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POLLINATION IN AN ENDEMIC AND THREATENED MONOECIOUS HERB *BEGONIA SATRAPIS* C.B. CLARKE (BEGONIACEAE) IN THE EASTERN HIMALAYA, INDIA

Subhankar Gurung, Aditya Pradhan & Arun Chettri

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Pollination in an endemic and threatened monoecious herb *Begonia satrapis* C.B. Clarke (Begoniaceae) in the eastern Himalaya, India

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Abstract: *Begonia satrapis* was studied for its pollination aspects at Sumbuk, Sikkim, India. The floral details and the foraging behaviour of insects visiting the flowers were examined to define the pollination syndrome and its functionality for the success of sexual reproduction in this species. The flowers do not produce nectar and offer only pollen as floral reward to foraging insects. Therefore, male flowers were foraged more for its pollen than the female flowers. There was a significant difference in the visit to male and female flowers by both *Apis florea* and *Bombus breviceps*, respectively. The bees spent more time on male flowers than on female flowers. The bees appear to rely on visual stimuli to visit male and female flowers. The plant produces abundant fruit and seed set in both hand and open-pollinations indicating that it is facultatively xenogamous. The female flowers lacking any reward resemble male flowers and in effect are pollinated by deceit.

Keywords: *Apis florea*, *Bombus breviceps*, deceit, northeastern India, Sumbuk.

***Nepali* सार:** भारतको उत्तर-पूर्वीय राज्य सिक्किमको सुम्बुकमा पाइने मंगरकाँजे (*Begonia satrapis*)को परागण प्रक्रिया माथीको विशेष अध्ययन हो। यहाँ पाइने यस प्रजातिको प्रजनन प्रक्रिया तथा स्त्री फूल अनि नर फूलको पराग मिलन प्रक्रियालाई बुझ्न, फूलको मुख्य भागको जाँच साथै फूलको पराग खान आउने किटपतङ्गको क्रिया कलापमाथी विशेष जाँच गरिएको छ।फूलमा रस नहुनाले गर्दा किटपतङ्गले परागको धुलोलाई नै टिप्ने गर्दछ। विशेष गरि नर फूलमा परागको धुलो पाइनाले गर्दा मौरी (*Apis florea*) अनि भमरा (*Bombus breviceps*)ले नर फूलमा मात्र अधिक समय बिताएको पायौं।आफ्नै हातले गरिएको परागण प्रक्रिया साथै प्राकृतिक स्वभावले हुने परागण (Open pollination)द्वारा अधिक मात्रामा फल र बिज लागेको हुनाले मँगरकाँजेको यस प्रजातिलाई *फ्याकलटेटिभ जिनोग्यामस्* भन्न सकिन्छ। मौरी अनि भमराले आफ्नो नजरमाथी भर परेर नै नर फूल र स्त्री फूलमाथी बसेको अवलोकन गर्यौं। स्त्री फूलमा किटपतङ्गको निम्ति कुनै रस अथवा आहार नभए पनि नर फूल झैं दुरुस्त देखिने हुनाले किटपतङ्ग झुक्किएर स्त्री फूलमा बस्दा नर फूलबाट टिपेको परागको धुलो छरिन्छ, यसो हुँदा फूलमा परागण प्रक्रिया सम्भव भएको पाइन्छ।

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Author details: Subhankar Gurung is currently doing his PhD in the Department of Botany, Sikkim University, India. His interest lies in studying the reproductive biology of plants with special emphasis on understanding the role of pollinators. Aditya Pradhan, a PhD scholar and a Junior Project Fellow (JPF), is studying the diversity and distribution of the genus *Begonia* in Sikkim Himalayas. Dr. Arun Chettri is a plant taxonomist/ecologist who has worked extensively on plant diversity, taxonomy, ecology and conservation of threatened plants in different forests types in northeastern India, in particular in Sikkim.

Author contribution: Study designed by SG; Data collected by SG, AP; analysis of data done by AC, and SG wrote the manuscript.

