

Yuhina nigrimenta Blyth (Zosteropidae) as a bird pollinator of *Brandisia hancei* Hook.f. (Scrophulariaceae) during winter

Yifan QIAN^{1,2,3}, Yun Xiang LI^{1,3}, Xuemei ZHANG³, Qiu Mei QUAN^{1,3,*}

¹College of Environmental Science and Engineering, China West Normal University, Nanchong, Sichuan, P.R. China

²Nanchong Environmental Monitoring Central Station, Nanchong, Sichuan, P.R. China

³Key Laboratory of Southwest China Wildlife Resources Conservation, College of Life Science, China West Normal University, Nanchong, Sichuan, P.R. China

Received: 26.10.2016 • Accepted/Published Online: 12.04.2017 • Final Version: 28.09.2017

Abstract: Winter-flowering plants commonly attract an insufficient number of pollinators. Harsh climate conditions, such as low temperature, make it impossible for insects to evolve as reliable pollinators. Birds can buffer against changes in weather to be more reliable pollinators for these kinds of plants, such as *Brandisia hancei* Hook.f. (Scrophulariaceae), a shrub endemic to the Northeast Sichuan Basin in China. In this study, the flower visitors, flowering phenology, pollinators, breeding system, and nectar availability of *B. hancei* Hook.f. were investigated. Results show that the flowering period duration was approximately 3 months for the whole population and more than 10 days per flower. *B. hancei* Hook.f. is known to be self-compatible but may need pollinators. According to our findings, the passerine bird *Yuhina nigrimenta* Blyth was the only visitor and pollinator attracted by the copious dilute nectar. The birds carried large pollen loads and exhibited high pollen deposition. The seed set from hand-selfing pollination ($87.68 \pm 0.89\%$) was similar to that of cross-pollination ($87.25 \pm 1.17\%$), with values significantly higher than those of the control ($65.63 \pm 4.15\%$). In addition, the fruit and seed sets of bagged flowers were considerably low, which suggested that *B. hancei* Hook.f. depends on flower visitors for pollination. Therefore, we conclude that flower longevity might be increased by attracting this pollinator. *B. hancei* Hook.f. can also reproduce via cross- and self-pollination. All these mechanisms may be regarded as an adaptation to adverse weather conditions.

Key words: *Brandisia hancei* Hook.f., Scrophulariaceae, *Yuhina nigrimenta* Blyth, winter flower, floral syndrome

1. Introduction

Tubular red and pink flowers often indicate bird pollination (Johnson et al., 2010). Three groups of birds, namely hummingbirds, honey-eaters, and sunbirds, are well-known pollinators of flowering plants (Proctor et al., 1996; Cronk and Ojeda, 2008). Other birds are rarely reported as pollinators of plants (Gu et al., 2009, 2010; Fang et al., 2012; da Silva et al., 2014), specifically those blossoming in erratic climates, such as winter-flowering plants. Animals cannot easily evolve as reliable pollinators under unpredictable weather, low temperature, rain, and snow (Vespisrini and Pacini, 2010). Birds can largely withstand changes in weather and be reliable pollinators.

Brandisia hancei Hook.f. flowers during winter (from November to January of the next year) and early spring (February). In the beginning of flowering, adverse environmental conditions including low temperature, rain, and snow occur. During early spring, the climatic conditions become mild, and many other plants start to bloom during the last weeks of the flowering of *B. hancei*

Hook.f. The flowers of *B. hancei* Hook.f. last for 10–11 days. Previous studies on *B. hancei* Hook.f. focused on its medicinal effects (Chinese Materia Medica Editorial Committee, 1999). This plant is rarely studied, except for the identification of its phenylethanoid glycoside content, which is an effective medicinal ingredient in *B. hancei* Hook.f. (He et al., 1990; He and Yang, 1991; He et al., 2000). Experimental data about its natural conditions are limited.

The present study aimed to analyze whether the tubular red flowers of *B. hancei* Hook.f. indicate bird pollination, identify the natural pollinator of *B. hancei* Hook.f. and the effect of climatic conditions on pollinator activity, and determine the breeding system of *B. hancei* Hook.f. and the effect of flowering time on reproductive success.

2. Materials and methods

2.1. Study site

The study site was located in the Jinchengshan National Forest Park, northeast of Sichuan, China (106°28'E,

* Correspondence: meimeiq@163.com

30°45'N, at an altitude of 650–780 m). This area experiences a subtropical monsoon climate. The annual average temperature is around 17.4 °C, and January is the coldest month. The average air temperature is around 5 °C. The mean annual sunshine duration is 1068.0 h, and the mean annual precipitation is about 987.2 mm. Fieldwork was conducted from mid-December 2012 to mid-March 2013; this period covered the flowering and fruiting peaks of *B. hancei* Hook.f. The most common vascular plants in the study area were trees, including *Pinus massoniana* Lamb. (Pinaceae), *Fagus sylvatica* L. (Fagaceae), *Cornus controversa* Hemsl. (Cornaceae), *Vernicia fordii* (Hemsl.) Airy Shaw (Euphorbiaceae), *Quercus fabri* Hance (Fagaceae), *Cornus wilsoniana* Wangerin (Cornaceae), and *Fraxinus chinensis* Roxb. (Oleaceae); shrubs, including *Rhododendron simsii* Planch (Ericaceae), *Buddleja officinalis* Maxim. (Loganiaceae), *Myrsine africana* Linn. (Myrsinaceae), *Symplocos setchuenensis* Brand (Symplocaceae), *Mahonia aquifolium* (Pursh) Nutt. (Berberidaceae), *Rubus ichangensis* Hemsl. & Kuntze (Rosaceae), *Rosa hugonis* Hemsl. (Rosaceae), *Eurya loquaiana* Dunn. (Theaceae), and *Acanthopanax trifoliatum* (Linn.) Merr. (Araliaceae); and herbs, including *Dryopteris labordei* (Christ) C.Ch. (Dryopteridaceae), *Capillipedium parviflorum* (R.Br.) Stapf (Gramineae), *Iris tectorum* Maxim. (Iridaceae), *Selaginella pulvinata* (Hook. & Grev.) Maxim. (Selaginellaceae), *Pilea notata* C.H.Wright (Urticaceae), *Cayratia japonica* (Thunb.) Gagnep. (Vitaceae), *Ophiopogon japonicus* (Linn.f.) Ker Gawl. (Liliaceae), *Arthraxon hispidus* (Thunb.) Makino (Gramineae), *Reineckea carnea* (Andrews) Kunth (Liliaceae), *Imperata cylindrica* (L.) Raeusch. (Gramineae), *Epimedium wushanense* T.S.Ying (Berberidaceae), and *Veronicastrum latifolium* (Hemsl.) T.Yamaz. (Scrophulariaceae).

