', respectively.

For the three plant species the following variables were calculated, considering censuses of 15 min: (i) number of flowers visited of each plant species by census; (ii) number of insect individuals that visited each plant species by census; and (iii) time spent by insects on flowers of each plant species (in seconds). The number of flowers visited by insects was standardized by the number of open flowers of each species at the moment of the observations. The time spent by insects was standardized by the total number of visited flowers. The activity data of the mean pollinators lacked normality and were compared among plant species using a Kruskal–Wallis test and a post-hoc Dunn test for variables (i) and (ii). A Welch *F*-test and a Tukey's test were used to compare the three species for the final variable.

Mean differences in the activity of pollinators when arriving to *T. guibertiana* from *S. diversifolium*, *O. agrophylla* or from 'none plant' and when going to these plants (or to 'none plant') from *T. guibertiana* were assessed using a one-way ANOVA as implemented in EcoSim 7.72 (Gotelli & Entsminger 2010). We used 10 000 randomizations and the level of significance of the hypothesis test was 0.05.

Comparison of the reproductive success of Tolumnia guibertiana in two populations

Just after the peak of flowering, male and female pollination success was calculated considering the presence/absence of pollinia in the clinandrium and the stigma of flowers of *T. guibertiana*, respectively (see Fig. 1c). In

plants with 1–3 flowers, the plant level value was equal to the mean of all its flowers. In contrast, in plants with >3 flowers, the data from three randomized flowers were used to infer the mean success of the plant. These data were used to compare the success (male and female components) among plants of different floral displays (i.e. number of flowers per plant) (using a Kruskal–Wallis test) and also to compare both populations (by means of a Mann–Whitney *U*-test). The significance level for the hypothesis tests was 0.05. For these comparisons we considered three forms of pollination success: the average number of pollinaria removed by plant (male success), the average number of pollinia deposited on the stigma per plant (early female success) and the number of flowers that initiated fruits (late female success). This last variable was noted in a visit at the end of the plant blooming season.

Results

Floral traits of Tolumnia guibertiana

The orchid *T. guibertiana* produced lax inflorescences with 1–10 flowers ($n = 171$). The mean floral lifespan of each flower was 19.8 ± 0.1 days. The flowers opened in succession, but all flowers of the inflorescence were open at the same time owing to their long lifespan.

The area of *T. guibertiana* flowers presented a halfway mean value between the area of the flowers of *O. agrophylla* and *S. diversifolium*. The orchid flowers from CSA were statistically bigger in size than the flowers of *O. agrophylla*, but smaller than the flowers of both populations of *S. diversifolium* ($F_{(4, 37.816)} = 55.827, P < 0.001, n = 17$; Fig. 2).

The mean value of the approximate area of the orchid callus was statistically similar to the central conjunct of *O. agrophylla* and statistically smaller with regard to the central conjunct of *S. diversifolium* ($F_{(4, 37.942)} = 115.689, P < 0.001, n = 17$; Fig. 2).

The column of *T. guibertiana* presented a remarkable tabula infrastigmatica that occupied the same place as the upper petal of *Stigmaphyllon* flowers and the base of the anthers in *Ouratea* flowers. Nevertheless, the tabula infrastigmatica presented a mean value statistically bigger than the analogous structures in the other two plants ($F_{(4, 39.251)} = 18,766, P < 0.001, n = 17$; Fig. 2).

The viscidium was directly exposed to the frons of the pollinator in *T. guibertiana*. Once removed by the pollinator, the pollinarium suffers a movement in an angle of nearly 71° in the stipe 406 ± 24 s after being removed. Two column wings flank the stigma (Fig. 1c,g) and direct the head of the arriving pollinators towards the stigmatic tissue and the clinandrium. In this way, both pollinarium adhesion to visitors and deposition onto the stigma of

