

A novel mutualism between an ant-plant and its resident pollinator

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Abstract Pollination systems in which the host plant provides breeding sites for pollinators, invariably within flowers, are usually highly specialized mutualisms. We found that the pollinating bee *Braunsapis puangensis* breeds within the caulinary domatia of the semi-myrmecophyte *Humboldtia brunonis* (Fabaceae), an unusual ant-plant that is polymorphic for the presence of domatia and harbours a diverse invertebrate fauna including protective and non-protective ants in its domatia. *B. puangensis* is the most common flower visitor that carries the highest proportion of *H. brunonis* pollen. This myrmecophyte is pollen limited and cross-pollinated by bees in the daytime. Hence, the symbiotic pollinator could provide a benefit to trees bearing domatia by alleviating this limitation. We therefore report for the first time an unspecialised mutualism in which a pollinator is housed in a plant structure other than flowers. Here, the cost to the plant is lower than for conventional brood-site pollination mutualisms where the pollinator develops at the expense of plant reproductive structures. Myrmecophytes housing resident pollinators are unusual, as ants are known to be enemies of pollinators, and housing them together may decrease the benefits that these residents could individually provide to the host plant.

Keywords *Braunsapis puangensis* · Domatia · *Humboldtia brunonis* · Myrmecophyte · Symbiosis · Western Ghats

Introduction

Pollination systems in which plants provide brood sites as rewards to their pollinators are usually housed in flowers and are also usually highly specialised (Sakai 2002; Dufaÿ and Anstett 2003). Such systems include ovule parasites, plants where pollinator larvae consume pollen and plants in which pollinators develop within decomposing flowers (Sakai 2002). We report for the first time that the caulinary domatia of the semi-myrmecophytic plant *Humboldtia brunonis* Wallich (Fabaceae, Caesalpinioideae) serve as brood sites for the pollinating bee *Braunsapis puangensis* Cockerell (Apidae, Xylocopinae, Allodapini). *H. brunonis* is unique in being polymorphic for the presence of domatia; although all trees produce extrafloral nectar, in the same population only some trees possess domatia. The plant is therefore referred to as a semi-myrmecophyte (Gaume et al. 2005b). *Humboldtia* is the only myrmecophytic genus in which a huge diversity of invertebrates including *Braunsapis* bees has been reported from domatia in at least two species: *H. brunonis* in India (Michener et al. 2003; Rickson et al. 2003; Shenoy 2003; Gaume et al. 2006) and *Humboldtia laurifolia* in Sri Lanka (Krombein et al. 1999). As *Braunsapis* are known pollinators in Asian forests (Momose et al. 1998; Corlett 2004), to test whether *B. puangensis* could be a reliable pollinator of its myrmecophytic host, we asked the following questions: (1) How common is *B. puangensis* in the domatia of *H. brunonis*? (2) What is the visitation pattern and pollen load of *B. puangensis* that frequent *H. brunonis* flowers? (3) Is *H. brunonis* solely dependent on insect visitors for pollination? (4) As *B. puangensis* is a diurnal visitor of flowers that have bloomed the previous evening, are flowers receptive in the day, and if so, are they predominantly pollinated in the day or at night?

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Materials and methods

H. brunonis is a common understorey tree in the low-elevation, wet evergreen forests of the Indian Western Ghats (Pascal 1988) and flowers from December to April. Each raceme has 25 to 30 flowers that bloom acropetally over 1–4 days, opening between 1500 and 1700 hours, with anthesis around 1800 hours and the stigma becoming simultaneously erect, allowing for the possibility of self-pollination. We sampled bees within domatia at three sites in Karnataka State: Bisle Reserve Forest (12°40' N, 75°40' E, 600 m asl) in May 2002, Talcauveri Reserve Forest (12°22' N, 75°31' E, 680 m asl) and Brahmagiri Wildlife Sanctuary (12°8.5' N, 75°46' E, 624 m asl) in October 2002. We investigated the pollination biology of *H. brunonis* between January and March 2003 and in December 2006 at Brahmagiri Wildlife Sanctuary.