2.2. Study species

B. hancei Hook.f. is a perennial evergreen shrub 2–3 m in height. This shrub is common on forest margins or cliff edges (Figure 1A). Leaves are opposite, thinly leathery, ovate or lanceolate, and integrifolious. The base of the leaf is nearly in cord form. The flowers are large, hermaphroditic, and produced singly on leaf axils. Dense grayish hairs are present on the five-lobed, bell-shaped calyx. The corolla is campanula-shaped, externally red, and with a wide bilobed upper lip and short trilobed lower lip; the two lateral lobes are reflexed, and the central lobe is folded upward.

2.3. Phenology

Our study was conducted during the blooming season of *B. hancei* Hook.f. in 2012 and 2013. The timing of flower developmental events, including the flowering phase, stamen and style elongation, anther dehiscence, and

presence of nectar, was recorded. In addition, 50 matured flowers from distinct plants ($n = 6$) were randomly collected and dissected for morphological measurements, including the length, height, and width of the corolla as well as the lengths of the stamen, pistil, and style. Measurements to the nearest 0.01 mm were obtained using a digital caliper (GuangLu, Guilin, China). Furthermore, 21 buds were collected and stored in 70% ethanol in individual Eppendorf tubes. The numbers of pollen grains and ovules per flower were counted using a binocular microscope (Leica DM500, Heerbrugg, Switzerland) and a binocular anatomical lens (Olympus SZ61, Tokyo, Japan), respectively.

2.4. Nectar properties

Flower buds from 10 plants were randomly selected, labeled, and bagged to assess the effect of flower age on nectar secretion patterns. Nectar volume and sugar concentration of each flower were measured from the first day until the end of anthesis. Additionally, 18 flowers from six plants at the stage of stigma development with high receptivity were selected, tagged, and bagged. Nectar production and sugar concentration were continuously measured in daytime (from 0900 to 1700 hours) and nighttime (from 1700 to 0900 hours the next day). For each flower, nectar volume was quantified using calibrated 10- μ L capillary tubes (Hirschmann Laborgerate, Eberstadt, Germany). Sugar concentrations were measured using a hand-held refractometer to the nearest 0.2% (Taihua, Chengdu, China). The caloric value per nectar sample was determined by transforming the sugar percentage in the nectar into mg/mL and multiplying by 4 calories (Kearns and Inouye, 1993).

2.5. Breeding system

A total of 150 *B. hancei* Hook.f. flowers from 10 plants were randomly selected and tagged. For the breeding system experiment, each plant was allocated to one of five pollination treatments, namely hand-crossing/bagged, hand-selfing/bagged, emasculated and open, autonomous selfing/bagged, and open control group. The treatments are described below.

2.5.1. Hand-crossing, bagged

A total of 30 unopened buds across five plants were opened and emasculated using tweezers. They were pollinated from a flower of a *B. hancei* Hook.f. plant growing at least 100 m away. Afterward, fine-mesh nylon bags were used to cover the flowers.

2.5.2. Hand-selfing, bagged

A total of 30 unopened buds across five plants were opened and emasculated using tweezers. These buds were pollinated from another flower on the same plant by using a toothpick. Afterward, fine-mesh nylon bags were used to cover the flowers.

2.5.3. Emasculated and open

A total of 30 unopened buds across five plants were tagged and emasculated using tweezers. These buds were left open to natural pollinators.

2.5.4. Autonomous selfing, bagged

A total of 30 unopened buds distributed across five plants were bagged with fine-mesh nylon bags to prevent animal visitors. No other manipulation was performed.

2.5.6. Open control

A total of 30 buds across five plants were marked with green twisted ties and left open to natural pollinators.

2.6. Pollen viability and stigma receptivity

After flower opening, pollen viability and stigma receptivity were examined daily until the end of anthesis by using acetocarmine and 30% hydrogen peroxide, respectively. Fully stained pollen grains were considered viable. The generation rate and quantity of bubbles on top of the stigma were used as a measurement standard; rapid generation and abundance of bubbles on the stigma indicate higher receptivity.

2.7. Flower visitors

During peak flowering of *B. hancei* Hook.f., visitors were observed from December 2012 to January 2013 (15–17 December, 24–26 December). Observations were performed in the daytimes of clear, windless, relatively warm days, totaling 51 h. The frequency of visitors, number of flowers visited, and time spent at each flower were recorded. Pollinating behavior was observed and photographed (Nikon DSLR, D7000, 16.2 million pixels). The morphological measurements (length, height, and width of bird beak) of specimens collected in the China West Normal University's herbarium were measured and compared with the flower morphology, because the birds were not captured.

2.8. Pollen deposition and pollen removal

A total of 50 buds were randomly selected, tagged, and bagged from five plants during peak flowering of *B. hancei* Hook.f. All bags were removed on the 1st day of flowering. The flowers were collected at a specific time every day until the end of anthesis. Stamens and pistils were separated and stored in individual Eppendorf tubes with 70% ethanol. Afterward, pollen depositions on stigma and pollen grains remaining on the visited and nonvisited anthers were counted under a binocular microscope (Leica DM500). In addition, 15 unbagged flower stigmas were collected at the end of anthesis. Pollen depositions with two different treatments were compared.

