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RESEARCH PAPER

Reproductive biology of two Himalayan alpine gingers (*Roscoea* spp., Zingiberaceae) in China: pollination syndrome and compensatory floral mechanisms

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ABSTRACT

According to the concept of pollination syndromes, floral traits reflect specialisation to a particular pollinator or set of pollinators. However, the reproductive biology of endemic, and often specialised, plants may require increased attention as climate change accelerates worldwide. Species of *Roscoea* endemic to the Himalayan region have striking orchid-like flowers with long corolla tubes, suggesting pollination by long-tongued insects. Until now, the reproductive biology of species of *Roscoea* has been poorly documented. We investigated the floral biology, breeding system and pollination ecology of *R. cautleoides* and *R. humeana*, from Hengduan Mountains, a global biodiversity hotspot in southwest China. We also tested whether floral longevity increases pollination success. Pollination experiments showed that the two species were self-compatible and depended on insects for fruit production. Over several flowering seasons we did not observe any potential pollinators with long tongues that matched the corolla tube visiting flowers in centres of distribution. The principal pollinators observed were pollen-collecting generalist bees, with low visitation frequencies. In general, members of the ginger family are characterised by short-lived (usually 1 day) flowers, but flowers of *R. cautleoides* and *R. humeana* last 8 and 6 days, respectively. Removing stigmas decreased fruit set in both study populations. Our results suggest that the original pollinators may have been long-tongued insects that are now absent from the Chinese Himalayas because habitats have responded to climate change. However, long-lived and self-compatible flowers, coupled with the presence of generalist pollinators, are traits that have allowed these gingers to reproduce and continue to persist in the alpine habitats.

INTRODUCTION

The relationship between plants and their animal pollinators has been widely used since the time of Darwin as a model system for the study of adaptation (Barrett 2010). Observations of pollination systems by Darwin (1862), Vogel (1954) and others suggested that different pollinators promoted selection for diverse floral forms, which produced an array of 'pollination syndromes' – suites of convergent floral traits that are adapted to their particular pollinators (reviewed in Fenster *et al.* 2004). The concept of pollination syndromes has been a fundamental subject in plant reproductive ecology because of its capacity to predict the pollinator type of a given species based on the basis of its floral traits (Vogel 1954; Faegri & van der Pijl 1979; Fenster *et al.* 2004). The most famous example is Darwin's prediction of the long-proboscid pollinator of a Malagasy orchid with an extremely long nectar spur (Darwin 1862). Many works that have followed these original studies have also strongly supported and crystallised this concept. However, whether pollination syndromes exist continues to be debated (*e.g.* Ollerton *et al.* 2007).

A number of specialised pollination systems have been described in global hotspots, where exceptional concentrations of endemic species are found, such as the Cape Floral Region of South Africa (Goldblatt & Manning 2000; Pauw 2006). Increasing evidence suggests that global change will have a significant impact on plant–pollinator interactions, and may result in biodiversity loss (reviewed in Alonso *et al.* 2010). Recent investigations indicate that pollinator populations across the globe are declining, especially in biodiversity hotspots (Mitchell & Ashman 2008). In addition, the exceptional concentration of endemic species present in such biodiversity hotspots suggests that these local species are facing a higher risk of extinction than more widespread taxa (Johnson 2004; Vamosi *et al.* 2006). Therefore, understanding specialised plant–pollinator interactions and floral adaptation is critical in a rapidly changing world from a conservation perspective (Kearns *et al.* 1998; Johnson 2004). In fact, little is known about the reproductive biology of the majority of plants in species-rich tropical countries where many such hotspots are located.