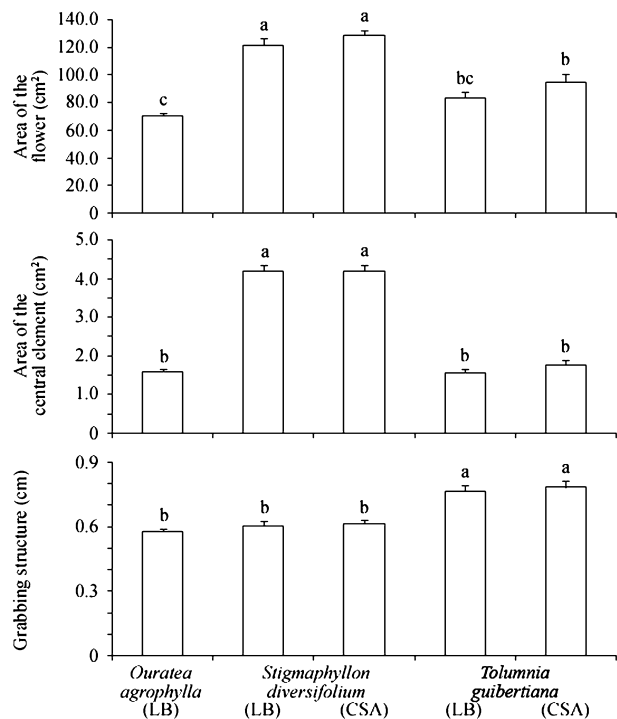


Fig. 2 Comparison of three morphological features of the flowers of *Tolumnia guibertiana*, *Stigmaphyllon diversifolium* and *Ouratea agrophylla* at La Bajada (LB) and Cabo de San Antonio (CSA), Península de Guanahacabibes, Cuba ($n = 17$ individuals for each species in each locality). The letters over the columns show the results of a Dunn’s multiple comparison Test. Different letters reveal significant differences ($P < 0.05$).

previously removed pollinia would be favored. Less than 1 day after pollination occurs, column wings plug the stigma wet surface, occluding it and preventing subsequent depositions of pollinia (Fig. 1g). No other changes were observed over the next 1–2 days, but after that flower color vanished and all pieces lost their turgidity, showing senescence.

Breeding system of Tolumnia guibertiana

This orchid depended completely on pollinators for sexual reproduction. At LB, none of the 98 bagged flowers suffered spontaneous self-pollination, whereas 15% of the 192 open flowers at this site produced fruits. Hand-pollination experiments clearly showed a self-incompatible condition. No fruit was initiated after geitonogamous pollinations, whereas the 91% of flowers pollinated with allogamous pollinaria set fruits ($n = 29$ and 32 flowers, respectively, from the same 13 plants).

Pollination ecology

Most floral visitors to *S. diversifolium* and *O. agrophylla* were shared with *T. guibertiana*. Bees were the predomi-

nant visitors to the three plant species. In particular, female bees *Centris poecila* were the main pollinators of *T. guibertiana* (Fig. 1d–f,h; Table 1).

Centris poecila visited *O. agrophylla* only for pollen. In contrast, the glandular flowers of *S. diversifolium* were mainly visited for gathering oils. In the orchid flowers, *C. poecila* scratched the internal erose margins of the labellum with its forelegs in the same way that they did in *S. diversifolium* floral elaiophores, without finding any collectable substance. During their visits they bite a particular part of the flowers with their mandibles and keep all their legs free to manipulate the floral structures. As previously suggested, the floral structures bitten by *Centris* bees in each plant were species-specific: the tabula infrastigmatica in the orchid flowers, the fleshy base of the flag petal in *Stigmaphyllon* and the conjunct of the stamens and the base of the ovary in *Ouratea*.

At LB, females of *C. poecila* preferred collecting pollen by buzzing on *O. agrophylla* instead of collecting the more accessible pollen of *Stigmaphyllon* flowers, which was also consumed by other less specialized visitors (Table 1).

On average, the time spent by *Centris* female bees in orchids was similar to the time spent in *Stigmaphyllon* flowers (Table 2). Some visits included the collecting of oils, but many were made by mistake during pollen seeking routines among *Ouratea* flower clusters. Thus the time spent on the orchid and on *Stigmaphyllon* was significantly shorter than the time spent on *Ouratea* flowers in which buzzing required more time (Table 2). The mid lobe of the orchid labellum was always divided into two portions much bigger than the lateral lobes (Fig. 1c). This structure possessed the same position and played the same role as the two lower petals of the flowers of *S. diversifolium* and *O. agrophylla* (Fig. 1a,c) in attracting insects and supporting their landings (Fig. 1d,g). In general, females of *C. poecila* showed a strong preference for yellow flowers. On average, the flowers of *O. agrophylla* were visited almost 10-fold more than the flowers of the other two species, between which there were no differences (Table 2). In addition, plants of *O. agrophylla* received on average more visits by bees than the other two plant species (Table 2).