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INTRODUCTION

Male and female flowers provide different levels of rewards to the pollinators. In fact, some female flowers do not produce a pollinator reward and are actually pollinated by deceit (Willson & Ågren 1989; Ågren & Schemske 1991). Deceit pollination can be considered as an extreme case of unreliable signalling in plants since flowers do not offer any reward while they benefit from pollinator visitation (Renner 2006). Intersexual mimicry drives the pollinators to visit the female flowers that do not provide any reward (Little 1983; Ågren & Schemske 1991). Mimicry hypothesis suggests that plants that exhibit intersexual mimicry experience selective advantage when pollinators pay more visits to reward-less female flowers which resemble reward-providing male flowers (Ågren & Schemske 1991). Female flowers in many monoecious species of the genus *Begonia* may attract pollinators by mimicking conspecific male flowers in which bees mistakenly visit unrewarding female flowers (Vogel 1998; Wiens 1978; Ågren & Schemske 1991; Schemske & Ågren 1995; Corff et al. 1998). This hypothesis is based on the remarkable similarity between unrewarding female flowers and the pollen-rich male flowers (Ågren & Schemske 1991). Although, mimicry hypothesis and the behaviour of pollinators have been studied in some species of *Begonia* (Ågren & Schemske 1991; Castillo et al. 2002; Wyatt & Sazima 2011), no detailed study of such kind has been done in *Begonia satrapis* C.B. Clarke (Begoniaceae), an IUCN Red Listed Endangered herb of eastern Himalaya (Adhikari et al. 2018).

Little (1983) suggested that in floral mimicry hypothesis the mimic and the model floral displays are similar, and the pollinators mostly visit the model more often than the mimic. Accordingly, we tested this hypothesis by studying the floral display of male and female phase inflorescences and the pollinator behaviour in *B. satrapis*. The study addressed the following questions: (i) are there any differences between the size and morphology of male and female flowers? (ii) do pollinators discriminate between male and female flowers? (iii) what is the success rate of intersexual mimicry to deceive the pollinators to effect pollination?

MATERIALS AND METHODS

Study site

This study was conducted in a private forest in Sumbuk which falls in the Rangit Valley, South District, Sikkim, eastern Himalaya ($27^0$06’18.90”N & $88^0$22’07.32”E, altitude 555m). The area experiences a maximum and minimum temperature of 26.9^{0}C and 17.3^{0}C, respectively, with an annual precipitation of 2,766mm. The study site comprises a sub-tropical type of forest where *B. satrapis* flourished in abundance along the margins of this forest which is close to human habitation. The forest surroundings comprised species of *Shorea robusta* C.F. Gaertn and *Schima wallichii* Choisy.

Statistical data analysis

Mann-Whitney U Test was performed to evaluate the difference in seed set rate in hand and open pollination of female flowers. Non-parametric t-test was used to evaluate the variation in morphological characters of male and female flowers. Data collected from different patches were pooled and subjected to a t-test to know whether the resulting variation levels are statistically significant or not. A t-test was performed between the open flowers and closed buds of both male and female flowers, respectively, to check if there is any chronological difference in the opening of male and female flowers.

Inflorescence sex ratio and floral morphology

A sample of 50 flowers, each for male and female sex, was used to record floral morphometrics. A sample of 50 plants was used to record the average number of male and female flowers produced in individual inflorescences. Anthesis schedule and flower lifespan were observed in the field itself.

Foraging activity and pollination

Pollinators were observed in three 3×1 m randomly chosen patches of *B. satrapis* which were reselected on 31 August, 1, 2 and 14 September 2017 (as per Ågren & Schemske 1991). The observation period for the day continued until the pollinators ceased to visit the flowers. Before making observations, number of open male and female flowers were counted within the monitored patch. A total of 1,013 open flowers were counted in each of the inflorescences of the monitored patch out of which 895 were male flowers and 118 were open female flowers. The foraging behaviour of pollinators that entered the patch was observed until they moved out