The genus *Roscoea* is a small genus of 18 species in the ginger (Zingiberaceae) family (Cowley 2007). The striking orchid-like flowers generally have long corolla tubes and provide nectar as a reward, suggesting a long-tongued insect floral syndrome. In addition, similar to the genus *Salvia* (Lamiaceae; Troll 1929; Classen-Bockhoff *et al.* 2003), the versatile anther is reversibly movable. However, in contrast to *Salvia* with elongated connectives, the thecae in the Zingiberaceae are elongated, pivoting around a thin joint. This anther structure was first described in *Roscoea* by Lynch (1882), who predicted that it is a mechanism for cross-fertilisation.

The Zingiberaceae is a large, predominantly tropical family of animal-pollinated pantropical monocotyledons, comprising about 1300 species in 52 genera (Kress *et al.* 2002). Floral morphologies are correlated with corresponding pollinator groups in many ginger species (reviewed in Sakai *et al.* 1999; Kress & Specht 2005). Despite our increasing knowledge of the reproductive biology of the ginger family (Li *et al.* 2001; Wang *et al.* 2004), only one detailed study has been conducted on the pollination system of the only true alpine genus, *Roscoea* (Zhang & Li 2008). Furthermore, most species of *Roscoea* are concentrated in the eastern Himalayas (Nepal to north India) and the Hengduan Mountains of southwest China (Cowley 2007). This area is widely regarded as a global biodiversity hotspot because of its high species diversity and endemism (Wilson 1992; Myers *et al.* 2000), but little information is available on reproductive biology of the endemic plant species in this area.

Here, we describe the floral biology, breeding system and pollination ecology of two sympatric species of *Roscoea*, *R. cautleoides* and *R. humeana*. We explore the possible adaptive significance of floral traits in relation to pollinator type and environmental conditions. According to the concept of pollination syndromes, floral trait combinations reflect the pollinator type. Thus, we predicted that long-proboscid insects should be the pollinators of long-tubed *Roscoea*, which was supported by preliminary observations in the eastern Himalayas (Fletcher & Son 1931; Dierl 1968). Through field observations and manipulated pollination experiments we tested the prediction that *Roscoea* species are currently, or were formerly, pollinated by a long-proboscid pollinator in the Hengduan Mountains.

MATERIAL AND METHODS

Study site and species

The research site was located at Ganhaizi (27°05′ N, 100°16′ E; 3120 m a.s.l.), a grassy and rocky slope on Mt. Yulong, 25 km north of Lijiang city, northwestern Yunnan Province, China. The average annual precipitation of this site is about 1600 mm, and about 80% of the rain falls between May and September as a result of the warm, wet air masses of the prevailing southwestern summer monsoons from the Indian Ocean; the average annual temperature is approximately 6 °C (1951–1981, Meteorological Department of Yunnan Province, unpublished observations). We also conducted manipulated pollination experiments on floral longevity in a second population located near Lijiang City at the Lijiang Alpine Botanical Garden (27°00′ N, 100°11′ E; 2830 m a.s.l.). Lijiang is located in the core area of the Hengduan Mountains region and is considered to have the highest diversity of *Roscoea* species (Ngamriabsakul *et al.* 2000; Wu & Larsen 2000; Cowley 2007; Zhang & Li 2008).

Roscoea humeana and *R. cautleoides* are perennial hermaphroditic herbs, reaching a height of 9–40 cm. At our study site, they are sympatric and usually co-flower from May to June. The two species of *Roscoea* are distinguished through both vegetative features and floral characteristics (Fig. 1A and B). *Roscoea humeana* has ovate, sessile leaves while *R. cautleoides* has narrowly lanceolate, petiolate leaves. In this research site, flowers of *R. humeana* are purple while flowers of *R. cautleoides* are bright yellow with a longer flora tube than *R. cautleoides* (Fig. 1 C). In addition, *R. humeana* generally flowers before the leaves emerge, while flowers of *R. cautleoides* are located at the apex of a leafy shoot. In spite of their floral and vegetative differences, a phylogenetic analyses of the ITS region suggested that *R. humeana* and *R. cautleoides* are closely related in a small unresolved clade of four species in the genus (Ngamriabsakul *et al.* 2000). Our research site is in the core of the distributional range of both species, which is restricted to the southeast part of Hengduan Mountains, *i.e.*, Northern Yunnan Province and neighbouring Sichuan Province.

