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Research Article

Pollination biology of *Rhododendron cyanocarpum* (Ericaceae): An alpine species endemic to NW Yunnan, China

Yong-Peng Ma[1], Zhi-Kun Wu[2], Kun Dong[3], Wei-Bang Sun[1]*, and Tobias Marczewski[4]

[1]Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China
[2]Lijiang Alpine Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[3]Yunnan Agriculture University, Kunming 650204, China
[4]University of Edinburgh, Edinburgh EH9 3JH, UK
*Author for correspondence. E-mail: wbsun@mail.kib.ac.cn. Tel./Fax: 86-871-5223630.
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Abstract The pollination biology of *Rhododendron cyanocarpum* (Franch.) W. W. Smith, an alpine species endemic to NW Yunnan, China, was investigated between 2007 and 2008. Floral traits including flowering time, floral morphology, petal color, and floral scents were assessed, and the associated pollinator assemblage and their foraging behavior were recorded. The flowering period of *R. cyanocarpum* ranges from late March to middle May and a single flower can last 8–10 days in its natural habitat. Flowers generally show marked herkogamy and the protruding style encourages pollinator foraging behavior that counteracts self-pollination. Floral scents comprise mostly aliphatics (64.37%) and terpenoids (29.88%), which make the flowers attractive for several insect groups, but a peak at 430 nm in the reflectance spectrum of petals suggest selection for the attraction of bumblebees. The two *Bombus* species, *B. festivus* and *B. richardsiellus*, representing 90% of total recorded visits, were the only insect species that can be considered to effectively pollinate *R. cyanocarpum*. Pollination treatments indicated the general self-compatibility of *R. cyanocarpum*; however, outcrossing seemed to be the dominant strategy, with low rates of self-fertilization providing a certain level of reproductive assurance in its alpine habitat.

Key words: alpine habitat, endemic species, floral scents, petal color reflectance, pollination biology, *Rhododendron*.

Reproduction is one of the key processes in seed-plant life-history, impacting directly on the evolutionary success of an individual. As many alpine plants are predominantly cross-pollinated, the behavior of pollinators can strongly affect pollen flow and reproductive success in flowering plant populations (Kudo, 1993; Bingham & Orthner, 1998; Escaravage & Wagner, 2004). Flowers are recognized and discriminated by pollinators according to specific signals such as color, scent (volatile compounds), size, and shape. At long distance, the size of the floral display can influence pollinator behavior, with larger flower displays generally attracting more visitors (Klinkhamer & de Jong, 1990; Stout, 2000, 2007; Delmas et al., 2014). At close range, flower color and nectar guides, as well as volatile compounds, nectar and pollen, supply cues to direct pollinator activity for rewards (Casper & La Pine, 1984; Sun et al., 2005). Hence, the diversity of cues used by plants to appear attractive to pollinators contributes to the reproductive success of entomophilous plants.

Rhododendron L. is one of the largest and most widespread woody plant genera, comprising over 1000 species, and is distributed from the northern temperate zone, throughout tropical southeastern Asia, to northeastern Australia (Chamberlain et al., 1996). In China, 562 species have been recorded, of which 405 are endemic, and a large part of this diversity is represented by subgenus *Hymenanthes* (Blume) K. Koch, which comprises 24 subsections with 225 species, the majority of which occur in China (Chamberlain, 1982; Fang & Min, 1995). *Rhododendron* species vary considerably in flower color as well as in number and length of stamens, occasionally showing dimorphism in stamen length within one flower (Escaravage & Wagner, 2004). A wide range of flower visitors, including bees, butterflies, and sunbirds, have been reported by studies outside the Sino-Himalayan region (e.g., Escaravage et al., 1997; Ng & Corlett, 2000; Mejías et al., 2002; Ono et al., 2008). Despite representing one of the centers of *Rhododendron* diversity, to our knowledge, data on pollinators and their foraging behavior is crucially lacking in the Sino-Himalayas (Milne et al., 1999; Ma et al., 2010). Therefore, it is the aim of the present study to advance the understanding of the reproductive biology of *Rhododendron* in this area, and provide a first assessment of potential key factors.

During 2007 and 2008, field experiments were carried out to investigate the pollination biology of *R. cyanocarpum* (Franch.) W. W. Smith in an alpine environment at an altitude of 3200 m in Huadianba of Cangshan Mountain. Data were gathered on: (i) flower visitors, effective pollinators, and their foraging behaviors; (ii) pollination associated floral characters—floral scents (volatile compounds), petal color, floral display sizes, and nectar; and (iii) the breeding system of *R. cyanocarpum*, using pollination experiments to better understand its reproductive strategies in alpine habitat.