3. Results

3.1. Phenology

Anthesis commenced in late November and was largely finished by February, although flowers were occasionally observed in mid-March. The peak of flowering occurred in mid-December and extended through late January of the following year. The fruit setting stage was from March to April. The duration of a single flower was approximately 11 days. All flowers were hermaphrodites and protandrous. The secretion of nectar occurred simultaneously with blooming, and anther dehiscence occurred about 2 days later. The style was curved at the beginning of blooming (Figure 1B, the black arrow's direction); it extended and gradually straightened when the anthers dehiscid. Four stamens were yellow (two stamens were long; the other two were short). The surface of the anthers was covered with dense grayish hairs (Figure 1B, the white arrow's direction). The number of pollen grains and ovules per flower was $304,567.55 \pm 4697.71$ ($n = 21$) and 405.50 ± 7.08 ($n = 21$), respectively. The pollen/ovule ratio was 752.51 ± 8.08 ($n = 21$), and the flat ovate capsule bore 236.75 ± 14.06 seeds ($n = 40$).

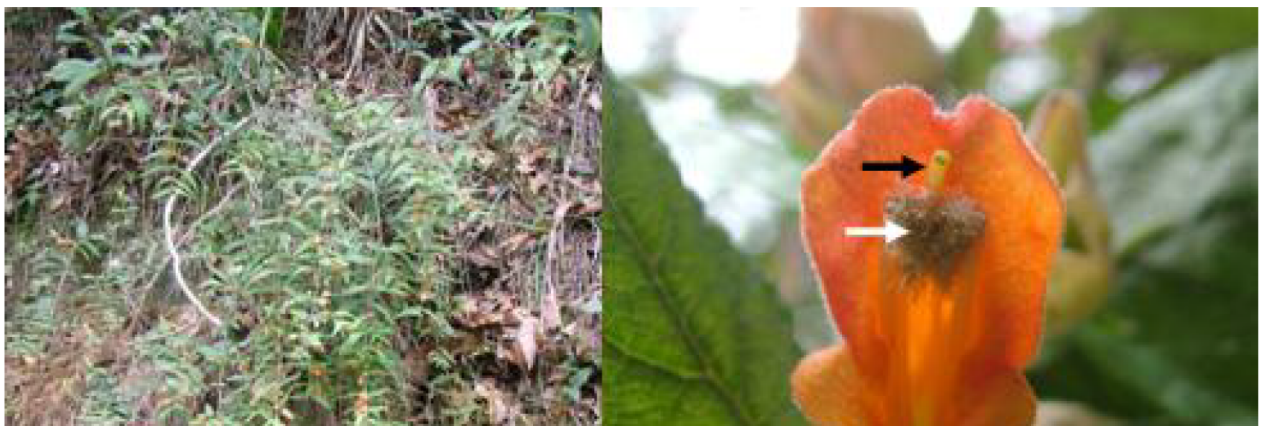


Figure 1. Habitat and flowers of *B. hancei*. Hook.f. A) The habitat of *B. hancei* Hook.f. B) Individual flower, the curved style at the beginning of blooming (the black arrow's direction) and the surface of anthers covered with dense grayish hairs (the white arrow's direction).

The average dimensions and standard errors for flowers are summarized in Table 1.

3.2. Nectar properties

In the stigma with high receptivity, the nectar secretion was $4.16 \pm 0.13 \mu\text{L}$ nectar and $14.5 \pm 0.2\%$ sugar concentration per day/flower. The mean sugar content and caloric value of nectar were $0.60 \pm 0.02 \text{ mg}$ and 2.40 ± 0.07 calories, respectively. The diurnal secretion (from 0900 to 1700 hours) of nectar per flower ($1.68 \pm 0.11 \mu\text{L}$, $n = 18$) was significantly higher than that of nocturnal production (from 1700 to 0900 hours the following day) ($2.47 \pm 0.10 \mu\text{L}$, $n = 18$) ($F = 29.536$, $df = 1$, $P < 0.001$). However, sugar concentrations during daytime and nighttime were not significantly different ($14.6 \pm 0.2\%$ vs. $14.4 \pm 0.3\%$, $n = 18$; $F = 0.774$, $df = 1$, $P = 0.385$).

Nectar secretion occurred after the flower bloomed and lasted until the stigma could not receive or germinate the pollen (Figure 2). The accumulated volume of nectar gradually increased ($F_{9, 50} = 756.363$, $P < 0.001$) until

the 9th flowering day. Nectar production decreased significantly at the end of anthesis. Nevertheless, the nectar sugar concentration was fairly constant throughout the field study ($\chi^2 = 11.261$, $df = 9$, $P = 0.258$, $n = 50$), and no reabsorption was observed.

3.3. Breeding system

Before the dehiscence of mature capsules with different treatments, the seeds fell and were collected (except for the 19 hand-crossing pollination and 20 hand-selfing pollination samples). Fruit and seed sets were then calculated. All flowers across all treatments produced capsules and seeds (Figure 3). Autonomous selfing produced generally small capsules, and its fruit setting was the lowest among all treatments.

B. hancei Hook.f. is self-compatible but depends on pollinators for increased reproductive output, which is the same result found by Ren et al. (2016). The seed set of hand-crossing pollination was similar to that of hand-selfing pollination ($\chi^2 = 0.178$, $df = 1$, $P = 0.673$) and significantly

Table 1. The morphological characteristics of corolla of *B. hancei* Hook.f.

N	LBU (mm)	LC (mm)	WC (mm)	HCM (mm)	WCM (mm)	LLS (mm)	LSS (mm)	LP (mm)	LS (mm)
50	23.81 ± 0.23	16.62 ± 0.14	11.38 ± 0.20	12.89 ± 0.15	7.74 ± 0.09	17.73 ± 0.14	15.98 ± 0.14	25.62 ± 0.24	20.27 ± 0.24

LBU – length from corollaceous base to upper lip, LC – length of corolla, WC – width of corolla, HCM – height of corollaceous mouth, WCM – width of corollaceous mouth, LLS – length of two long stamens, LSS – length of two short stamens, LP – length of pistil, LS – style length; “±” refers to SE.