We noted the behavior and visitation time of *B. puangensis* and other diurnal visitors at 22 *H. brunonis* inflorescences on ten trees over 3 days (0900–1800 hours at 30-min intervals). For determining pollen loads, haphazardly collected floral visitors were individually placed in vials of formalin/acetic acid/alcohol solution for 12 h and transferred to 70% ethanol. Each vial was subjected to mild sonication followed by centrifugation after bee removal. The pollen pellet obtained was dissolved in 50 µl of distilled water, transferred to a slide, and air-dried. This procedure was repeated thrice to complete pollen transfer. Pollen were stained with basic fuchsin in gelatin (Kearns and Inouye 1993) and categorised as *H. brunonis* or “other” pollen. We observed 22 inflorescences on 12 trees for two nights (1830–0630 hours) at 10-min intervals to record nocturnal visitors.

As *H. brunonis* pollen is plentiful and presented on free-standing exertile anthers, we tested for wind pollination by bagging flowers with fine mesh bags (1×1-mm mesh size) a day before flower opening. Control inflorescences were covered with particle-proof bags (brown paper bags) to detect spontaneous self-pollination. As pollinating activity

had been observed only during the day (Gaume et al. 2005a) while the flowers open in the evening, hand pollinations using self- and cross-pollen were performed both in the evening (1800–2000 hours) and the next morning (0900–1100 hours) to determine if pollination is possible in the day. In all cases, recipient flowers were emasculated before hand pollination, and excess pollen was deposited to ensure sufficient pollen for fruit formation. To test for apomixis (reproduction without fertilization), flowers were emasculated before anther dehiscence and bagged without further treatment. To determine whether natural pollination occurs diurnally or nocturnally, we bagged one set of inflorescences in the day and exposed them in the night [natural pollination (night)] and vice-versa for diurnal pollination [natural pollination (day)] with another control set of inflorescences open over day and night [natural pollination (day+night)]. The percentage of flowers initiating fruits (fruit set) was determined 2 weeks after each treatment.