Material and Methods

Study species and study site

Rhododendron cyanocarpum is an endangered species (Wang & Xie, 2004; Gibbs et al., 2011), endemic to NW Yunnan, China. It has only been recorded on Cangshan Mountain, which is part of the Hengduan Massif in the eastern Himalayas (Duan et al., 1994). Experiments for the present study were carried out at a site located on Cangshan Mountain near Huadianba (25°52′N, 99°59′E), situated at an altitude of approximately 3200 m.

Individuals of *R. cyanocarpum* are evergreen shrubs that only occur in an alpine environment at altitudes above 3000 m (Chamberlain, 1982; Wu, 1986; Wang & Xie, 2004). Inflorescences are typically umbelliform or racemose and contain five to nine flowers (Fig. 1: A; Fig. 2: A). The pink, rarely white, pentamerous flowers have five equally sized nectar pouches on the base of every petal. As is usual in the genus, the flowers tend to slight zygomorphy, with the 10 stamens and the style curving toward one marginally larger petal. The stamens are shorter than the style, and have apically porose anthers, containing pollen grains that are interconnected by sticky viscin threads (King & Buchmann, 1995).

Floral traits

We herein only examined floral traits potentially relating to pollination processes of *R. cyanocarpum*. To determine the number of flowers per inflorescence, four inflorescences from each of 10 individuals were randomly selected, and in total 40 inflorescences were thus counted. These 40 inflorescences were also used to determine inflorescence flowering time by measuring the time interval between the opening of the first flower up to the wilting of the last flower in the inflorescence. For every subsequent set of measurements a further 20 flowers or buds were randomly chosen from 10 individuals (two flowers per individual). Most of the manipulations and measurements were carried out following Ng & Corlett (2000). Flowering time per flower was examined by labeling a flower bud just before it opened and observing it every day until the corolla wilted. Using calipers, we measured the length of the pedicel, length of calyx lobes, corolla-length, stamen-length, style-length, and the closest distance between stigma and stamens. For measurements of nectar volume and corresponding sugar concentration, flower buds likely to open the next day were enclosed in plastic bags to prevent evaporation, and disturbance by visitors. The following day after the buds had opened, at around 10:00, the volume of nectar was directly measured by extracting it from the nectar pouches with a calibrated capillary of 100 μL (Sigma Chemical Co., St. Louis, MI, USA). Sugar concentration was afterwards obtained using a hand-held refractometer (Eclipse; Bellingham & Stanley Ltd., Basingstoke, UK), measured as grams of sugar per grams of nectar, and expressed as percentage (e.g., Kearns & Inouye, 1993; Barrios & Koptur, 2011).

Floral scents

Floral scent compositions could potentially mediate plant–pollinator interactions (Raguso, 2008). To collect volatile compounds, three young inflorescences, comprising together 25 flowers, were enclosed in Tedlar bags (DuPont, Wilmington, DE, USA), starting from 12:00, which corresponds to the time of highest pollinator activity (Fig. S1). Following the protocol of Chen et al. (2012), using a pump (inlet flow rate 300 mL/min) volatiles were then drawn for 3 h from the enclosures into cartridges containing the adsorbent Porapak Q (150 mg, mesh 60/80; Waters Associates, Inc., Milford, MA, USA). Prior to use, the adsorbent cartridges had been cleaned with 2 mL diethyl ether and dried with nitrogen gas. For analysis the trapped volatiles were eluted with 400 μL dichloromethane and the concentration was subsequently increased by reducing the original volume 1:5 through evaporation at room temperature, aided by a gentle stream of nitrogen. Then 720 ng of *n*-nonane was added to each sample as standard for later quantification, and the samples were stored at −20 °C.

The extracts from the inflorescences were subsequently analyzed using a gas chromatograph (HP 6890; Agilent Technologies, Santa Clara, CA, USA), equipped with an HP-5MS column (30 m × 0.25 mm, 0.25 μL film thickness), and linked to a mass spectrometer (HP 597; Agilent Technologies). Helium was used as carrier gas at a flow rate of 1 mL/min, and injector temperature was set to 250 °C. Column temperature was 40 °C at injection, and was increased to 250 °C at a rate of 3 °C/min. Compounds were identified by comparing mass spectra and retention times with values of reference compounds in the

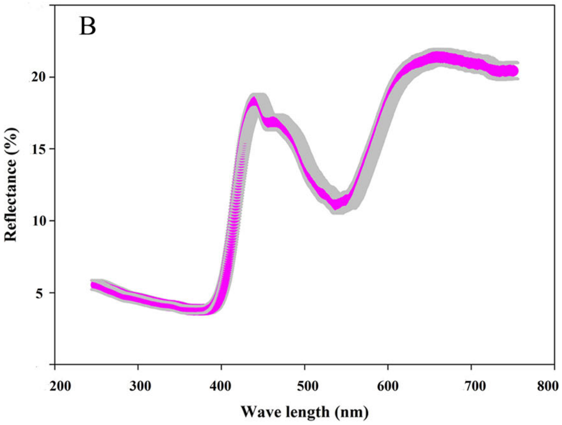

Fig. 1. Typical inflorescence of *Rhododendron cyanocarpum* showing the characteristically pink flowers (**A**), and the reflectance curve obtained measuring 40 petals (**B**), with the corresponding standard deviation shown as a gray area.