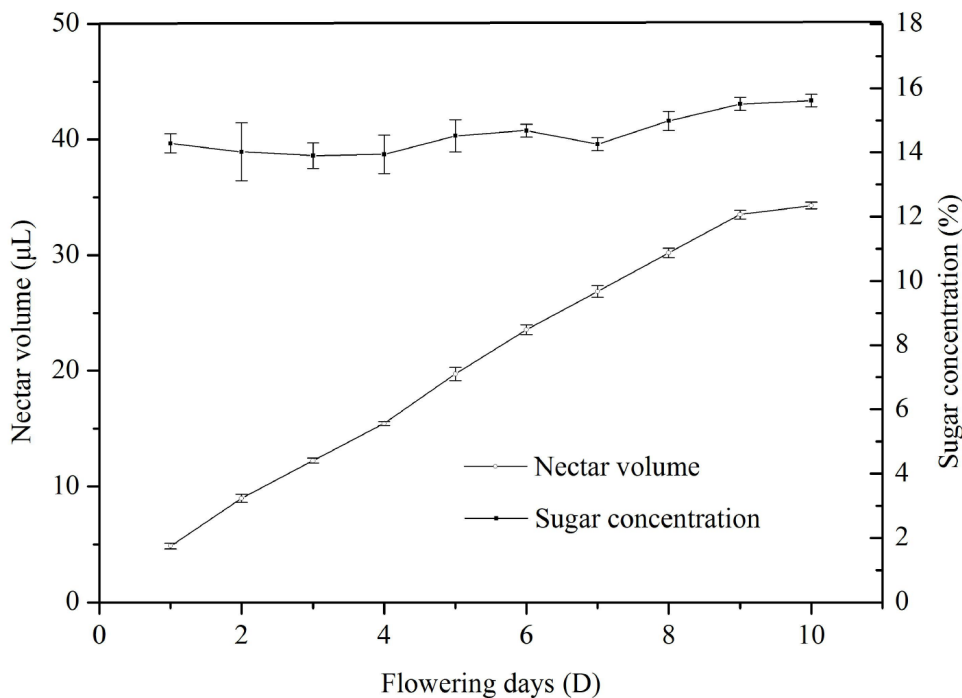


Figure 2. Changes in nectar volume and sugar concentration of *B. hancei* Hook.f. during anthesis. All error bars in this paper denote the mean ± SE.

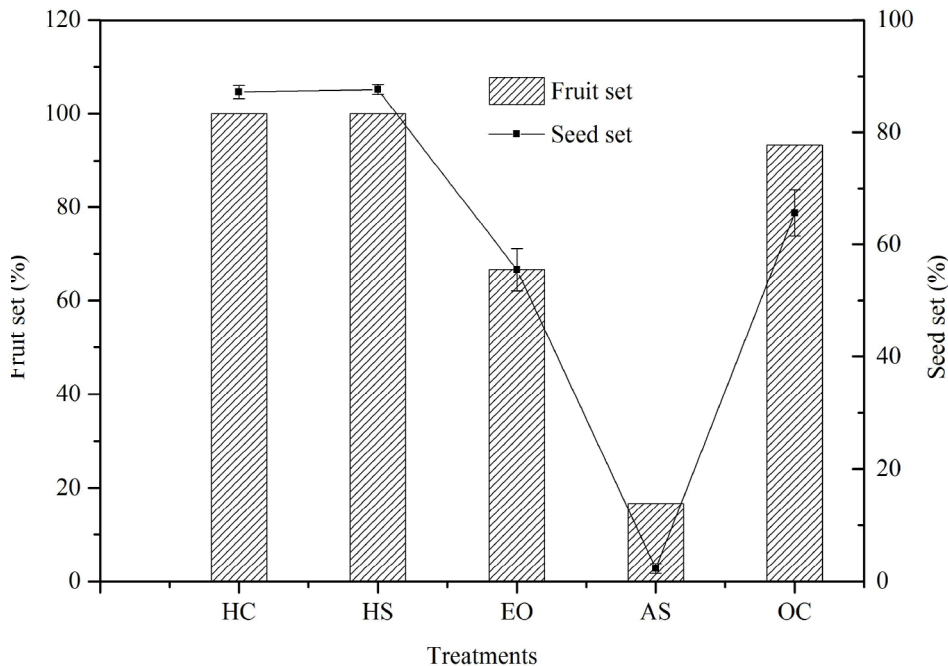


Figure 3. The fruit set and the seed set of *B. hancei* Hook.f. with different treatments. HC – hand-crossing, bagged; HS – hand-selfing, bagged; EO – emasculated and open; AS – autogamous selfing, bagged; OC – open control.

higher than that of the open control treatment ($\chi^2 = 18.423$, $df = 1$, $P < 0.001$) (Figure 3). The seed sets between the emasculated treatment (bore 55.48% fertile seeds) and open control treatment (bore 65.63% fertile seeds) were significantly different ($\chi^2 = 4.373$, $df = 1$, $P = 0.037$). The autonomous selfing treatment scarcely bore fertile seeds and displayed a significantly lower seed set than the other treatments ($\chi^2 = 58.490$, $df = 4$, $P < 0.001$).

3.4. Flower visitors

The only species recorded visiting *B. hancei* Hook.f. during the field observation period was *Y. nigrimenta* Blyth (black-chinned Yuhina, Sylviidae) (Figure 4). *Y. nigrimenta* Blyth is a common resident bird that prefers to forage in groups in mountainous areas. The average lengths of beaks (12.84 ± 0.17 mm, $n = 18$) were shorter than the average lengths of the protruding sections of the styles (20.27 ± 0.24 mm, $n = 50$) and long stamens of flowers (17.73 ± 0.14 mm, $n = 50$).

Y. nigrimenta Blyth typically perched on the branch and probed all “fresh-looking” flowers (Figure 4). These birds consume nectar by introducing the beak into the floral cavity; the forehead touches the stigmas or anthers and completes the pollination. The daily activity of the birds usually occurred between 1000 and 1100 hours and was not affected by weather conditions (Table 2). The duration of one visit varied from 2 s to 6 s ($n = 163$).

