

Pollination and breeding system of *Canna paniculata* (Cannaceae) in a montane Atlantic Rainforest: asymmetric dependence on a hermit hummingbird

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ABSTRACT

We studied the pollination biology of *Canna paniculata* (Cannaceae), a plant species common in the Atlantic Rainforest of southeastern Brazil. The species presents specialized ornithophilous flowers, which in our study area are solely pollinated by the hermit hummingbird *Phaethornis eurynome*. Although *C. paniculata* is capable of bearing fruit after self-pollination, it requires pollinators for reproduction. We discuss the importance of hermit hummingbirds for the reproduction of specialized ornithophilous plants such as *C. paniculata*, including their asymmetric dependence on hermit hummingbirds - core pollinators in Neotropical forest ecosystems.

Keywords: hummingbirds, ornithophily, *Phaethornis eurynome*, Serra do Mar, Zingiberales

Hummingbirds arrived to South America some 22 million years ago (McGuire *et al.* 2014) and have since become the most important avian pollinator group in the Neotropics (Cronk & Ojeda 2008). As a result of this strong mutualistic association between hummingbirds and plants, numerous plant groups have achieved remarkable diversity (e.g., Schmidt-Lebuhn *et al.* 2007). More comprehensive information on pollination and reproductive biology for plants belonging to some of these groups are now available, such as the study by Matallana *et al.* (2010) for Bromeliaceae. Zingiberales is another monocot plant clade in which bird pollination is common (Cronk & Ojeda 2008), and although the pollination systems for some of the families within this group have been thoroughly studied (e.g., Costaceae, Kay & Schemske 2003; Heliconiaceae, Stiles 1975; Zingiberaceae, Sakai *et al.* 1999) data are still lacking for other groups. *Canna* L. is the only genus in Cannaceae and constitutes a conspicuous element in forests of the New World, where it is native, and in the Asian Paleotropics, where it has been introduced by humans (Prince 2010). The center of diversity of the family is South America (Prince 2010),

and the species exhibit highly modified flowers, with the development of a colorful androecium and gynoecium with petaloid structures (Glinos & Cocucci 2011). Through a process known as “secondary pollen presentation”, the region below the apical and at the side of the lateral portion of the stigma acts as the pollen-dispensing structure, which demonstrates the unusual mechanism by which plants of the family achieve pollination (for details see Glinos & Cocucci 2011). Nevertheless, besides the aforementioned study, which detailed the functional adaptation of this unusual floral morphology for *Canna indica* L. (Glinos & Cocucci 2011), we are unaware of other detailed studies on the pollination and reproduction for other species in the *Canna* family. Here, we report the pollination biology of *Canna paniculata* Ruiz & Pav. from a montane Atlantic Rainforest area in southeastern Brazil. This species occurs in scattered localities at low to mid elevation (<2,000 m) throughout the wetter areas of the Neotropics, ranging from Panama in the north to southeastern Argentina (Prince 2010).

We studied *C. paniculata* at the Santa Virgínia Field Station (23°20'10”S and 45°8'46”W, 916–950 m above sea

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level), located in Serra do Mar State Park in the state of São Paulo, Brazil, where *C. paniculata* is particularly common. Fieldwork was conducted during the flowering season of *C. paniculata* (June–September) in 2012 and 2013. Flowers were accompanied during the anthesis and collected for morphological measurements ($n = 10$, one for each individual). Individuals were defined as clumps at least 5 m apart as the plant presents clonal growing. For all floral measurements, we used a digital caliper (error = 0.01 mm). In order to characterize the breeding system of *C. paniculata*, we conducted controlled pollination experiments with the following treatments: 1) manual “cross-pollination” (crossing between flowers from different individuals); 2) manual “self-pollination” (pollination within the same flower); 3) “spontaneous self-pollination” (flowers kept isolated in nylon mesh bags); 4) “agamospermy” (flowers emasculated before opening and kept isolated); and 5) marked control

flowers open to visitation to estimate “natural pollination”. All treatments were distributed as much as possible among 22 individuals within the study area, and only flowers at the first day of opening were used in the treatments. The fruit set was evaluated two months after the experiments. In order to quantify the volume of nectar produced and its concentration at the end of the day (~17:00), flowers were bagged before opening with nylon mesh bags and nectar was measured using a microsyringe and a pocket refractometer (Eclipse® 0–50 brix; $n = 17$ flowers from 12 individuals). We also conducted 60 hours of plant focal observations in eight individuals from 06:00 to 18:00 to identify the pollinators of *C. paniculata*.