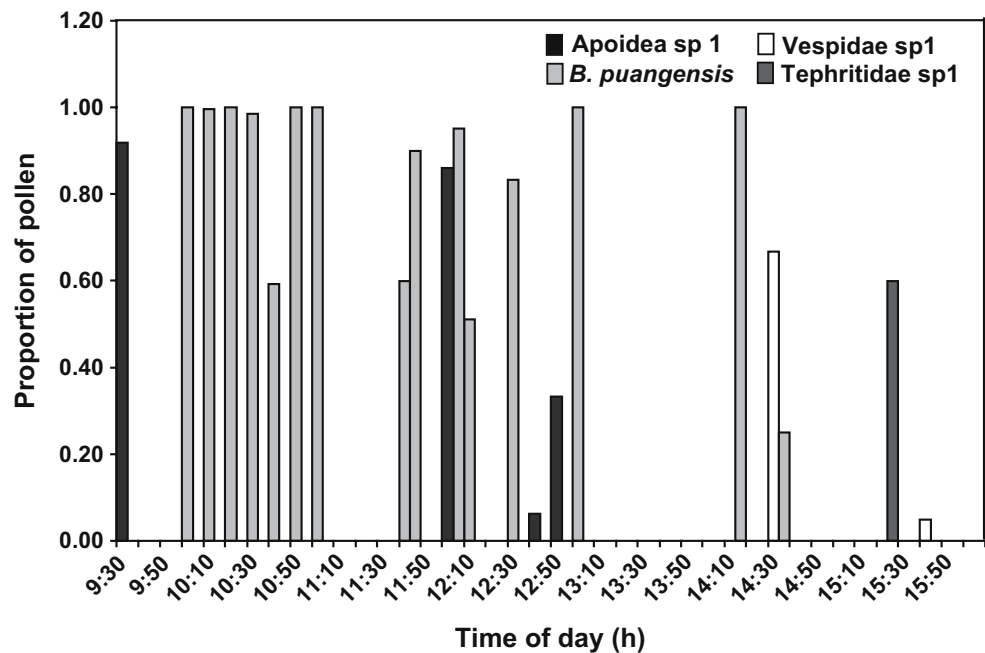
Results

B. puangensis bees and brood occupied 38.5% domatia-bearing trees ($n=26$) and 6.2% domatia ($n=290$) in Bisle Reserve Forest, 13.3% trees ($n=30$) and 2.5% domatia ($n=198$) in Talcauveri Reserve Forest and 3.3% trees ($n=30$) with 0.7% ($n=141$) domatia occupancy in Brahmagiri Wildlife Sanctuary. The entrance to all domatia containing bees was modified from the regular, narrow, self-opening slit to a round opening [diameter= 0.3 ± 0.1 cm ($n=15$)] that facilitated head passage of the bees. All *H. brunonis* domatia could be potentially occupied by *B. puangensis*, as there was no difference in length and breadth at the widest part of domatia occupied by bees (length= 9.7 ± 2.4 cm, breadth= 0.5 ± 0.1 cm, $n=15$) and those occupied by ants (length= 8.6 ± 1.1 cm, breadth= 0.5 ± 0.1 cm, $n=15$) [*t*-test for independent samples; length: $t_{30}=1.53$, $p=0.136$;

Table 1 Pollen loads on insect visitors to *H. brunonis* flowers

Insect species	<i>Humboldtia brunonis</i> pollen grains	Non- <i>Humboldtia brunonis</i> pollen grains	Percent of individuals carrying <i>Humboldtia brunonis</i> pollen	Proportion of <i>Humboldtia brunonis</i> pollen carried by insect
	Mean±SD	Mean±SD		Mean±SD
Bees				
<i>Braunsapis puangensis</i> ($n=16$)	148.5±217.7	4.6±6.6	93.8	0.79 ± 0.31
Superfamily Apoidea sp1 ($n=5$)	227.2±418.2	33.2±31.1	100	0.44 ± 0.42
Wasps				
Family Vespidae sp1 ($n=4$)	0.5±1.0	1±0.8	25	0.17 ± 0.33
Flies				
Family Tephritidae sp1 ($n=2$)	6±8.5	4±5.7	50	0.30 ± 0.42

Fig. 1 Visitation pattern and proportion of *H. brunonis* pollen carried by individual daytime floral visitors



breadth: $t_{30}=0.20$, $p=0.841$]. Both ants and bees were co-residents in 53.3% of trees, while bees occurred without ants in only 6.7% of trees; in the rest, bees co-occurred with other invertebrates. Of 15 *B. puangensis* bees observed at *H. brunonis* flowers, 6 touched both stigma and stamens, 4 touched only stamens, and 5 touched neither. The bees captured at *H. brunonis* flowers carried pollen from other flowers but in lower quantities [paired t -test, $t_{16}=2.62$, $p=0.019$, Table 1]. *B. puangensis* visited flowers regularly from morning to mid-afternoon unlike other visitors whose visits were few and sporadic (Fig. 1). They also consistently carried a high proportion of *H. brunonis* pollen during these visits [significantly more than the wasp (Mann–Whitney $U=6.5$, $n_1=16$, $n_2=4$, $p=0.016$), though not significantly different from the other bee ($U=18$, $n_1=16$, $n_2=5$, $p=0.067$); Table 1].

The requirement of pollinators for fruit set was established by the exclusion of wind pollination (Table 2). There

was a significant difference between fruit set of cross- and self-pollination treatments and between that of cross- and natural-pollination treatments (Fisher's exact tests) indicating that *H. brunonis* is cross-pollinated and pollinator-limited in this site (Table 3). Furthermore, the fruit sets from the night and day cross-pollinations were not significantly different, indicating no decline in stigma receptivity or pollen quality in the day. Natural fruit set mainly resulted from daytime pollination; there was no difference between the fruit sets of flowers subjected to natural pollination (day+night) and natural pollination (day) (Table 3). No pollinators were observed during the night.

Discussion

B. puangensis was the predominant floral visitor and pollinator of *H. brunonis* at the Brahmagiri site where it

Table 2 Pollination in *H. brunonis* (fruit set under various pollination treatments)

Treatment	Time of day (h)	No. of trees	No. of flowers	No. of fruits	No. of experimental inflorescences/tree	Fruit set (%)
Wind pollination (mesh bag)		7	314	0	1–4	0
Wind pollination (control: brown bag)		5	281	0	2–4	0
Hand self-pollination	1800–2000	7	64	0	1–2	0
	0900–1030	8	168	1	1–3	0.6
Hand cross-pollination	1800–2000	7	73	5	1–2	6.8
	0900–1100	5	69	4	1–3	5.8
Natural pollination (day)	0630–1830	11	337	7	1–3	2.1
Natural pollination (night)	1830–0630	5	324	1	1–5	0.3
Natural pollination (day and night)		6	571	13	1–5	2.3
Apomixis		8	116	1	1–2	0.9