3.5. Pollen deposition and pollen removal

B. hancei Hook.f. produced a large amount of pollen per flower ($304,567.55 \pm 4697.709$, $n = 21$). The pollen load transfer to the stigma and pollen removal from anthers gradually increased daily during the flowering period (Figure 5). Hence, pollen deposition and pollen removal showed a significant correlation ($r_s = 0.806$, $P = 0.002$). The difference in pollen deposition was not significant between natural (827.40 ± 77.763 , $n = 15$) and late anthesis ($\chi^2 = 3.350$, $df = 6$, $P = 0.764$, $n = 47$). However, the number of natural pollen depositions was significantly higher than that during early anthesis ($\chi^2 = 18.258$, $df = 4$, $P = 0.001$, $n = 34$).

3.6. Pollen viability and stigma receptivity

Pollen viability was high at the beginning of anthesis and peaked on the 4th day (Figure 6). Hereafter, pollen viability reduced gradually. The stigma showed no receptivity on the 1st day of anthesis. One day later stigma receptivity gradually became stronger. Stigma receptivity from the 4th to the 6th day was higher than during the other days (Figure 6). Afterward, stigma receptivity gradually weakened until complete disappearance.

4. Discussion

Plant interactions with pollinators are often affected by abiotic conditions (Heinrich and Raven, 1972; Herrera,



Figure 4. *Y. nigrimenta* Blyth visiting flowers.

Table 2. Visitation frequency of *Y. nigrimenta* Blyth to *B. hancei* Hook.f. flowers.

Dates	Session time (total)	Weather conditions	Visiting time	Number of individuals observed	Number of flowers visited during observation	Time per flower (s)		
						Average	Shortest	Longest
15 December 2012	0900–1730 (8.5 h)	Partly cloudy	1053 hours	6	23	3	2	5
16 December 2012	0900–1730 (8.5 h)	Partly cloudy	1058 hours	8	20	3	2	5
17 December 2012	0900–1730 (8.5 h)	Partly cloudy	1059 hours	9	30	3	3	6
24 December 2012	0900–1730 (8.5 h)	Cloudy to overcast	-	0	0	0	0	0
25 December 2012	0900–1730 (8.5 h)	Cloudy and rainy	1034 hours	5	15	2	2	3
26 December 2012	0900–1730 (8.5 h)	Cloudy and rainy	1036 hours	6	18	3	2	4

1996; Herrera et al., 2001; Totland, 2001). In many species, the long lifespan of the flower exposed to pollinators achieves cost-efficient seed production (Schemske et al., 1978; Herrera, 2002; Valtuena et al., 2008). Long lifespan is also important for understanding the ecology of pollination as a dynamic process (Primack, 1985). The optimal pollen vector is the agent that most effectively transfers pollen and produces the maximum seed set throughout the entire flowering period (Stiles, 1978). Winter pollination is also abrasive from an environmental point of view because this period suffers from the worst weather conditions (cold, low temperature, frequent rainfall, or occasional snowfall). Therefore, flower longevity is considered a mechanism to avoid intra- or interspecific competition and obtain reproductive assurance during an adverse season for

pollinators (Herrera, 1982; Rathcke, 2003). When flower longevity is extended, the number of pollen depositions on the stigma significantly increases. *B. hancei* Hook.f. flowers displayed considerable longevity (approximately 3 months for the whole population, and more than 10 days per flower), while those of another shrub, *Buddleja officinalis* Maxim. (Loganiaceae), were small (single flower, approximately 2–5 mm), with flowering lasting for approximately 4–5 days. This longevity extended the exposure of flowers and increased the pollination success when pollinators were scarce during winter. Moreover, the *B. hancei* Hook.f. population was small and presented low density. Figure 5 shows that the pollen deposition of *B. hancei* Hook.f. significantly increased through extension of flowering duration; the number

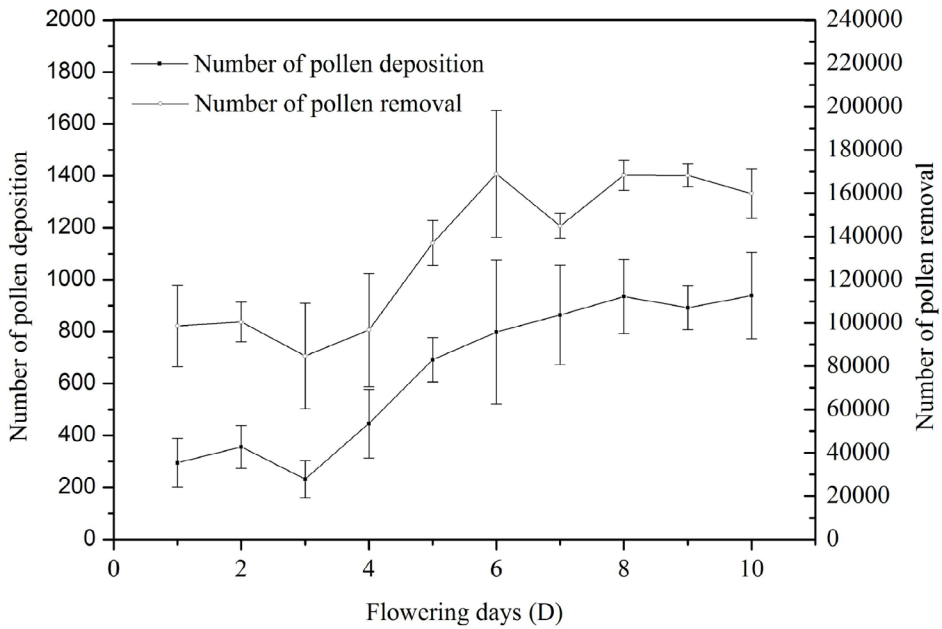


Figure 5. Number of pollen depositions on the stigma and number of pollen removals from the anthers after *Y. nigrimenta* Blyth visited *B. hancei* Hook.f. flowers.