By analyzing the foraging sequence of *C. poecila* bees coming to *T. guibertiana* flowers, we found that movements from *O. agrophylla* to *T. guibertiana* were more frequent, and movements between *T. guibertiana* flowers followed. The movement pattern was significantly different from random ($F = 17.05$, $P < 0.0001$; Fig. 3).

A similar behavior was observed in relation to visits after contact with the orchid. In most cases bees left the orchid flowers to visit a flower of *O. agrophylla*, whereas the choice of a conspecific flower (always on a different individual) was significantly lower ($F = 13.94$, $P < 0.0001$; Fig. 3).

Table 1 Visitor spectrum of the studied plants at La Bejada, Cuba (LB), in March–May 2007

Visitors	Foraging activity	Buzzing behavior	Plant species				Total visits
			<i>Ouratea agrophylla</i>	<i>Stigmaphyllon diversifolium</i>	<i>Tolumentia guibertiana</i>		
			Direct contacts (n)	Effective visits (%)	Direct contacts (n)	Effective visits (%)	
Coleoptera							
Undetermined (1 sp.)	EP	No	0	—	1	—	1
Hymenoptera							
Apidae							
<i>Centris aethiops</i> (♂)	CS	No	2	100.0	0	—	2
<i>Centris poecila</i> (♀)	CPO	Yes	2587	99.9	200	99.5	2820
<i>Melissodes</i> sp. (♀)	CP	Yes	29	100.0	0	—	31
<i>Xylocopa cubacola</i> (♀)	CP	Yes	235	88.7	5	100.0	342
Megachilidae							
<i>Megachile</i> sp. (♀)	CP	No	3	100.0	0	—	3
Vespidae (2 spp.)	N	No	7	—	0	—	7
Lepidoptera							
Hesperiidae (2 spp.)	N	No	2	0	3	—	5

For each insect the total number of contacts with flowers of each plant species and the proportion of these contacts in which the sexual organs of the flowers were touched is given. CP, collecting pollen; CPO, collecting pollen and oils; CS, consuming oils; EP, eating pollen; N, looking for nectar.

Table 2 Characterization of the activity of the females of the solitary bee *Centris poecila* in flowers of *Tolumnia guibertiana* and its two putative model plants at La Bajada (Cuba)

Plant species	Flowers visited / census†	No. visiting bees /Census†	Time spent on flowers (s)‡
<i>Ouratea agrophylla</i>	1.3 ± 1.8 ^a	3.6 ± 3.3 ^a	3.6 ± 2.0 ^a
<i>Stigmaphyllon diversifolium</i>	0.1 ± 0.2 ^b	1.5 ± 1.6 ^b	2.9 ± 2.3 ^{ab}
<i>Tolumnia guibertiana</i>	0.07 ± 0.1 ^b	1.5 ± 1.8 ^b	2.6 ± 1.5 ^b
	$H = 223.4$	$H = 17.9$	$F_{2,194} = 3.972$
	$P < 0.001$	$P < 0.001$	$P < 0.05$
	$n = 40$	$n = 40$	$N_O = 134, N_S = 40, N_T = 23$

† Data obtained from 15 min pollinator censuses. ‡ Calculated as the time elapsed between arrival to the first flower and departure from the last flower of the same species divided by the total number of conspecific flowers. Results are given as mean ± standard deviation. Different letters reveal statistical differences ($P < 0.05$).