C. paniculata presents red-orange flowers with traces of yellow (Fig. 1A) and individual clumps offer 4.3 ± 3.9 (range of 1–20) flowers per day during the flowering period. The opening of flowers started early in the morning



Figure 1. A, *Phaethornis eurynome* (Lesson 1832), visiting the flower of *Canna paniculata* Ruiz & Pav. Note the head of hummingbird contacting the apical receptive part of the stigma (arrow) when approaching the flower. B, When the bill is completely inserted in the corolla, the hummingbird's head makes contact with the flattened part of the style (arrow), which acts as secondary pollen presenter. C, In detail, the long and curved bill of *P. eurynome*.

before sunrise (~06:00) and lasted until the afternoon of the second day (~16:00), i.e., flowers lasted roughly 1½ days. As reported for other members of the family (Glinos & Cocucci 2011), *C. paniculata* showed secondary pollen deposition at the side of the flattened style, where pollen is deposited by the single theca in an elliptical clump. Flowers measure ca. 6 cm in total length, but the actual restriction to the pollinator, i.e., the corolla tube, amounts to 4.07 ± 1.03 cm in length with 0.51 ± 0.12 cm of opening. Controlled pollination experiments showed that *C. paniculata* is able to produce fruits after self-pollination, although the fruit set is less than half in comparison to cross-pollinated flowers (Table 1). Moreover, this species requires pollinators to bear seeds, as no fruit was set after spontaneous self-pollination or agamospermy. Flowers exposed to natural pollination had two times more fruit set than self-pollinated flowers, but 13.8% less than cross-pollinated flowers (Table 1). Nectar production amounted to 45.0 ± 34.5 µl, with sugar concentration of $23.4\% \pm 3.11\%$. During focal observations, the only pollinator observed was the Scale-throated hermit, *Phaethornis eurynome* (Lesson 1832), which visited individuals of *C. paniculata* 84 times (1.40 ± 0.94 visits/hour). This species seemingly acted as a “tripliner”, returning at the same clump of flowers at roughly regular intervals (see Stiles 1975). When approaching the flower, the hummingbird first touched the tip of the stigma (i.e., the receptive region, see Fig. 1A, marked with an arrow) with its head, and subsequently, the clump of pollen deposited in the flattened style was pressed against the head of the pollinator (Fig. 1B, marked with an arrow). During our fieldwork, we also noted that stingless bees, *Trigona* sp., robbed nectar from approximately 20.8% (n = 250) of flowers.

Despite being a widespread group in the tropics, this is only the second detailed report on pollination and breeding biology for a *Canna* species. Hummingbird pollination seems to be common for species in the genus, but presumably the one species occurring in North America, *C. flaccida* Salisb., is pollinated by nocturnal moths (Prince 2010). *C. indica* is also pollinated by a single species of hummingbird in Argentina, the Blue-tufted starthroat, *Heliomaster furcifer* (Shaw 1812), from the Mountain gems clade (Glinos & Cocucci 2011). The prevalence of hummingbird pollination (or other birds in introduced areas) in Cannaceae requires

further investigation. The identity of *C. paniculata*'s sole pollinator and external morphological characteristics of the flowers conform to the classical notion of ornithophily, and this is reinforced by the presence of abundant and diluted nectar similar to other ornithophilous species (Cronk & Ojeda 2008). Moreover, bird pollination in other groups within Zingiberales resembles the adaptations found in *C. paniculata*. For instance, in *Costus* L. (Costaceae), adaptation to hummingbird pollination is achieved by narrow, long tubular flowers with brightly colored bracts (yellow, orange, or red), which present copious amount of nectar (Kay & Schemske 2003). Similar traits are found for hummingbird pollinated Heliconiaceae in wet forests of Costa Rica (Stiles 1975). In the Bornean Zingiberaceae, sunbird-pollinated species also presented long tubular corollas with conspicuous colors (often red), and with copious production of more diluted nectar in relation to insect pollinated species (Sakai *et al.* 1999). Altogether, these parallels reinforce the association of some prominent floral traits to specialized bird pollination within Zingiberales.