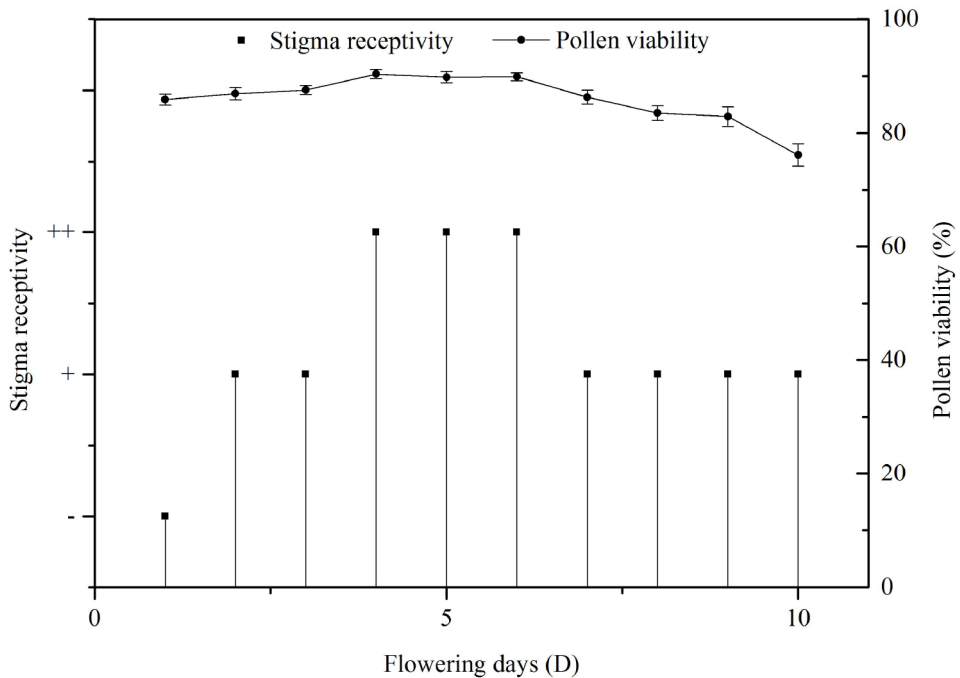


Figure 6. Changes in pollen viability and stigmatic receptivity of *B. hancei* Hook.f. during anthesis. “-” means stigma had no receptivity, “+” means stigma had receptivity, “++” means stigma had high receptivity.

of pollen depositions in nature (827.40 ± 77.76 , $n = 15$) was threefold that of the initial flowering (the first 3 days of flowering, 289.38 ± 45.66 , $n = 13$). Flower longevity increases with low temperatures and wet environments;

this observation was confirmed in some species, e.g., *Pyrola* Linn. (Ericaceae), *Trillium* Linn. (Orchidaceae), and *Cypripedium* Linn. (Orchidaceae) (Primack, 1985); *Loranthus acaciae* Zucc. (Loranthaceae) (Vaknin et al.,

1996); *Retama sphaerocarpa* (L.) Boiss. (Fabaceae), *Cytisus multiflorus* (L'Hér.) Sweet (Fabaceae), and *Cytisus striatus* (Hill) Rothm. (Fabaceae) (Rodríguez-Riaño et al., 1999); *Tristerix corymbosus* Quinral (Loranthaceae) (Aizen, 2003); and *Kalmia latifolia* L. (Ericaceae) (Rathcke, 2003). The *B. hancei* Hook.f. flower is large in size and unscented; it also presents red long-tubular corolla, diurnal anthesis, and anthers covered with dense hairs; these characteristics are considered those of avian pollination in other species (Endress and Steiner-Gafner, 1996; Sánchez-Lafuente, 2005; Rodríguez-Rodríguez and Valido, 2008). According to our observations, the only visitor of *B. hancei* Hook.f. flowers in our study site was *Y. nigrimenta* Blyth; we did not observe other pollinators during the entire flowering time. Ren et al. (2016) reported that *Apis cerana* Fabricius (Apidae) was the only flower pollinator of *B. hancei* Hook.f., and visitation rates were very low. This may be due to observation in different areas (Sichuan and Guizhou) or during different times (December 2012 and March 2012). Bird-pollinated flowers are known to secrete relatively dilute nectars (Nicolson, 2002). *B. hancei* Hook.f. secreted a relatively dilute floral nectar (with concentrations averaging $14.5 \pm 0.2\%$). The nectar served as food for *Y. nigrimenta* Blyth. When the bird accessed the flower and consumed the nectar, its forehead touched the stigma and anthers, thereby completing the pollination. Other adaptations of the flower to low-frequency pollination include the copious and diluted production of nectar. This adaptation is persistent in the flower to ensure a significant and predictable nectar production. The diluted and copious nectar is a characteristic of ornithophilous species (Stiles, 1981; Proctor et al., 1996; Pellmyr, 2002; Cronk and Ojeda, 2008). Furthermore, the long-tubular flowers of *B. hancei* Hook.f. and the humid climate contribute to reducing nectar evaporation and stabilizing nectar quality. Given the low temperatures during winter, subtropical Asia presents lower insect activities than that of the threshold. Therefore, only a few insects are observed under such conditions, despite the abundance of pollinating species such as honeybee, bumblebee, sweat bee, and hoverfly (Wang et al., 2012) acting as pollinators or visitors for *Epimedium wushanense* T.S.Ying (Berberidaceae). In addition, *Buddleja officinalis* Maxim. (Loganiaceae) and *Mahonia aquifolium* (Pursh) Nutt. (Berberidaceae) were visited by butterflies and hawkmoths, respectively, during warm seasons in our study sites. By contrast, birds are endothermic; they forage in cool conditions and maintain high activity levels. Nevertheless, passerine birds such as *Y. nigrimenta* Blyth are not usually specific flower visitors; these birds are not typically insectivorous or frugivorous in terms of feeding habits or morphology (Ortega-Olivencia et al., 2005; Surhone et al., 2010). No study has addressed this bird's flower-visiting behavior. However,

our observations and pollination experiment results suggest that *Y. nigrimenta* Blyth acted as an agent of pollen transfer when insect pollinators were scarce in winter.