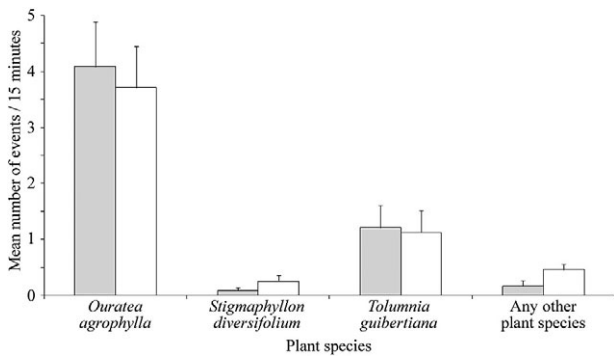


Fig. 3 Plant species visited in La Bajada (Cuba) by female *Centris poecila* bees before (black bars) and after (white bars) visiting flowers of *Tolumnia guibertiana*. The values are presented as the mean number of events (± standard deviation) by census ($n = 25$).

Comparison of the reproductive success of Tolumnia guibertiana in two populations

The natural reproductive success of this orchid, in terms of pollinia exported or received per plant, did not differ between populations (male success: $U = 3842.0, P > 0.05$; female success: $U = 3805.5, P > 0.05, N_{LB} = 99$ and $N_{CSA} = 74$; Fig. 4). Both populations also presented a similar percentage of fruits initiated per plant ($U = 3805.5, P > 0.05, N_{LB} = 99$ and $N_{CSA} = 74$; Fig. 4).

Discussion

Breeding system and floral traits ensuring allogamic offspring

Self-incompatibility was recorded for the first time in *T. guibertiana*. This condition, although rare among deceptive Orchidaceae (Dressler 1993; Roberts 2003; Jersáková et al. 2006), is reported in more than 73% of Oncidiinae members (Charanasri & Kamemoto 1977; Singer & Koehler 2003). Among the genera closely related to *Tol-*

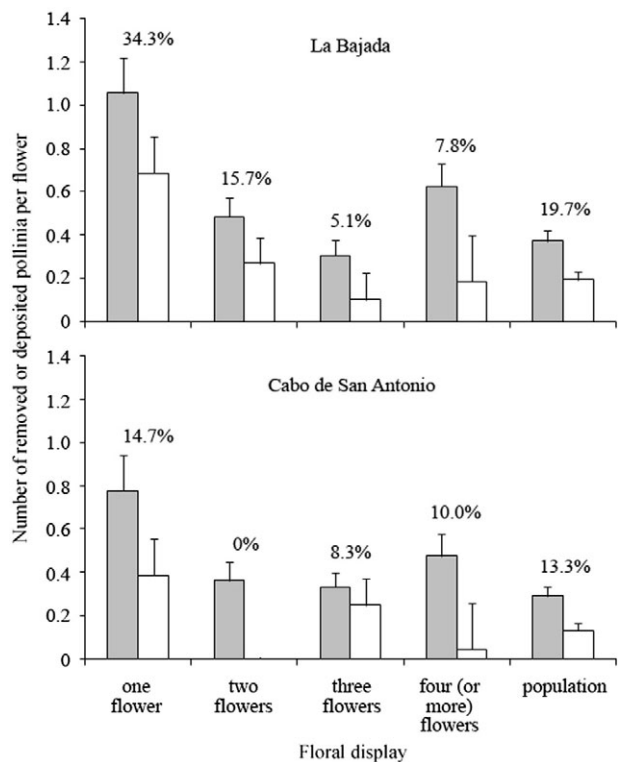


Fig. 4 Comparison of male (white bars) and early female pollination success (grey bars) among plants with different floral displays at the two populations, La Bajada (LB) and Cabo de San Antonio (CSA). Late female pollination success evaluated as the percentage of fruit initiated is shown as the number of fruits initiated over the columns.

umnia (i.e. *Oncidium*, *Trichocentrum* and *Cohniella*) self-incompatibility is apparently also frequent (East 1940; Charanasri & Kamemoto 1977; Ackerman 1995; Abdala-Roberts et al. 2007). However, this condition is also controversial (Pemberton 2008) because some species of *Oncidium* s. l. that have been reported as self-incompatible present some populations containing both self-

incompatible and self-compatible individuals (Scott 1864 in Van der Pijl & Dodson 1966; Abdala-Roberts *et al.* 2007; Pemberton 2008).