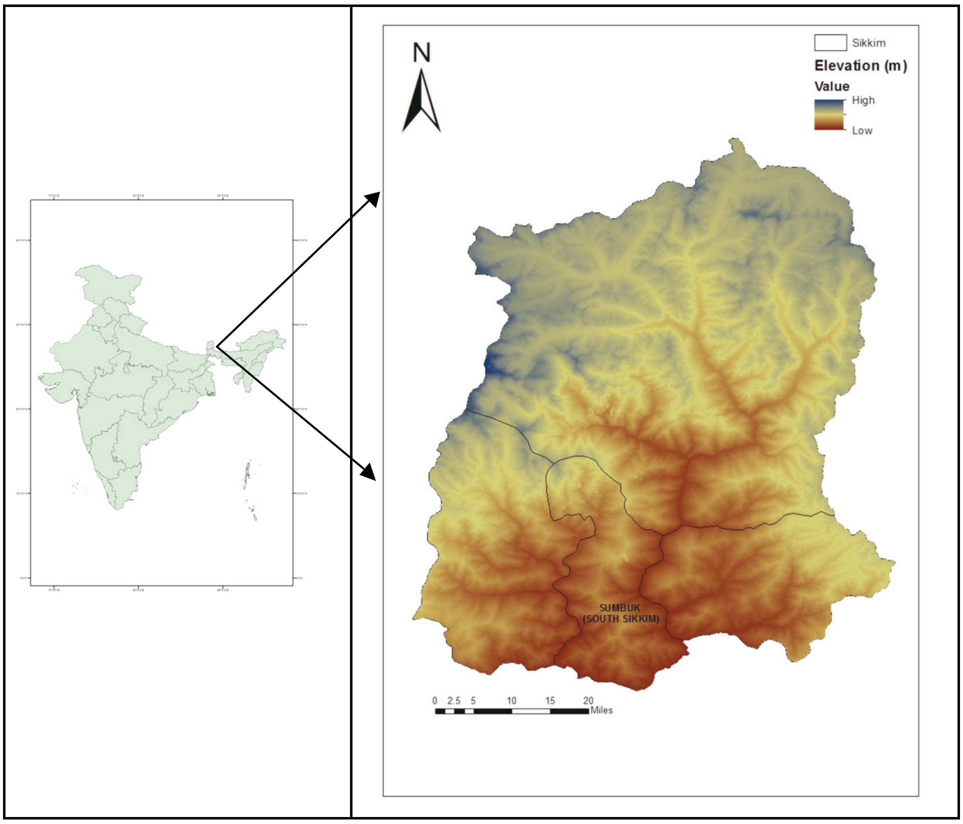

Figure 1. Study site in South Sikkim, the eastern Himalaya.

of it. The number of visits to both the male and female flower were recorded. The time spent on each flower was recorded with a stopwatch in each monitored patch (Male: N=50; Female: N=14). A minimum count of 50 visits to the flowers were kept mandatory in each monitored patch. To test if pollination occurs by wind, several of the buds (N=25) were bagged with mosquito nets which were made into small bags to exclude visitors in order to record the fruit and seed set rate. Similarly, another set of buds (N=25) were bagged and tagged with ribbons to test apomixis.

Apis florea and *Bombus breviceps* were the most frequent pollinators throughout this study. A few rare visits by two other unidentified bee species were noted. The observations were made only on *A. florea* and *B. breviceps* as other foragers visited the flowers rarely.

Hand pollination

Female buds (N=50) were bagged and tagged with a coloured ribbon a day before their anthesis and were hand pollinated on the following day. Hand pollination was performed by brushing the fresh dehisced anther against the stigma of a different plant. Similarly, female buds (N=50) were tagged with a different coloured ribbon and were left for open pollination. The matured fruits were collected to record the number of seeds produced against the number of ovules produced per flower. The pollen limitation was estimated as the ratio of hand cross pollination to open pollination (Larson & Barrett 2000). The scale ranged from 0–100 where 0 indicated no pollen limitation to 100 indicating pollen limitation. All the tagged flowers could not be retrieved at the time of their collection due to anthropogenic activities at the study site.

RESULTS

Floral morphological details

The flowers of *B. satrapis* bloom during July–October. The flowers are open from 05.00–06.00 h. A female flower lasts for 7–9 days while a male flower lasts for almost 15 days. The inflorescence is a cymose with male and female flowers with pink tepals (Image 1). Male flowers comprise four tepals (2+2) while female flowers comprise five tepals (2+3). The outer tepals of male flowers were significantly longer (1.5 ± 0.24 vs. 1.2 ± 0.17 cm; t=1.55e-09, $p<0.05$) and wider