Hand cross-pollination produced a seed set ($87.25 \pm 1.17\%$) almost equal to that of the hand self-pollinated ($87.68 \pm 0.89\%$) flowers of *B. hancei* Hook.f. ($\chi^2 = 0.178$, $df = 1$, $P = 0.673$). Meanwhile, the autonomous selfing treatment scarcely bore fertile seeds (only 16.67%) and a seed set ($2.32 \pm 0.89\%$) significantly lower than those of other treatments ($\chi^2 = 58.490$, $df = 4$, $P < 0.001$), thereby indicating self-compatibility. In addition, intact flowers presented slightly higher seed sets than emasculated flowers (Figure 3); this result may indicate that *B. hancei* Hook.f. could increase its reproductive success with self-compatibility. This conclusion is consistent with another study of *B. hancei* Hook.f. by Ren et al. (2016) in Guizhou Province. Autogamy provides reproductive assurance for plants during scarcity of pollinators (Lloyd and Schoen, 1992) or adverse environmental conditions (Vogler and Kalisz, 2001; Goodwillie et al., 2005). Nonetheless, approach herkogamy and protandry limit selfing (Bertin and Newman, 1993). In our study, protandry might have provided a barrier to autogamy in *B. hancei* Hook.f. The stigma was also extended beyond the anthers (separation of 2.51 ± 0.12 mm ($n = 30$) between anthers and stigma), and a few capsules were produced when the pollinators were excluded. However, artificial selfing produced abundant capsules when the stigmatic receptivity was high during anthesis; this stage was used in the pollination trial. Therefore, cross-fertilization is sometimes the preferred approach for producing suitable offspring by avoiding inbreeding depression (Jarne and Charlesworth, 1993), pollen discounting (Holsinger et al., 1984), or ovule discounting (Lloyd, 1992). Nevertheless, many species tend to exhibit a mixed mating system (Lloyd, 1979; Barrett and Eckert, 1990; Vogler and Kalisz, 2001; Goodwillie et al., 2005) under poor pollination conditions. Consequently, the mixed mating system of *B. hancei* Hook.f. might be a reproductive assurance mechanism to produce abundant capsules under severe environmental conditions.

On the basis of our results, *Y. nigrimenta* Blyth may be regarded as an effective pollinator of *B. hancei* Hook.f. and their relationship suggests a good example for pollination syndromes. *Y. nigrimenta* Blyth is an effective pollinator of *B. hancei* Hook.f. Flower longevity increases the reproductive output by attracting the bird pollinator. Furthermore, abundant capsules can be produced through self-compatibility when pollinators are scarce. These results reveal that a nectar-feeding bird plays a role in the current maintenance of plant populations and possibly in the evolution of some flower traits. However, although this species is widely distributed in central-southwestern China, the coevolution relationship between the bird and *B. hancei* Hook.f. is still unknown. Our observations of

winter-flowering *B. hancei* Hook.f. are red long-tubular corolla and secretion of relatively dilute nectars, which are typical traits of bird pollination (Johnson et al., 2010). Nectar sugar concentration of $14.5 \pm 0.2\%$ is similar to that of bird-pollinated flowers (10%–15%) (Nicolson, 2002). It appears that the reproduction of *B. hancei* Hook.f. depends largely on the services of a single bird species, *Y. nigrimenta* Blyth. Its attractiveness for *Y. nigrimenta* Blyth is due to the copious production of nectar and a long-lasting flowering period. We did not observe other pollinators,

e.g., *A. cerana* Fabricius (Ren et al., 2016), so this is the first study to suggest *Y. nigrimenta* Blyth as a pollinator of *B. hancei* Hook.f. Although bird pollination is best known in hummingbirds, sunbirds, and honeyeaters, it also occurs on an opportunistic basis in a varied assortment of birds (Ortega-Olivencia et al., 2005). This was the first study reporting this bird species as a pollinator. These findings may provide crucial data for understanding the reproductive strategy of winter-flowering *B. hancei* Hook.f. and its coevolution with this bird pollinator.