Although both *C. indica* and *C. paniculata* have similarly elongated corolla flowers, differences in pollinating hummingbird species imply distinct areas of pollen deposition. For a non-hermit species, *H. furcifer* has a long bill of 2.8 ± 0.2 cm in length, enabling it to access the nectar in the flower. However, because its bill is straight (Glinos & Cocucci 2011), during its visits the bill is tightly encased in the slightly curved floral tube, and pollen is deposited on the hummingbird's bill when it forces its way out of this “entrapment” (Glinos & Cocucci 2011). In *C. paniculata*, pollination is carried out by the hermit *P. eurynome* with a curved bill of 3.4 ± 0.1 cm (Vizentin-Bugoni *et al.* 2014; Fig. 1C), which promotes a good fit to the long, slightly curved corolla of the flower. While visiting the flower, the head of the hummingbird often touches the receptive part of the stigma first, and thereafter it comes into contact with the pollen (Fig. 1A, C). Although this does not ensure cross-pollination, since an individual plant can present more than one open flower at a time, chances of self-pollination are at least diminished.

Considering the breeding system, *C. paniculata* can be regarded as self-compatible (with an Index of Self-Incompatibility of 0.36—estimated as the division of the fruit set through self-pollination by cross-pollination, as in Wolowski *et al.* 2013), similar to other groups of monocots strongly associated to hummingbird pollination such as the bromeliads (Matallana *et al.* 2010) and hummingbird pollinated plants in general (Wolowski *et al.* 2013). Nevertheless, *C. paniculata* requires pollinators to set fruits, characterizing its dependence on pollen vectors. Naturally pollinated (i.e., control) flowers show *P. eurynome* as a relatively good pollinator, being able to set more fruits than when self-pollinated, even though pollination success is not as high as hand cross-pollination. The distinctive triplining behavior of hermit hummingbirds, as we may also infer for *P. eurynome*, is

Table 1. Fruit set after controlled pollination experiments and natural pollination in *Canna paniculata* Ruiz & Pav. (Cannaceae) at the Santa Virginia Field Station, Serra do Mar State Park, São Paulo, Brazil.

Pollination treatment	% (Flowers)
Cross-pollination	53.8 (n = 52)
Self-pollination	19.2 (n = 52)
Spontaneous self-pollination	0.0 (n = 65)
Agamospermy	0.0 (n = 58)
Natural pollination	40.0 (n = 70)

expected to increase the rates of outcrossing (Stiles 1975), which also characterizes hermit hummingbirds as relatively efficient pollinators. This is further supported if one considers that for *C. indica* pollinated by *H. furcifer*, a presumably territorial hummingbird, fruit set in control flowers was only 20% in comparison to 86% in hand crossed flowers (Glinos & Cocucci 2011). These results illustrate the link between distinct hummingbird behaviors (e.g., territorialism) and pollination success (Justino *et al.* 2012).

At the community level, in the studied montane Atlantic Rainforest site, *P. eurynome* is a “core” hummingbird pollinator, interacting with more plant species than any other hummingbird species, and being the sole pollinator of many long-tubed flowers; at least 24 other plant species, 15 of which have overlapping flowering with *C. paniculata* (Vizentin-Bugoni *et al.* 2014). Similar community organization is found in lowland Atlantic Forest areas, where the Saw-billed hermit *Ramphodon naevius* (Dumont 1818) is solely responsible for the pollination of the more specialized long-tubed flowers (Sazima *et al.* 1995). Also in the Neotropical savanna, where patches of forest habitats are found embedded in the landscape, similar organization can be seen, where the Planalto hermit *P. pretrei* (Lesson & Delattre 1839) interact with the more specialized flowers (Maruyama *et al.* 2014). In summary, this suggests that there is an asymmetrical interaction between hermit hummingbirds and the plant species they pollinate. While many plant species (such as *C. paniculata*) depend on only one hermit hummingbird species for their reproduction, each plant species alone potentially has less importance for the hermit hummingbirds that pollinate them. Although this asymmetry between interacting plants and hummingbirds might vary among communities (see Maruyama *et al.* 2013), it should have strong implications for the structure and dynamics of the entire plant-hummingbird community, and hence, deserves further investigations.

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