Most orchids are hermaphroditic and self-incompatibility is the most effective way to avoid selfing (Matton *et al.* 1994; Ortíz-Barney & Ackerman 1999; Wallace 2003). In this context, self-incompatibility could be particularly necessary when pollen vectors are promoting high levels of self-pollination and geitonogamous pollination (Matton *et al.* 1994; Alcántara *et al.* 2006). In many *Oncidium*-like orchids, self-incompatible systems and their maintenance have been associated with the behavior of their pollinators (mainly *Centris*, *Epicharis*, *Tetrapedia* and *Trigona* spp.). In general, these bees exploit exhaustively the floral sources of a certain area before moving to another, resulting in a high number of geitonogamous pollinations (Alcántara *et al.* 2006). This behavior has also been described both for the interaction between these bees with orchids (Dodson 1962; Parra-Tabla *et al.* 2000) and with malpighiaceae species (Sigrist & Sazima 2004), including an experimental survey in which oil-gathering bees over-visited flowers artificially eglandulated (Sazima & Sazima 1989).

Despite its genetic and ecological advantages, self-incompatibility could also represent an important reproductive cost when the plant is pollinator limited (Larson & Barrett 2000). In fact, self-incompatibility has been frequently associated with very low levels of fruit set (Neiland & Wilcock 1998), particularly within Oncidiinae (Dodson 1962; Singer & Cocucci 1999). In such cases, geitonogamous pollinations cause an unnecessary waste of energy and resources. Thus, in rewardless, pollinator-limited and self-incompatible orchids, like *T. guibertiana*, additional pre-pollination barriers are expected to arise to favor outcrossing. These mechanisms reinforce temporal or spatial differentiation between the two sexual functions of each flower (Roberts 2003). Among these mechanisms, only protandry has been reported in a few species of the genera *Notylia* and *Macradenia* in Oncidiinae (Singer & Koehler 2003).

In *T. guibertiana*, the post-pollination plugging of the columnar wings (see Fig. 1g) probably helps to separate the sexual functions within the same flower. Thus, occlusion of the stigma makes over-pollination of the same flower in the next one–two subsequent days difficult, but this does not affect the male function during that period. Almost 70% of the monitored individuals of both populations produced one or two flowers. Consequently, in these individuals this mechanism is enough to avoid completely (in monofloral plants) or to reduce to half (in bifloral ones) the possibility of a second visitation to the same plant by the same bee. Accordingly, this mechanism could favor a better distribution of the potential effective visits among a certain group of flowering plants. This contriv-

ance has not been previously suggested for Oncidiinae orchids, despite the fact that the presence of columnar wings is a common feature of the subtribe (Dressler 1993).

In contrast, among orchids the proper deceptive condition has been interpreted as a feature preventing over-visitation (Jersáková *et al.* 2006; Johnson & Morita 2006). This was corroborated for the rewardless *T. guibertiana* (see Table 2 and the scarce rate of consecutive visits to conspecifics in Fig. 3).

Finally, pollinarium bending reduces the occurrence of self-pollination in orchids (i.e. Peter & Johnson 2006 and references therein; Jersáková *et al.* 2006). In the case of *T. guibertiana*, the mean value of the time required by the removed pollinarium to its bending and the adoption of a right position of pollinia (406 s) is 1.5-fold more than the interval bees spent on an orchid plant (249 s). Thus, this mechanism could be an effective temporal barrier to immediate geitonogamous pollinations in this species, as has been demonstrated for many temperate orchid species (Darwin 1888; Kullenberg 1961; Johnson & Nilsson 1999; Johnson & Edwards 2000; Peter & Johnson 2006). Nevertheless, the information available about this issue in tropical orchids is very scarce, and this is the first report of these values for the Oncidiinae subtribe.

Pollination ecology

Our study is congruent with the previously mentioned dependence of *Tolumnia* species on *Centris* bees as pollinators (Van der Pijl & Dodson 1966; Nierenberg 1971; Ackerman *et al.* 1997). At LB, *T. guibertiana* attracted females of *C. poecila*, sometimes during their oil-collecting activity on *S. diversifolium*. Moreover, the behavior of *C. poecila* females while visiting both plant species was similar in frequency and intensity (Table 2); and also in relation to the time they spent in the flowers of these two plants (see Table 2). These results are consistent with the role of Malpighiaceae as model plants for most *Oncidium*-like orchids, as has been proposed many times (Van der Pijl & Dodson 1966; Nierenberg 1971; Van der Cingel 2001; Powell *et al.* 2003; Pemberton 2008; Renner & Schaeffer 2010), but scarcely documented (Carmona-Díaz & García-Franco 2009; Pemberton 2010).