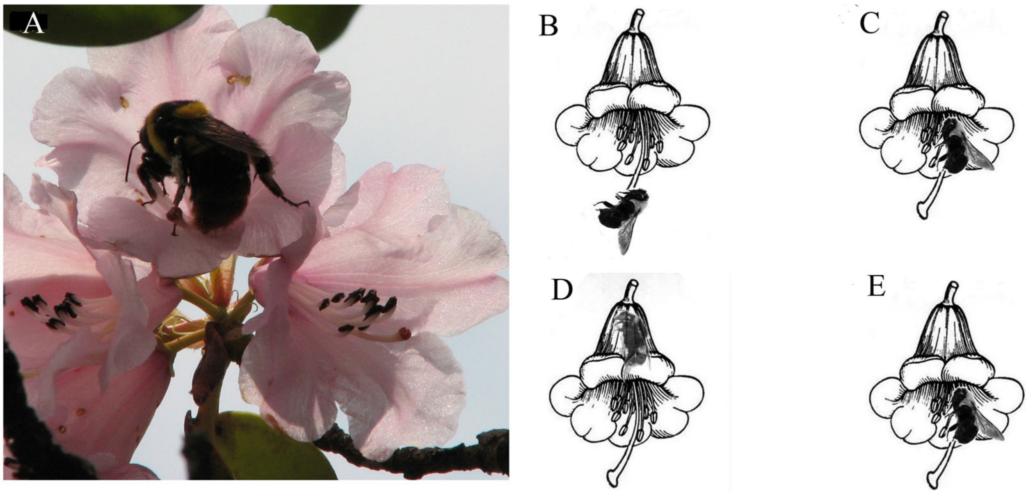

Fig. 2. Foraging behavior of *Bombus* species. **A,** *Bombus richardsiellus* on a flower of *Rhododendron cyanocarpum*. **B–E,** Schematic highlighting important stages of foraging behavior of the two bumblebee species *B. festivus* and *B. richardsiellus* on *R. cyanocarpum*: (**B**) for landing, the style is used as an aid, and the stigma is touched before the anthers; (**C**) advancing along the style, the bumblebee passes the anthers; (**D**) having reached the nectar pouches at the bottom of the flower; and (**E**) when exiting the flower the bumblebee passes the anthers a second time and takes off before reaching the end of the style, hence avoiding coming into contact with the stigma.

National Institute of Standards and Technology (US Department of Commerce) Standard Reference Database (http://webbook.nist.gov/chemistry/) and on a chemical suppliers' website (http://www.lookchem.com/).

Petal color analysis

The reflectance spectrum of petal color could also act as a signal for attracting potential pollinators (Dyer et al., 2012). To assess light reflection patterns of petals at different wavelengths, we obtained spectral data from petals using an S2000 miniature fiber optic spectrometer with a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). All measurements were carried out in the range from 250 to 750 nm, using 0.38-nm increments. Differences in the reflectance pattern have been found in certain plants (Frey et al., 2011), but preliminary testing for changes in the reflectance pattern along the same petal did not show any differences, and therefore only one measurement per petal was taken. In total, 40 petals from different flowers, obtained from 10 individuals, were analyzed to assess potential differences between plants.

Pollination treatments

To investigate self-compatibility and effectiveness of possible modes of pollination, flowers were subjected to different types of treatments intended to mimic several scenarios: (i) parthenogenesis test, in which flower buds were emasculated and bagged; (ii) autonomous self-fertilization test, in which flower buds were bagged; (iii) to determine self-compatibility, flowers were randomly chosen to hand self-pollinate with the pollen from the same flower; (iv) to detect geitonogamous pollination, flower buds were randomly selected to hand pollinate with pollen from another flower within the same individual; (v) to test pollinator-mediated cross-pollination, flower buds were randomly chosen and emasculated; (vi) hand cross-pollination test, in which flower buds were randomly selected to subject hand cross-pollination with pollen from different individuals at least 10 m apart from experimental plants; (vii) to examine whether pollen limitation existed, supplemental pollination was carried out; and (viii) randomly selected flowers were not manipulated as control. Pollination treatments were carried out in April 2007 and 2008, and fruits produced by these flowers were then counted the following October in each year. Fruit set was calculated as the ratio of mature fruits to the total number flowers that were initially treated, which is a good indicator for pollination success in species with large number of seeds per fruit, such as *Rhododendron* (e.g., Kudo, 1993; Ma et al., 2010).