Flower biology

We conducted our phenological observations in Ganhaizi in 2004. We randomly selected 63 individuals of each species on which we counted inflorescences per plant and flowers per inflorescence; one unopened bud was selected in each individual for recording flower longevity. The length of the corolla tube was measured on 30 flowers per species using a vernier caliper. To measure the nectar concentration and volume, we haphazardly selected approximately 60 flowers of each species (one flower from each plant) and bagged them in nylon net bags before the flowers opened. After anthesis, six flowers of each species were chosen and removed from plants at 09:00, 12:00 and 15:00 h on three consecutive days to measure nectar secretion. Nectar was drawn with 5- and 10-μL SIGMA 'micro-cap' calibrated capillary tubes (Sigma-Aldrich, St. Louis, MI, USA). Volume was determined by measuring the length of the filled tube and converting the length measurements to microliters. The nectar sucrose concentration was measured using a hand-held, temperature-compensated refractometer (eclipse; Bellingham & Stanley Ltd., Turnbridge Wells, Kent, UK). We also quantified pollen grain and ovule numbers for the two *Roscoea* species by selecting 30 flower buds per species and fixing the dissected anthers and ovaries separately in FAA solution. We used a haemocytometer to estimate pollen number per flower following the methods of Dafni (1992). The number of ovules in each ovary was carefully counted under a dissecting microscope. For each flower, the pollen/ovule ratio (P/O) was calculated.

Manipulated pollination experiments

In 2004, we investigated the breeding system of *R. cautleoides* and *R. humeana* using five different pollination treatments. More than 180 individuals with unopened flowers were haphazardly selected; each treatment involved at least 30 plants. The five treatments were: (i) bagged – flowers were left un-emasculated and covered with fine mesh bags to exclude pollinators; (ii) emasculated – buds were emasculated and covered with fine mesh bags; (iii) hand out-crossed – plants were kept covered with fine mesh bags, flowers were emasculated

Fig. 1. Plants, flowers and pollinators of *R. cautleoides* and *R. humeana*. A: Plant of *R. cautleoides*. B: Plant of *R. humeana*. C: Flowers of *R. cautleoides* (right) and *R. humeana* (left); bar = 10 mm. D: Pollen-collecting bee *Andrena* sp. visiting a flower of *R. cautleoides*. E: Pollen-collecting bee *Lasioglossum* sp. visiting a flower of *R. humeana*.

before anthesis, then the fresh flowers were hand-pollinated with fresh pollen from other individuals several meters away; (iv) hand self-pollinated – plants were kept covered with fine mesh bags, flowers were emasculated before anthesis, then the fresh flowers were hand-pollinated with pollen from the same plant; and (v) control – plants were left unbagged and thereby exposed, permitting insects to visit the flowers. To assess whether the amount of pollen transferred was a limiting factor in female fecundity of flowers of *R. cautleoides* and *R. humeana*, we also surveyed the natural fruit set and seed set in wild populations, in a random sample of 100 individuals during the flowering season of 2004 and 2006. Treatments 1 and 2 were used to test for autogamy and apomixis, respectively. Treatment 3 and 4 were used to compare the effect of out-crossed *versus* self-pollination. Treatment 4 was also used to test for self-compatibility. Treatment 5 was used as a natural control. Because percentage of fruit set did not conform to ANOVA assumptions, we employed the contingency Kruskal–Wallis test to check for significant differences in fruit set per treatment for each species, and 2 × 2 contingency tables to compare their means. A one-way ANOVA was used to test for significant differences in seed number per fruit among treatments for each species.