Another model plant, *Ouratea agrophylla*, morphologically similar to the two above-mentioned species (see Fig. 2), offers pollen as a reward and also receives intense visitation from *C. poecila* females (see Tables 1,2). In that foraging activity in *O. agrophylla*, *C. poecila* also contacts in an effective manner the orchid flowers (see Table 2). Bees of the genus *Centris* need floral oils for brood provisioning, nectar for daily energy, and pollen as a protein source for their brood and themselves (i.e. Aguiar & Gaglianone 2003). Foraging preferences of *Centris* for *O. agrophylla* at

LB are probably reinforced by the polliniferous condition and local abundance of this plant.

Many other examples of Batesian mimicry involving an orchid and more than one model plant from different families (even with quite diverse symmetries) have been published (Bierzychudek 1981; Dafni & Ivri 1981b; Johnson 1994; Pansarin *et al.* 2008). According to this, *T. guibertiana* and its putative models could be another example of a more plastic pollination strategy as part of a floral mimicry complex for which the traditional strict limits between the Batesian mimicry and the generalized food deception could be reconsidered (i.e. Johnson *et al.* 2003a; Peter & Johnson 2006, 2008; Jersáková *et al.* 2009).

Reproductive success of Tolumnia guibertiana: comparison between populations and relative species

In the present study, both populations of *T. guibertiana* presented the same reproductive fitness. This could be a consequence of similar abundances of *Centris* bees at both sites. The opposite was found as the main cause of an irregular pattern of pollination success in three populations of *T. variegata* in Puerto Rico (Sabat & Ackerman 1996; Ackerman *et al.* 1997). The authors did not find other floral or environmental traits directly related to the pollination success in many of the locations and years evaluated for this species. In *T. guibertiana*, we also found at both sites that individuals with different floral displays did not differ in their pollination success (see Fig. 4). Considering the gregarious habit of these twig epiphytes, maybe the importance of floral display on reproduction must be evaluated at a plant cluster level instead of at an individual level. At the moment many attempts using diverse frameworks and statistical approaches in *T. variegata* have only shown that the reproductive scenario governing the fitness of the orchid is complex and very labile depending on locality, micro-habitat and year (Sabat & Ackerman 1996, Ackerman *et al.* 1997; Morales *et al.* 2010).

The observed male success values (see Fig. 4) were similar to those reported for other rewardless species of Oncidiinae (6.5–20% in *Oncidium scandens* and 15–25% in *Oncidium sphacelatum* in Florida; Parra-Tabla *et al.* 2000 and Pemberton 2008, respectively) and much higher than other species with *Oncidium*-like flowers (Tremblay *et al.* 2005; Damon & Salas-Robledo 2007), many of which derived a fruit set near or equal to zero (e.g. Damon & Salas-Robledo 2007). Among the studies focusing on the effect of mimicry pollination success, Carmona-Díaz and García-Franco (2009) reported in Mexico that *Oncidium cosymbephorum* C. Morren presented higher fruit sets when exposed to *Centris* bees in co-occurrence of flowered shrubs of *Malpighia glabra* L. The fruit set values reported for co-occurring patches were very similar to

those reported for *T. guibertiana* (above the columns in Fig. 4). On the contrary, the fruit set values reported in the absence of *M. glabra* (between 0 and 1) are similar to the values reported for other isolated individuals of *Oncidium*-like species (Damon & Salas-Robledo 2007), suggesting a positive effect of concurrent rewarding flowers.

Within *Tolumnia*, the allogamous and also deceptive *Tolumnia variegata* differs from *T. guibertiana* by being apparently not mimetic (Ackerman *et al.* 1997). We found that the pollination success of *T. guibertiana* in co-occurrence with the proposed model plants is comparatively much higher than that presented for its Caribbean sister species. This higher pollination success suggests that *T. guibertiana* may be obtaining an additional advantage of mimicry to attract and mislead local *Centris* bees.

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