Observation of flower visitors

Flower visitors were observed for at least 20 h each year during the flowering period (8–12 April, 2007 and 10–14 April, 2008). Observations were made each day between 10:00 and 16:00. It should be noted that if it was raining, field observations were stopped. We recorded the type of flower visitors that either touched the anthers or the stigma of a flower. All visitors that met these criteria were captured, anesthetized with ether, and pinned onto cardboard for later taxonomic identification in the laboratory. Insects that both picked up pollen and deposited it on a receptive stigma were classified as effective pollinators, as suggested by Stout (2007).

Effect of inflorescence size on visitation rates

To study the potential of different flower display sizes to attract pollinators, we chose three random plots of 5×5 m, in 2007, and in each removed the flowers from all *R. cyanocarpum* plants, excepting three individuals in each plot. The three individuals with remaining flowers were then manipulated to have 20, 40, and 60 inflorescences, respectively, with each inflorescence containing five flowers. In these plots, we recorded the number of pollinator visits to the manipulated individuals between 10:00 and 14:00 on four successive sunny days.

Influence of nectar presence on visitation rates

In many plant species, it has been shown that nectar presence can influence pollinator visitation rates (e.g., De Jong et al., 2011). To examine whether this would also be the case for *R. cyanocarpum*, pollinator activity was recorded in 2007 in four 4×4-m plots, in which flowers had been removed from all but two individuals of *R. cyanocarpum*. These two individuals had each been manipulated to have 20 inflorescences with each inflorescence comprising five flowers. The nectar was removed from all flowers from one individual using a syringe, whereas the other individual was not manipulated as control. Bumblebee visits to these individuals were then recorded between 10:00 and 14:00 on four successive sunny days.

Data analysis

Significance of differences between fruit set in the pollination experiments was tested using a simple χ^2-test. For all other treatments involving parametric tests, a possible deviation of the data from the normal distribution was assessed with a one-sample Kolmogorov–Smirnov test (Ma et al., 2012). For the comparison of visitation rates for different nectar treatments we assessed significance with Student's *t*-test, and for all other comparisons involving more than two treatments, a one-way ANOVA was used. All of these tests were carried out in SPSS 15.0 for Windows (SPSS, Chicago, IL, USA).

Results

Floral traits

The flowering period of *Rhododendron cyanocarpum* extended from late March to mid-May during our investigations in the field. The flowering time of an inflorescence could last 12–14 days, while the lifetime of a single flower was usually 8–10 days. The flowers are proterandrous, and occasionally some anthers mature, and release pollen, before the flowers have fully opened. In the flower bud, the stigma is almost entirely separated from the anthers by the folded petals, and cross-fertilization at this stage is unlikely. Furthermore, the stigma surface only becomes wet and receptive after the corolla has opened, but it then remains wet throughout anther dehiscence (Ma Y., 2008, pers. obs.).

Inflorescences comprised on average six flowers (6.00 ± 0.99, $n = 40$). The 10 stamens show marked differences in length, with the shortest stamen (2.1–3.2 cm) of one flower always being significantly shorter than the longest (3.5–4.7 cm, Student's *t*-test, $t = 13.4$; $P < 0.001$). However, in all flowers, the style raises the stigma significantly over the longest stamens (0.67–0.95 cm, Student's *t*-test, $t = 5.54$; $P < 0.001$, Table 1), indicating marked herkogamy and additionally fulfilling an apparently important role for pollinators as landing aid (see pollinator behavior). Flowers contained nectar volume of 76 ± 18 μL ($n = 20$), with a sugar concentration of $11.53 \pm 1.83\%$ ($n = 20$).

Table 1 Measurements of morphological flower characters of *Rhododendron cyanocarpum*

Character	Length measured (cm)		
	Range	Mean	SD
Pedicel	1.10–1.35	1.27	0.10
Calyx	0.40–0.70	0.52	0.09
Corolla	3.60–5.50	4.58	0.55
Stamens			
Shortest	2.10–3.20	2.54	0.31
Longest	3.50–4.70	4.11	0.42
Style	3.26–4.50	3.96	0.42
Shortest distance stigma–stamen	0.67–0.95	0.81	0.08

Sample size for all measurements, $n = 20$.

Volatile compounds in flowers and petal color reflectance

In total, 21 volatile compounds were identified in extracts taken from *R. cyanocarpum* flowers (Table 2), accounting for 96.73% of the total mass of extracts. Most of the detected compounds belong to either aliphatics (64.37%), or terpenoids (29.88%). Among these the four most abundant substances, representing together 49.01%, are diacetone alcohol, decanal, nonanal, and linalol (see Table 2).