Pollinator visitation

We observed flower visitors to *R. cautleoides* and *R. humeana* in 2004, 2006 and 2007 at the Ganhaizi site. For each year, during the peak flowering season, we chose three clear sunny days to observe the pollinators from 09:00 to 17:00 h each day. In addition, insect activity at the flowers was surveyed during other experimental manipulations. Because a previous study suggested that the long-tubed flowers of several species of *Hedychium*, another genus in the Zingiberaceae, were pollinated shortly after dusk by hawkmoths (Gao J.-Y., unpublished observation), we made observations on each of the *Roscoea* species on three continuous clear nights in the flowering seasons of 2004 and 2007. We observed nocturnal visitors from 19:00 to 22:00 h each night using an ATN Night Shadow (ATN Corp., San Francisco, CA, USA) and two flashlights (with red cloth covering the head) synchronously at three sites in the population. To investigate whether nocturnal long-tongued insects (moths) are pollinators of long-tubed *Roscoea* species after our initial observations in 2004, in 2007 we conducted pollination treatments on 20–30 additional randomly selected individuals of each species with open flowers using fine nylon bags. For each individual, we bagged one inflorescence in daytime from 08:00 to 22:00 h to isolate day pollinators and removed the nets when we ended the night observation each day. We checked the anthers and stigmas using a handheld magnifier to determine whether flowers were visited during the evening by potential night pollinators.

We also made observations on *R. cautleoides* on three continuous days from 08:00 to 20:00 h in 2008 at Lijiang Alpine Botanical Garden using a Sony Digital video (Shanghai Suo-Gang Electronics Co., Ltd., Shanghai, China) (night vision mode) and flashlights. To investigate whether nocturnal long-tongued insects are pollinators for *R. cautleoides*, we conducted two pollination treatments on 62 randomly selected individuals of *R. cautleoides* using fine-nylon bags. On half of those individuals, we bagged flowers in daytime from 08:00 to 20:00 h to isolate diurnal pollinators and removed the nets from 20:00 to 08:00 h in the next day to expose the inflorescences to potential nocturnal pollinators. To confirm seed set did not occur through accidental pollination by insects that entered the bags, other selected flowers were treated as a control and bagged for the duration of flowering. All of the different types of flower visitor were photo-recorded, and the main visitors were collected and deposited in the insect collections of XTBG. The voucher specimens of plants were deposited in HITBC.

Floral longevity and pollination success

To measure the effect of floral longevity on pollination success, we experimentally tested whether the long duration of open flowers increases fruit set by removing stigmas of *R. cautleoides* to reduce functional floral longevity. We randomly tagged more than 180 individuals with unopened flowers from the Ganhaizi population in 2006. One new flower (in anthesis) on each individual was tagged during the experiment. Plants with newly opened flowers were evenly and randomly assigned among three different pollination treatments: (i) stigmas of tagged flowers were removed after they had been receptive for 1 day; (ii) stigmas of tagged flowers were removed after they had been receptive for 4 days; or (iii) stigmas were left intact as a natural control. Stigmas were removed with scissors just below the receptive surface, and the scissors were sterilised with ethanol after each use. All flowers were exposed to natural pollination. We repeated the experiment in the botanical garden population in 2007.

To ascertain whether stigma clipping was harmful to normal fruit and seed development (Rathcke 2003), 50 plants with buds were selected from the botanical garden population and prevented from receiving visitors by covering with nylon mesh bags for the duration of flowering. Plants were then evenly and randomly assigned among two anther removal treatments (one flower per plant): (i) pollinated then treated – flowers were out-crossed in the afternoon when pollinators were not seen, and their stigmas were removed the following morning; and (ii) control – flowers were out-crossed and stigmas were left intact. To determine whether stigma removal would preclude subsequent pollination and fertilisation, as assumed, we also removed 10 stigmas of virgin flowers and immediately added pollen to the remaining styles. Fruit and seed sets were compared between these treatments.