References

- Aizen MA (2003). Influences of animal pollination and seed dispersal on winter flowering in a temperate mistletoe. *Ecology* 84: 2613-2627.
- Barrett SCH, Eckert CG (1990). Variation and evolution of plant mating systems in seed plants. In: Kawano S, editor. *Biological Approaches and Evolutionary Trends in Plants*. New York, NY, USA: Academic Press, pp. 229-254.
- Bertin RI, Newman CM (1993). Dichogamy in angiosperms. *Bot Rev* 59: 112-152.
- Chinese Materia Medica Editorial Committee (1999). *Chinese Materia Medica*. Shanghai, China: Shanghai Science and Technology Press.
- Cronk Q, Ojeda I (2008). Bird-pollinated flowers in an evolutionary and molecular context. *J Exp Bot* 59: 715-727.
- da Silva LP, Ramos JA, Olesen JM, Traveset A, Heleno RH. (2014). Flower visitation by birds in Europe. *Oikos* 123: 1377-1383.
- Endress PK (1996). *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge, UK: Cambridge University Press.
- Fang Q, Chen YZ, Huang SQ (2012). Generalist passerine pollination of a winter-flowering fruit tree in central China. *Ann Bot-London* 109: 379-384.
- Goodwillie C, Kalisz S, Eckert CG (2005). The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu Rev Ecol Evol* 36: 47-79.
- Gu L, Luo Z, Zhang D, Renner SS (2010). Passerine pollination of *Rhodoleia championii* (Hamamelidaceae) in subtropical China. *Journal of Tropical and Subtropical Botany* 42: 336-341.
- Gu L, Zhang DX (2009). A review on ornithophily in the Chinese flora. *Biotropica* 17: 194-204.
- He ZD, Lau KM, Xu HX, Li PC, Pui-Hay But P (2000). Antioxidant activity of phenylethanoid glycosides from *Brandisia hancei*. *J Ethnopharmacol* 71: 483-486.
- He ZD, Wang DZ, Yang CR (1990). Phenylpropanoid glycosides from *Brandisia hancei*. *Acta Botanica Yunnanica* 12: 439-446.
- He ZD, Yang CR (1991). Brandioside, a phenylpropanoid glycoside from *Brandisia hancei*. *Phytochemistry* 30: 701-702.
- Heinrich B, Raven PH (1972). Energetics and pollination ecology. *Science* 176: 597-602.
- Herrera CM (1982). Seasonal variation in quality of fruits and diffuse coevolution between plants and avian disperser. *Ecology* 63: 773-785.
- Herrera CM (1996). Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd DG, Barrett SCH, editors. *Floral Biology*. New York, NY, USA: Chapman and Hall, pp. 65-87.
- Herrera CM (2002). Censusing natural microgametophyte populations: variable spatial mosaics and extreme fine-graininess in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Am J Bot* 89: 1570-1578.
- Herrera CM, Sánchez-Lafuente AM, Medrano M, Guitián J, Cerdá X, Rey PJ (2001). Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). *Am J Bot* 88: 1025-1032.
- Holsinger KE, Feldman MW, Christiansen FB (1984). The evolution of self-fertilization in plants: a population genetic model. *Am Nat* 124: 446-453.
- Jarne P, Charlesworth D (1993). The evolution of selfing rates in functionally hermaphrodite plants and animals. *Annu Rev Ecol Syst* 24: 441-466.
- Johnson KA, McQuillan PB, Kirkpatrick JB (2010). Bird pollination of the climbing heath *Prionotes cerinthoides* (Ericaceae). *Int J Plant Sci* 171: 147-157.
- Kearns CA, Inouye DW (1993). *Techniques for Pollination Biologists*. Boulder, CO, USA: University Press of Colorado.
- Lloyd DG (1979). Some reproductive factors affecting the selection of self-fertilization in plants. *Am Nat* 113: 67-79.
- Lloyd DG (1992). Self- and cross-fertilization in plants. II. The selection of self-fertilization. *Int J Plant Sci* 153: 370-380.
- Lloyd DG, Schoen DJ (1992). Self- and cross-fertilization in plants. I. Functional dimensions. *Int J Plant Sci* 153: 358-369.
- Nicolson SW (2002). Pollination by passerine birds: why are the nectars so dilute? *Comp Biochem Phys B* 131: 645-652.
- Nicolson SW, Fleming PA (2003). Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Syst Evol* 238: 139-153.

- Ortega-Olivencia A, Rodríguez-Riaño T, Valtuena FJ, López J, Devesa JA (2005). First confirmation of a native bird-pollinated plant in Europe. *Oikos* 110: 578-590.
- Pellmyr O (2002). Pollination by animals. In: Herrera CM, Pellmyr O, editors. *Plant Animal Interactions: An Evolutionary Approach*. Hoboken, NJ, USA: Blackwell Science Press, pp. 157-184.
- Primack RB (1985). Longevity of individual flowers. *Annu Rev Ecol Syst* 16: 15-37.
- Proctor M, Yeo P, Lack A (1996). *The Natural History of Pollination*. New York, NY, USA: Harper Collins Publishers.
- Rathcke BJ (2003). Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *Am J Bot* 90: 1328-1332.
- Ren YQ, Xu YP, Zhang T, Ma YP, Marczewski T (2016). Growth discrepancy between filament and style facilitates self-fertilization in *Brandisia hancei* (Paulowniaceae). *Plant Spec Biol* 31: 153-158.
- Rodríguez-Riaño T, Ortega-Olivencia A, Devesa JA (1999). Reproductive phenology in three Genisteae (Fabaceae) shrub species of the W Mediterranean Region. *Nord J Bot* 19: 345-354.
- Rodríguez-Rodríguez MC, Valido A. (2008). Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: experimental evidence from *Isoplexis canariensis* (Scrophulariaceae). *Am J Bot* 95: 1408-1415.
- Sánchez-Lafuente AM, Guitián J, Medrano M, Herrera CM, Rey PJ, Cerdá X (2005). Plant traits, environmental factors, and pollinator visitation in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Ann Bot-London* 96: 845-852.
- Schemske DW, Willson MF, Melampy MN, Miller LJ, Verner L, Schemske KM, Best LB (1978). Flowering ecology of some spring woodland herbs. *Ecology* 59: 351-366.
- Stiles FG (1978). Temporal organization of flowering among the hummingbird foodplants of a tropical wet forest. *Biotropica* 10: 194.
- Stiles FG (1981). Geographical aspects of bird-flower coevolution, with particular reference to Central America. *Ann MO Bot Gard* 68: 323.
- Surhone LM, Tennoe MT, Henssonow SF (2010). *Black-Chinned Yuhina*. Saarbrücken, Germany: Betascript Publishing.
- Totland Ø (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology* 82: 2233-2244.
- Vaknin Y, Tov YY, Eisikowitch D (1996). Flowering seasonality and flower characteristics of *Loranthus acaciae* Zucc. (Loranthaceae): implications for advertisement and bird-pollination. *Sex Plant Reprod* 9: 279-285.
- Valtuena FJ, Ortega-Olivencia A, Rodríguez-Riaño T, López J (2008). Reproductive biology in *Anagyris foetida* L. (Leguminosae), an autumn-winter flowering and ornithophilous Mediterranean shrub. *Bot J Linn Soc* 157: 519-532.
- Vogler DW, Kalisz S (2001). Sex among the flowers: the distribution of plant mating systems. *Evolution* 55: 202-204.
- Vespirini JL, Pacini E (2010). Pollination ecology in sympatric winter flowering *Helleborus* (Ranunculaceae). *Flora* 205: 627-632.
- Wang Y, Quan QM, Li YX (2012). Effects of flowering period on nectar secretion and fruit set of *Epimedium wushanense* (Berberidaceae). *Plant Diversity and Resources* 34: 471-477.