The reflectance spectrum of the 40 measured petals showed extremely low variation between plants (gray area in Fig. 1: B), all clearly showing a marked peak in the reflectance spectrum at 430 nm (Fig. 1: B).

Mating system

Emasculated, bagged flowers showed no fruit set at all (Table 3), so that it can be assumed that parthenogenesis does not occur in *R. cyanocarpum*. However, fruits were obtained from selfed, hand-pollinated flowers (19.4%), giving evidence for general self-compatibility of *R. cyanocarpum*. Fruit set in bagged flowers (2.1%) was significantly lower compared to natural conditions ($\chi^2 = 328.3$, $P < 0.001$), suggesting that foraging pollinators might also facilitate the transfer of pollen within the same flower. The potentially important role of pollinators for the process of selfing becomes evident from the large fruit set obtained from geitonogamous pollination (83.3%), which is not significantly different from the fruit set obtained by hand cross-pollination (88.6%, $\chi^2 = 0.291$, $P = 0.589$). No significant differences in fruit set were observed between bee pollinated emasculated flowers (56.7%) and the control (non-manipulated, bee pollinated flowers, 64.2%, $\chi^2 = 1.854$, $P = 0.173$). However, supplementing older flowers with pollen led to significantly increased fruit set (86.7%) compared to the control (64.2%, $\chi^2 = 8.605$, $P = 0.003$ for pollen supplementation vs. control; Table 3), suggesting that flowers are pollen limited. A further line of evidence for this limitation stems from the observation that all hand-pollination treatments, excepting selfing within the same flower, produced similarly high fruit set (83.3%, 86.7%, 88.6%, $\chi^2 = 0.736$, $P = 0.692$; Table 3), all higher than obtained by bee-mediated pollination alone.

Pollinator observations

During the two flowering periods in 2007 and 2008, flowers of *R. cyanocarpum* were visited by eight species of insects, representing four orders: Lepidoptera, Diptera, Coleoptera, and Hymenoptera (Table 4). Of these, however, only two species were identified as effective pollinators, as defined by Stout (2007). Our observations suggest that pollination of *R. cyanocarpum* is nearly exclusively effected by *Bombus festivus* and *B. richardsiellus*. In total, we recorded 158 flower

Table 2 Volatile compounds detected in extracts from flowers of *Rhododendron cyanocarpum*

No.	Compound	CAS	t_x	t_n	t_{n+1}	KI	%
Aliphatics							64.37
1	Diacetone alcohol	123-42-2	6.69	5.62	8.91	632.52	15.39
2	Decanal	112-31-2	20.86	20.69	23.76	1005.54	12.77
3	Nonanal	124-19-6	17.31	17.17	20.69	903.98	10.85
4	Methyl heptenone	110-93-0	12.38	8.91	12.97	885.47	6.27
5	Dodecane	112-40-3	20.64	17.17	20.69	1298.58	3.80
6	Ocimene	13877-91-3	15.08	12.97	17.17	1050.24	2.91
7	Tridecanal	10486-19-8	19.66	17.17	20.69	1370.74	2.53
8	Hexanal	66-25-1	5.46	3.42	5.62	692.73	2.26
9	Octanal	124-13-0	13.09	12.97	17.17	802.86	2.25
10	Tetradecane	629-59-4	26.51	23.76	26.55	1498.57	1.41
11	Propyl acetate	109-60-4	3.69	3.42	5.62	512.27	1.19
12	3-Hexanol	623-37-0	5.37	3.42	5.62	688.64	0.98
13	2-Hexanone	591-78-6	5.21	3.42	5.62	681.36	0.95
14	3-Hexanone	589-38-8	5.10	3.42	5.62	676.36	0.81
Terpenoids							29.88
15	Linalol	78-70-6	17.18	17.17	20.69	1000.28	10.00
16	Eucalyptol	470-82-6	14.27	12.97	17.17	1030.95	6.08
17	β-Selinene	18423-23-9	28.94	26.55	29.12	1593.00	4.63
18	Sabinene	3387-41-5	11.72	8.91	12.97	1069.21	4.12
19	α-Pinene	80-56-8	10.01	8.91	12.97	1027.09	4.08
20	α-Selinene	473-13-2	29.16	29.12	31.53	1501.66	0.97
Benzenoids							2.48
21	Dibutylhydroxytoluene	128-37-0	29.52	29.12	31.53	1516.60	2.48
	Total						96.73

Kovats index (KI) was calculated according to the formula of Van Den Dool & Kratz (1963) as given in Chen et al. (2012): $KI = 100n + 100(t_x - t_n)/(t_{n+1} - t_n)$, where n is the number of carbon atoms in the n-alkane eluting immediately before the compound of interest. CAS, Chemical Abstracts Service registry number; t_n, retention times of the n-alkanes eluting immediately before; t_{n+1}, retention times of the n-alkanes eluting immediately after; t_x, retention time of the compound.

visits by these two species, representing approximately 90% of all visits by insects. Although *Papilio krishna* would qualify as an effective pollinator, as pollen was transferred to the stigma, we only recorded one visit in two flowering seasons, and therefore discount this species as an important pollen vector for *R. cyanocarpum*. *Bibio vestitus*, *Metasyrphus nitens*, honeybees, and one species of Coccinellidae were not considered as pollinators as they did not touch the stigma in the process of visiting flowers.