RESULTS

Floral biology

Phenological investigations of *R. cautleoides* and *R. humeana* at Ganhaizi indicated that the flowering seasons of the two species overlapped, and lasted from early May to late June, with peak flowering occurring from late May to the middle of June. *Roscoea cautleoides* usually has one to four slightly scented flowers per inflorescence (Table 1), with only a single flower open at a time; *R. humeana* produced more flowers per inflorescence than *R. cautleoides*, which were unscented, with one or more flowers open at the same time (range 1–10; Table 1). Our field observations of flowering at the Ganhaizi site indicated that flowers of *R. cautleoides* and *R. humeana* usually begin anthesis in the early morning and last for 8 days and 6 days, respectively (Table 1). Anthers of both *Roscoea* species dehisce when the flowers have completely opened. Stigmas of both species produced stigmatic fluid and were apparently receptive from anthesis until the flowers began to wilt.

Both *Roscoea* species have long, slender corolla tubes, with those of *R. humeana* longer than *R. cautleoides* (Fig. 1C; Table 1). *Roscoea humeana* produced more nectar than *R. cautleoides* ($F_{1,102} = 69.4$, $P < 0.001$), and nectar sucrose concentration was also significantly different between them ($F_{1,101} = 6.68$, $P < 0.05$). The numbers of pollen grains produced per flower in *R. cautleoides* and *R. humeana* from the Ganhaizi populations were 8348 ± 439 and 11,801 ± 527 (n = 30), respectively; the number of ovules was 79 ± 5 and 70 ± 4 (n = 30), respectively. Hence, the pollen:ovule ratio (P/O) of *R. cautleoides* was 117 ± 10 (n = 30) and that of *R. humeana* was 185 ± 16 (n = 30).

Manipulated pollination experiments

Neither emasculated nor bagged plants of *R. cautleoides* set fruits, indicating that apomixis and autonomous self-pollination do not occur in this species (Fig. 2). *R. cautleoides* did not show any reduction in fruit set in hand self- pollination *versus* hand cross-pollination ($\chi^2 = 0.81$, df = 1, $P > 0.25$; Fig. 2). Statistical analysis of seed set (seed number per fruit) following self-pollination and cross-pollination also indicated no significant difference ($F_{1,61} = 0.245$, $P = 0.62$; Fig. 3). These results demonstrate that *R. cautleoides* has a fully self-compatible breeding system. However, open-pollinated plants had significantly lower fruit set ($\chi^2 = 78.9$, df = 2, $P < 0.001$;

Table 1. Floral characters (mean ± 1SE) of *Roscoea cautleoides* and *R. humeana* based on samples from Ganhaizi populations. Numbers in parentheses are sample sizes.

		species	
character	unit	*R. cautleoides*	*R. humeana*
flowers/inflorescence	#	2.0 ± 0.1 (63)	4.0 ± 0.2 (63)
flower longevity	day	7.7 ± 0.1 (56)	5.9 ± 0.1 (52)
nectar volume	µl	1.4 ± 0.1 (59)	13.7 ± 4.1 (56)
sugar concentration	%	38.8 ± 1.3 (28)	32.1 ± 2.6 (28)
length of corolla tube	mm	33.3 ± 1.0 (30)	89.6 ± 2.2 (30)
pollen/flower	n	8343 ± 439 (30)	11,801 ± 527 (30)
ovules/flower	n	79 ± 5 (30)	70 ± 4 (30)
P:O ratio		117 ± 10 (30)	185 ± 16 (30)