Table 3 Pollination in *H. brunonis* (comparison of fruit set between different pollination treatments; Fisher's exact tests)

Treatment 1	Fruit/ flower ratio	Treatment 2	Fruit/ flower ratio	<i>p</i> - value
Cross-pollination (day+night)	9/142	Self-pollination (day+night)	1/232	<0.001
Cross-pollination (day+night)	9/142	Natural pollination (day+ night)	13/571	0.0127
Cross-pollination (1800–2000 hours)	5/73	Cross-pollination (0900–1100 hours)	4/69	0.2600
Natural pollination (day)	7/337	Natural pollination (night)	1/324	0.0344
Natural pollination (day)	7/337	Natural pollination (day+ night)	13/571	0.1828

was the least abundant in the domatia, indicating that *H. brunonis* could be even more assured of its pollination services at other sites where the bee was much more common in the domatia. Another study also found *Braunsapis* sp. to be common occupants of *H. brunonis* domatia in the Western Ghats (Rickson et al. 2003). We also found *B. puangensis* and its brood parasite *Braunsapis bislensis* (Michener et al. 2003) occupying domatia even in the non-flowering season of *H. brunonis*, indicating that *Braunsapis* is domiciled within the tree. The numerous woody domatia on this understorey tree could provide abundant nesting sites for the bees whose use of the domatia is probably governed by other resident invertebrate taxa, especially ants (Fonseca 1999). For example, our preliminary unpublished data suggest that bees are co-residents with timid rather than aggressive ants in trees.

In obligate myrmecophytes, mature individuals are inhabited by a single ant species behaving as a mutualistic symbiont (Heil and McKey 2003). However, *H. brunonis* is an unspecialized myrmecophyte whose domatia are occupied by at least 20 ant species across its geographical range whose benefits to the plant are variable (Gaume et al. 2005a; Shenoy 2003; Shenoy and Borges, unpublished data). Being such an unspecialized myrmecophyte has advantages and disadvantages. The advantage of non-specialization is the benefit from invertebrates other than ants, which may offer unconventional services such as pollination. The disadvantage, however, may be the use of plant resources (housing spaces or food) by parasitic partners that do not reciprocate benefits to the host plant (Yu 2001; Gaume et al. 2005a). However, being polymorphic for domatia, *H. brunonis* offers an ideal system to investigate the selective advantage conferred by domatia. Earlier studies suggested that although anti-herbivore

protection by resident ants is a major benefit mediated by domatia, it might not be the only one, as trees occupied by even non-protective ants produced more fruit than trees without domatia in the same environmental conditions (Gaume et al. 2005b). Additional explanations for the enhanced fruit set of plants with domatia have included the possibility of trophic exchanges between plants and domatia occupants (Sagers et al. 2000; Gaume et al. 2005b). With the discovery of a resident pollinator within domatia, we now hypothesize that *B. puangensis* could confer a specific advantage to *H. brunonis* plants having domatia by alleviating pollinator limitation. Plants with domatia could thus benefit from the double advantage of being protected by symbiotic ants and being pollinated by symbiotic bees. As this resident pollinator is not housed in all individuals of the plant species, this interaction is an unspecialized mutualism perhaps in its early stages. The geographic variation in occupancy of domatia by *Braunsapis* suggests spatial variation in the strength of the pollination mutualism (*sensu* Thompson 2005), as also exists for the ant-protection mutualism in this species (Shenoy and Borges, manuscript in preparation). A resident pollination mutualism in a myrmecophyte is unusual, as ants are known to be potential enemies of pollinators and are known to intimidate pollinators (Janzen 1977; Willmer and Stone 1997). Pollinators did not visit *H. brunonis* inflorescences if more than four individual ants were present on them (Gaume et al. 2005b). Such ant–pollinator conflicts create special constraints for the simultaneous evolution of a resident pollination mutualism and myrmecophytism. Further studies on such conflicts would be fruitful in understanding the evolution of potentially antagonistic effects in this atypical transitional myrmecophyte, a member of the primitive legume tribe Detarieae.

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