More detailed documentation of the foraging behavior of bumblebees during their flower visits showed that the foraging time per flower ranged from 5 to 15 s. A visit generally began with the bumblebee approaching the style, which is protruding from the flower, and using it as a landing aid. In this process, the bumblebee would effectively deposit pollen on the stigma before entering the flower (Fig. 2: A, B). This behavior is further encouraged by the flower as the petals are too soft and untextured to provide the bumblebee with a suitable landing surface. After having successfully landed on the style, the bumblebee would then use it to enter the corolla, and on its way toward the nectar pouches pass the anthers (Fig. 2: C). Already at this stage, most individuals of bumblebee were nearly entirely covered in pollen. Having reached the bottom of the corolla, the bumblebee would then remain to gather nectar from the base of the petals (Fig. 2: D). After completion of the foraging the bumblebee would then pass

Table 3 Breeding system of *Rhododendron cyanocarpum*

Pollination treatments	Inflorescences	Flowers	Fruits	Fruit set (%)
Parthenogenesis test	26	105	0	0.0
Autonomous self-fertilization test	46	242	5	2.1
Self-pollination within a flower	12	62	12	19.4
Geitonogamous pollination	16	84	70	83.3
Pollinator-mediated pollination test	20	67	38	56.7
Hand cross-pollination	12	44	39	88.6
Pollen limitation test	12	60	52	86.7
Control	24	106	68	64.2

Fruit set was calculated by dividing the number of treated flowers by the number of obtained fruits.

Table 4 Insects observed visiting flowers of *Rhododendron cyanocarpum*

Order	Family	Species	Effective pollinator
Lepidoptera	Papilionidae	*Papilio krishna*	No
		Sp.	No
Diptera	Bibionidae	*Bibio vestitus*	No
	Syrphidae	*Metasyrphus nitens*	No
Coleoptera	Coccinellidae	Sp.	No
Hymenoptera	Apidae	*Apis cerana*	No
		Bombus festivus	Yes
		Bombus richardsiellus	Yes

the anthers a second time on the way out, thus being efficiently loaded with pollen for the next flower (Fig. 2: E). It is noteworthy here that when exiting the bumblebee would mostly not come into contact with the stigma again, as a suitable take-off point seems to exist near the height of the three longest stamens, enabling the pollinator to leave the flower before reaching the stigma. Therefore, pollinator-mediated self-pollination within flower should generally be avoided.

Response to different flower display sizes

Visitation rates to plants with 60, 40, and 20 flowers were 1.02 ± 0.16, 0.56 ± 0.13, and 0.38 ± 0.15 visits per individual per hour, respectively (mean $\pm$ SD) (Fig. 3: B). The visitation rates to plants with the lowest and highest flower counts were significantly different (ANOVA, $F = 19.472$, $P = 0.001$). However, assuming a random foraging behavior would lead to the null expectation that rates should scale to flower number, hence that with no further effect three times more visitors can be expected on a plant with 60 as opposed to 20 flowers. After taking this scaling into account the visitation rates between the treatments are not different (ANOVA, $F = 1.437$, $P = 0.287$).

Response to nectar depleted flowers

The visitation rate of pollinators to flowers from which the nectar had been removed was 0.24 ± 0.03 visits per individual per hour (mean $\pm$ SD), while the rate for control flowers was 0.41 ± 0.11. These two rates are significantly different (Student's *t*-test, $t = 3.108$, $P = 0.021$, Fig. 3: A), and as expected suggests that pollinators prefer flowers that promise a higher reward.