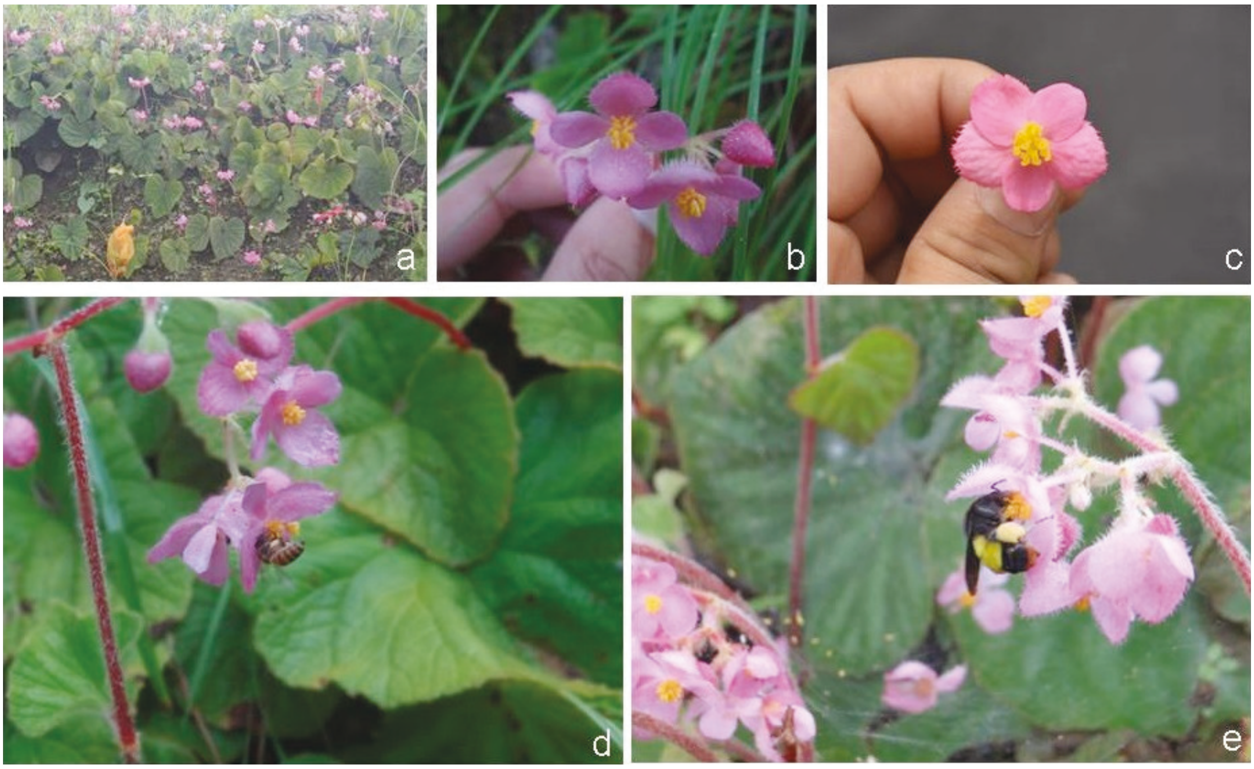

Image 1. *Begonia satrapis*: a—habitat | b—male flower | c—female flower | d—*Apis florea* | e—*Bombus breviceps*. © Subhankar Gurung.

(1.2 ± 0.16 vs. 1.0 ± 0.16 cm; t=4.05e-04, p<0.05) than those of the female flowers. In addition to the two large tepals, the inner tepals were significantly longer (1.2 ± 0.17 vs. 1.0 ± 0.18 cm; t=1.46e-06, p<0.05) than the female flowers while the inner tepal width showed no significant difference (0.6 ± 0.17 vs. 0.7 ± 0.19; t=0.13, p<0.05, NS) in both male and female flowers. The male flowers produced 34.9 ± 4.1 stamens which are 4.3 ± 0.8 mm in length. The anthers are rimose and each one produced 3,761.3 ± 1,409.4 pollen grains. The anther and stigma of a male and female flower are yellow in colour and are located in the centre of the flower respectively. *B. satrap*is is protandrous and exhibits temporal separation in the production of male and female flowers. The inflorescence was considered as a male phase inflorescence when a larger number of male flowers were open compared to the number of female flowers. The number of open male flowers produced per inflorescence was 3.36 ± 1.84, N=50 and that of female flowers was 0.34 ± 1.17, N=50. The number of open male flowers and closed male flower buds per inflorescence did not differ (t=0.39, p>0.05, NS) whereas difference was observed between open female flowers and closed female flower buds per inflorescence in a population (t=0.0001, p<0.05).

Foraging activity and pollination

During nine hours of observation on pollinators' behaviour on *B. satrapis* a total of 458 male and 14 female flowers were visited by *A. florea* and 100 male and seven female flowers were visited by *B. breviceps* inside the monitored patches (Image 1). The flowers were foraged by bees as and when they were open and continued foraging activity until 12.00h. *Apis florea* and *B. breviceps* showed strong preference to male flowers than female flowers (Table 1). The number of flower visits in the monitored patches by *A. florea* varied between male (91.6 ± 49.8) and female flowers (2.8 ± 2.2; *t*=0.007, *p*<0.05). Similarly, 107 flowers visited by *B. breviceps* varied between male (20 ± 8.3) and female flowers (1.4 ± 1.3; *t*= 0.005, *p*<0.05). *A. florea* and *B. breviceps* spent more time on male flowers than on female flowers due to pollen collection activity (Table 2). *Apis florea* used their legs to remove the pollen while *B. breviceps* performed vibration to collect pollen. Both bee species discriminated female flowers after making first visit to them. The foraging activity of *A. florea* was slower than *B. breviceps* while there was no significant difference in time spent on male flowers (t=0.31, p>0.05, NS) and female flowers(t=0.13, p>0.05, NS) by both bee species.