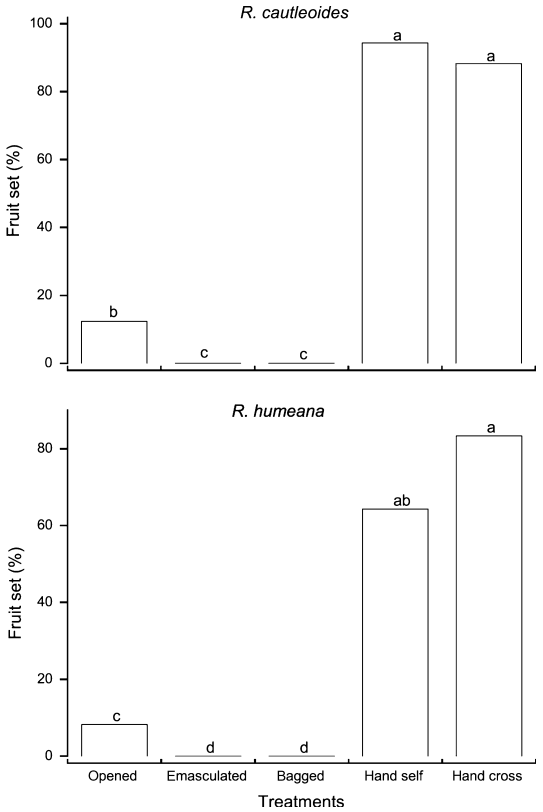

Fig. 2. Effect of pollination treatments on the fruit set of *R. cautleoides* and *R. humeana*. Significant differences are connected by different letters ($P < 0.05$).

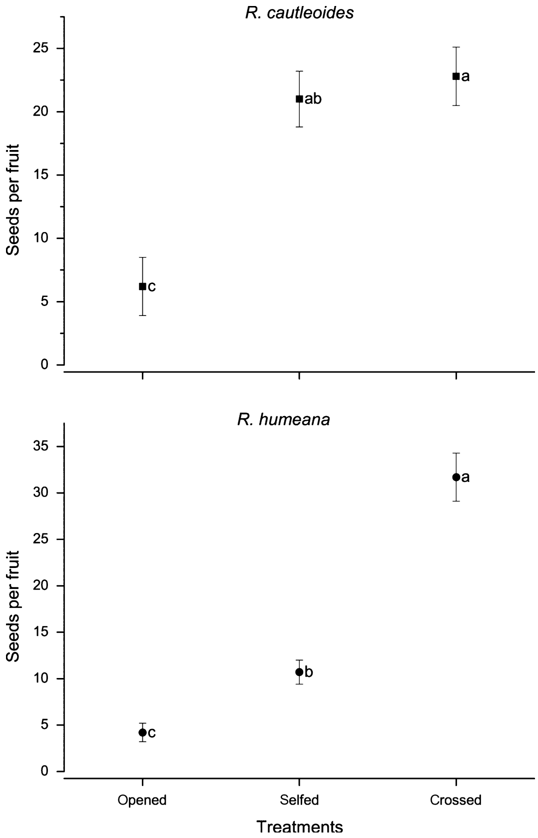

Fig. 3. The effect of pollination treatments on the number of seeds per fruit of *R. cautleoides* and *R. humeana*. Significant differences are connected by different letters ($P < 0.05$).

Fig. 2) and seed set than hand self- or cross-pollinated ones ($F_{2,90} = 16.5$, $P < 0.01$; Fig. 3). In 2006, natural fruit set of *R. cautleoides* was 10.1% ($n = 378$ flowers from 220 individuals), seed set was 8.2 ± 2.0 (range 1–46, $n = 40$).

Neither emasculated nor bagged plants of *Roscoea humeana* set fruit, indicating apomixis and autonomous self-pollination does not occur in *R. humeana* (Fig. 2); *R. humeana* had lower fruit set after hand self-pollination than hand cross-pollination, but the difference was not statistically significant ($\chi^2 = 2.81$, $df = 1$, $P < 0.05$; Fig. 2). However, *R. humeana* produced significantly fewer seeds after self- pollination than cross-pollination ($F = 43.97$, $df = 1$, $P < 0.001$; Fig. 3). These results indicate that the breeding system of *R. humeana* is partially self-incompatible. Similarly, open-pollinated plants had significantly lower fruit set ($\chi^2 = 77.3$, $df = 2$, $P < 0.001$; Fig. 2) and lower seeds set than hand self- and cross-pollinated plants ($F_{2,95} = 65.3$, $P < 0.001$; Fig. 3). In 2006, natural fruit set of *R. humeana* was 11.2% ($n = 896$ flowers from 258 individuals); seed set was 10.6 ± 1.2 (range 1–64, $n = 54$).