Discussion

Floral scent, petal color, and effective pollinators

To our knowledge, only one other study exists that analyzed floral scents of *Rhododendron*. Tasdemir et al. (2003) studied volatile compounds from five species of *Rhododendron* native to Turkey, including floral extracts from three species: *R. ponticum* L., *R. luteum* C. K. Schneid., and *R. ungernii* Trautv. They observed substantial intraspecific variations in volatile composition, and comparing the volatile spectrum of *R. cyanocarpum* to their results agrees with this statement. While some of the volatiles identified in this study also occur in *R. luteum* (linalol, methyl heptenone, eucalyptol, α-pinene, and hexanal) or *R. ungernii* (methyl heptenone and hexanal), the three most abundant in *R. cyanocarpum* (diacetone alcohol, decanal, and nonanal) were not present in the species studied in Tasdemir et al. (2003), or only in much lower quantity (*R. ponticum*, nonanal 0.9%). It is remarkable that the biggest overlap of *R. cyanocarpum* volatiles seems to be with *R. luteum*, which is in a different subgenus (*Pentanthera*), whereas *R. ponticum* and *R. ungernii* belong to the same subgenus as

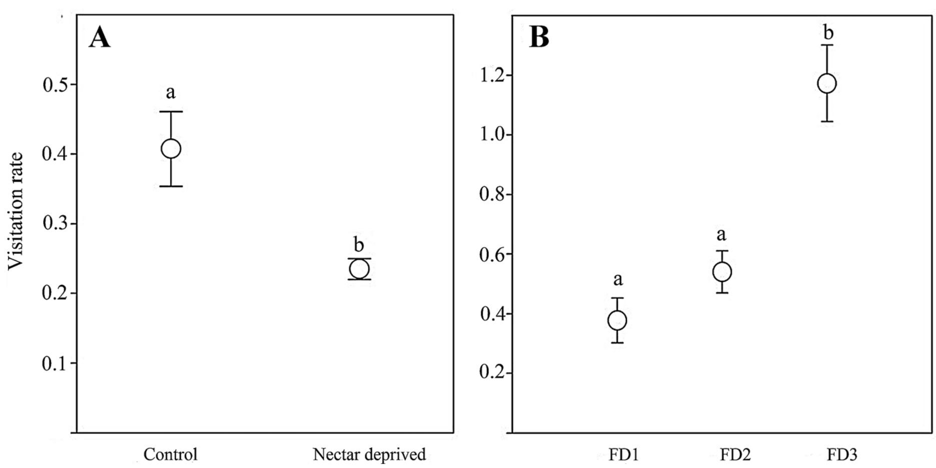

Fig. 3. Visitation rates (visits per plant per hour) of bumblebees to individuals of *Rhododendron cyanocarpum*. Influence of (**A**) nectar removal (control flowers had normal amounts of nectar), and (**B**) floral display size. FD1, individuals with 20 inflorescences; FD2, individuals with 40 inflorescences; FD3, individuals with 60 inflorescences.

R. cyanocarpum (*Hymenanthes*). Therefore, factors (e.g., ecological- and pollinator-related selective pressures) other than evolutionary relatedness might contribute to the volatile composition of *R. cyanocarpum*. However, limited data in either pollination or ecology of these *Rhododendrons* prevent further comparison relating to floral scents.

Monoterpenes, such as limonene, myrcene, and α-pinene, are the most common compounds in floral scents in angiosperms, especially for plant species with diverse pollinating insect taxa including bees, Lepidoptera, beetles, and fly species (e.g., Knudsen et al., 1993; Knudsen, 2002 and references therein). The composition of floral scents of *R. cyanocarpum*, which mostly comprise aliphatics (64.37%, Table 3) and terpenoids (29.88%), therefore implies a generalist strategy for pollinator attraction (Tollsten et al., 1994; Knudsen, 2002; Knudsen et al., 2006). Floral visitors of *R. cyanocarpum* support this hypothesis, as insects from the orders Lepidoptera, Diptera, Coleoptera, and Hymenoptera (Table 4) were observed visiting the flowers. These four orders of insects have been reported to be frequent visitors of *Rhododendron* flowers in similar environments worldwide (Hong Kong, Ng & Corlett, 2000; the Alps, Escaravage & Wagner, 2004).

However, although most flowers are visited by a diversity of potential pollinators, only few of these actually effect pollination, and hence only the two visiting bumblebee species, *Bombus festivus* and *B. richardsiellus*, can be considered to act as pollinators for *R. cyanocarpum*. Furthermore, *Bombus* accounted for 90% of all visits, further emphasizing the importance of this insect genus for *Rhododendron*, as concluded by several other studies (Ng & Corlett, 2000; Mejías et al., 2002; Escaravage & Wagner, 2004). The circumstances in which we carried out pollinator observations in both years, for only 1 week during April, likely led to a certain bias in recorded species, and more flower visitors may have been recorded if the observations would have been carried out for longer. However, looking at the species recorded in the other studies suggests that the addition of more species would not have added any further effective pollinators. The dependency of *Rhododendron* species on bumblebees as pollinators is most pronounced in sub-alpine to alpine habitats (Escaravage & Wagner, 2004), owing to their temperature tolerance, as they have been reported to forage under much harsher conditions than most other pollinators (Ng & Corlett, 2000; Escaravage & Wagner, 2004).