Table 1. Number of *A. florea* and *B. breviceps* visits to male and female flowers of *B. satrapis*.

	Number of flowers in a patch		Number of flowers visited			
			A. florea		*A. breviceps*	
Patch	Male	Female	Male	Female	Male	Female
1	177	7	60	2	22	2
1	177	7	175	6	32	0
2	173	26	48	2	15	2
3	197	16	83	4	10	3
2	173	63	92	0	21	0

Table 2. Time (in seconds) spent on male and female flowers of *B. satrapis* by *A. florea* and *B. breviceps*.

	A. florea		*B. breviceps*	
	Male	Female	Male	Female
Patch	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	4.9 ± 3.4	0.7 ± 0.3	1.1 ± 0.6	1.4 ± 0.1
1	4.6 ± 2.9	0.4 ± 0.1	1.2 ± 0.7	-
2	3.1 ± 2.4	0.6 ± 0.3	1.9 ± 1.2	1.0 ± 0.2
3	5.4 ± 3.2	0.7 ± 0.6	2.0 ± 1.6	1.0 ± 0.1
2	2.5 ± 1.4	-	1.4 ± 0.7	-
	4.1 ± 2.7	0.6 ± 0.3	1.5 ± 0.9	1.2 ± 0.1

Note: The visits to flowers outside the patch were also considered for better results.

Hand pollination

Although fruit set was observed in both hand pollinated and open pollinated flowers, hand pollination between male and female flowers of different plants resulted in an increase in seed set (95.4 ± 8.1, N=17) than open pollination (81.1 ± 17.2, N=17) and showed a significant difference between the two (U=0.001, $p<0.05$). Fruit and seed set was absent in both wind pollination and apomixis. The estimated pollen limitation (1.17) indicated that the pollinators deposit adequate pollen in its natural environment.

DISCUSSION

B. satrapis is a monoecious species with both male and female flowers borne in the same inflorescence whose morphological characters varied significantly between male and female flowers in a population. The larger tepals of male flowers appear to be an adaptation to provide visual stimuli to pollinator bees to locate the flowers that provide the reward (Ågren & Schemske 1991). The anthesis of male flowers prior to female flowers enable the pollinators to habituate themselves to the forage source and visit rewardless female flowers by deceit when available on the same or different inflorescences of the same or different conspecific plants. Similar observations were reported in *Jacaratia dolichaula* (Bawa 1980) where pollinators first encountered the rewarding male flowers and therefore, reduced the chances of discrimination of early flowering rewardless female flowers (Corff et al. 1998). The stigmas of female flowers are yellow and strongly resemble male flowers. The female flowers attract pollinators by mimicking male flowers (Wiens 1978; Ågren & Schemske 1991). The rimose anthers are grouped in large number which facilitate pollen collection by vibration (Wyatt & Sazima 2011). The two important foragers *A. florea* and *B. breviceps* showed more preference to the male flowers than the female flowers. This possibly could be because of the pollinators ability to recognize unrewarding flowers (Wyatt & Sazima 2011). It was observed that *A. florea* seemed diffident to collect pollen from a male flower immediately after its visit to a male flower. The lower rate of visitation and the hesitation shown in collecting pollen could be because of the lack of fragrance which has been experimentally proven to be an important aspect to encourage landing on a female flower (Lunau 1991; Schemske & Ågren 1995). Despite the negligible visits to a female flower a high seed set was observed in flowers left for open pollination which could be because of pollination by vibration (Wyatt & Sazima 2011). When a pollinator performs vibration while it visits a female flower by deception it transfers a large load of small and powdery pollen to the stigma (Wyatt & Sazima 2011). A single visit is adequate to deposit a large amount of pollen load to the stigma (Wyatt & Sazima 2011). Seed set in hand pollination was significantly higher than that of open pollination. The lack of pollen limitation, however, indicates that the pollinators are efficient in depositing pollen for successful seed set. Since wind pollination and apomixis are absent in *B. satrapis,* it becomes evident that it depends largely on pollinators for its successful sexual reproduction.

CONCLUSION

It is apparent from our study that the pollinators prefer to visit the polleniferous male flowers more than the rewardless female flowers. The imperfect discrimination by the pollinators by the number of "mistaken" visits to female flowers, however, guarantees pollination by deceit. Further, abundant fruit and seed set indicate that pollinators are efficient in contributing to the production of fruit and seed in *B. satrapis*. The study indicates that *A. florea* and *B. breviceps* are the principal pollinators of *B. satrapis* and hence, this plant is melittophilous.

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Editorial

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