Floral visitors

Observation of visitors to flowers of *Roscoea* at the Ganhaizi site indicated that visitors were absent in the early- to mid-flowering season; pollen-collecting bees [*Andreana* sp. (Fig. 1 D) and *Lasioglossum* sp. (Fig. 1E)] were the principal pollinators of *R. cautleoides* and *R. humeana* during the late-flowering season, respectively; their visitation rates were quite variable during the observed blooming period. For example, in 2004 *Andreana* sp. visited *R. cautleoides* at a rate of 0.102 ± 0.03 visits per flower·h^{-1}, and *Lasioglossum* sp. visited *R. humeana* at a visitation rate of 0.113 ± 0.03 visits per flower·h^{-1}. We also found that pollen-collecting bees (*Apis* sp.) visited flowers of *R. humeana* in the 2006 and 2007 flowering seasons, and transferred pollen within and between flowers. A short-tongued bumblebee (*Bombus* sp.) occasionally (six times in all observations) visited both *R. humeana* and *R. cautleoides* and probed the flowers for nectar. No nocturnal visitors were observed to visit the flowers of *R. cautleoides* or *R. humeana* in any flowering season during our night observations. Among 87 flowers exposed to potential nocturnal pollinators, 10 were visited by ineffective pollen thieves, because some pollen grains were removed from anthers, but the stigmas did not receive any pollen grains. In 2008, 31 nocturnal bagged flowers and 31 flowers bagged for the duration of flowering set only one fruit.

Floral longevity and pollination success

At the Ganhaizi population, the flowers of *R. cautleoides* with 1- and 4-day functional floral longevities had 7.3% and 20.7% fruit set, respectively; whereas control flowers with natural longevity had 22.2% fruit set (Fig. 4). In the botanical garden population, flowers of *R. cautleoides* had 8.0% and 18.0% fruit set when floral longevities were reduced to 1 and

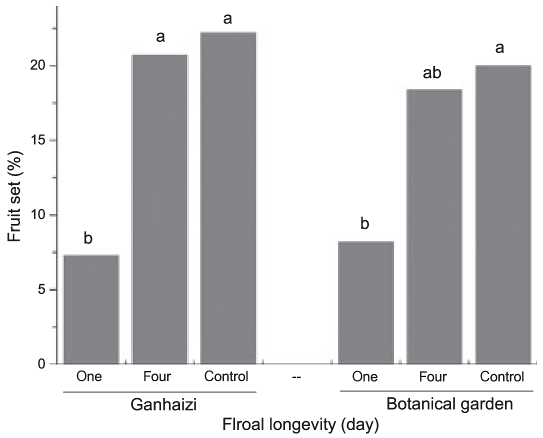

Fig. 4. Fruit set of *R. cautleoides* flowers for natural floral longevity and with reduced longevity (1 and 4 days) after clipping stigmas. Significant differences are connected by different letters (P < 0.05).

4 days, respectively; whereas control flowers had 20% fruit set (Fig. 4). Stigma removal did not affect fruit and seed development. The mean fruit set in flowers that were hand-pollinated and stigmas removed on the following day was not significantly different from the mean fruit set of hand-pollinated flowers in which the stigmas were not removed ($P = 0.965$); seed set between them was not significantly different ($P = 0.326$; Table 2). Virgin flowers in which the stigmas were removed and the styles pollinated directly, produced no fruit (Table 2), suggesting stigma removal precluded subsequent pollination and fertilisation, as assumed.