Bumblebees generally have three types of photoreceptor cells that they use to discriminate objects at close range (Giurfa et al., 1996, 1997; Frey et al., 2011). Their discriminatory maxima are UV (340 nm), blue (430 nm), and green (540 nm) (Peitsch et al., 1992). Spectrographic analyses showed a clear peak in the reflectance spectrum of petals at 430 nm, which therefore falls into one of the key recognition spectra of bumblebees, likely leading to a higher attractiveness of flowers to this insect group. The prevalence of visits from the two *Bombus* species (90% of overall visits) also supports this.

We conclude that although the floral scents play an important role in attracting pollinators, other floral characteristics such as coloration serve to narrow down the specificity of the pollination syndrome, along with flower characteristics such as size, shape, and style position further increasing pollinator specificity by inducing the observed landing and take-off behavior of *Bombus*, and preventing other insects from contacting the anthers due to their relatively small size.

As could be expected, visitation rates in *R. cyanocarpum* were unambiguously affected by nectar content of flowers, but the role of different flower display sizes is more difficult to assess. Measuring visitation rate by flower would result in attributing no effect on pollinators by flower display size. However, from the viewpoint of the plant, it might make a difference, as the visits received per plant are significantly increased. This means that, with regard to strong pollen limitation, which can be expected considering the extremely low visitation rates (0.38–1.02 visits per plant per hour), the plant could ensure a higher number of visits for the cost of producing more flowers, of which non-pollinated ones might later be aborted.

Breeding system

In general, long-lived woody plant species that have multiple flowering periods throughout their lifetime show low levels of self-compatibility (Rambuda & Johnson, 2004). However, the potential for self-fertilization originating from geitonogamous pollen transfer is substantial in *R. cyanocarpum*, as showcased by the high fruit set obtained from this treatment (83.3%, Table 3). Conditions for this mode of selfing to occur are likely to be favored by the foraging behavior of pollinators, as single plants display a large number of flowers, aggregated in inflorescences, and bumblebees often minimize interflower travel by preferentially foraging in adjacent flowers (Klinkhamer & de Jong, 1993; Goulson, 2003; Stout, 2007). Hence, selfing is likely to occur to a certain extent, but to what extent selfed seed and the resulting plants contribute to future generations cannot be deduced from fruit set alone, as this would need a more careful assessment of predictors such as inbreeding depression and parentage analysis. Some studies deduce a measure of inbreeding depression directly from the fruit set obtained by self-pollination compared to that of cross-pollination (e.g., Martínez-García et al., 2012). This would in this case be relatively low, however, this does not seem appropriate, as inbreeding depression should also take into account the viability of seeds and fitness of the resulting offspring (Charlesworth & Willis, 2009). It should be pointed out that fruit set with regard to corresponding seed set have been reported to be substantially different in other species of *Rhododendron* (Ng & Corlett, 2000). With regard to offspring fitness, it can be assumed that considerable ecological selection acts during seedling recruitment and survival (Ng & Corlett, 2000; Milne et al., 2003; Hirao, 2010). Hence, to assess inbreeding depression meaningfully, the survival during these two crucial stages would have to be taken into account. A possible scenario is that *R. cyanocarpum* is generally self-compatible, providing a moderate amount of reproductive assurance, as suggested by slightly higher fruit set in control flowers compared with emasculated flowers, but that under normal circumstances outcrossed offspring is fitter, and that under less restrictive pollinator availability selfed offspring is unlikely to become established. This could also explain the low abortion rates of selfed fruits, if nearly all parental individuals resulted from outcrossed seed themselves, and taking into account the very high number of seed per fruit in *Rhododendron*, a few seeds within each selfed fruit can still have overall high genetic heterozygosity and viability, potentially counteracting fruit abortion. Another aspect of self-compatibility, which we touched on above, is the often difficult conditions for pollinators in high alpine environments. This can lead to pollen limitation, which in turn may lead to conditions that can favor self-compatibility (Escaravage & Wagner, 2004). A survey of 258 species of angiosperms showed that 62% of species were pollen limited at some time during their life-history or in some locations throughout their occupied range (Burd, 1994). Moreover, pollen limitation occurs more frequently in woody plant species than in herbaceous species, due to larger floral displays reducing pollinator visits received by each flower (Larson & Barrett, 2000). Our results clearly indicate that *R. cyanocarpum* was, to a certain extent, pollen limited during two flowering periods, and there might be years in which this could be even more pronounced, depending on temperature and weather conditions important for pollinator activity. The extended time that the flowers of *R. cyanocarpum* remain open, 10 days, is a strong indicator that they adopt a sit-and-wait strategy to increase exposure to pollinators (Ashman & Schoen, 1994), as often observed in alpine plants.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12114/suppinfo:

Fig. S1. Bumblebee activity during anthesis in *Rhododendron cyanocarpum* observed in the field from 10:00 to 16:00 in April 2007.