DISCUSSION

Absence of long-proboscid pollinators

In accordance with the concept of pollination syndromes, the long corolla tube has been hypothesised to be associated in nature with a long-tongued (or long-billed) pollinator (Darwin 1862). In this aspect, moths and butterflies have been found to be the principal pollinators of long- and slender-tubed flowers of some species of *Hedychium* (Zingiberaceae), a closely related genus, although not sister to *Roscoea* (Kress *et al.* 2002). *Roscoea cautleoides* and *R. humeana* have long, slender corolla tubes (Fig. 1C) and can provide large quantities of nectar as rewards, suggesting a typical long-tongued insect floral syndrome. In addition, because of the lever-like stamens of flowers of *Roscoea*, Lynch (1882) predicted that pollinators follow a specific behaviour when visiting a flower: an insect seeking nectar will push against the elongated thecae, causing the anther to swing towards the insect's back, thus forcing the stigma to take up pollen (which came from another flower) from the insect; while pollen from the anther is placed on the pollinators' back. In the Himalayas of India and Nepal, Fletcher & Son (1931) and Dierl (1968) briefly described long-proboscid flies, *Corizoneura longirostris*, visiting flowers of *R. purpurea* for nectar, which strongly supported the concept of this pollination syndrome. Surprisingly, we found that short-tongued bees foraging for pollen were the most frequent visitors to flowers of *R. cautleoides* and *R. humeana* in our 3-year observations, *i.e.*, we did not observe pollinators with long-probosces that match the lengths of the corolla tubes. Our pollination experiments provided no evidence that night-flying moths are the current pollinators of the long-tubed study species during the 4 years of our observations. Occasional pollination by short-tongued bees was achieved because of the close proximity in the flower of pollen and stigma, coupled with the absence of self-incompatibility.

Table 2. Fruit set (%) and seed set (means ± SE) of pollinated then stigma clipped, stigma clipped then pollinated and control flowers of *Roscoea cautleoides.*

treatment	N	fruit set	significance	seed set	significance
control	24	91.7	n.s.	27.3 ± 3.8	n.s.
pollinated then clipped	23	91.3			
control	24	91.7	not tested	32.7 ± 3.7	not tested
clipped then pollinated	10	0		0	

n.s., no significant difference.

The absence of long-proboscid pollinators led us to conclude, for several reasons, that the original long-proboscid pollinators of these long-tubed flowers of *Roscoea* have been ecologically lost, at least in the Chinese Himalayas. First, long-proboscid flies were observed visiting flowers of *R. purpurea* for nectar in the Himalayas of India and Nepal (Fletcher & Son 1931; Dierl 1968). In addition, Ngamriabsakul *et al.* (2000) suggested, based on phylogeny and distribution of the genus, that *Roscoea* species possibly originated in northeast India and spread east along the nearest mountain ranges. Therefore, it is possible that long-proboscid flies were the original pollinators of *Roscoea* species. Second, although 15 species of long-tongued flies in five genera of two families are known from the Himalayan region of China, they have not been recorded in the Hengduan Mountains region (see The Comprehensive Scientific Expedition to the Qinghai-Xizang Plateau, CAS 1993; Goldblatt & Manning 2000). Bond (1994) suggested that the breakdown of a mutualism might occur when pollinators are lost from a highly specialised pollination system. Two examples are the breakdown of a Mediterranean buzz-pollinated plant *Cylamen persicum* (Primulaceae; Schwartz-Tzachor *et al.* 2006) and the Chinese tropical plant *Tacca chantrieri* (Zhang *et al.* 2005). Loss of long-tongued pollinators is mirrored in another group, *Pedicularis*, which has variable corolla tube length but in which bumblebees are the pollinator (Huang & Fenster 2007). However, it may require long-term observations to actually demonstrate pollination of a specialised flower by moths, such as the long-spurred Malagasy orchid flower. Our current study on *Roscoea* reflects only our relatively limited observations, and further field studies should be conducted to confirm our results.

Pollinator changes and compensatory floral mechanisms

When the original pollinators of a plant species disappear and/or lose their attraction to the flowers, unspecialised visitors may fill this vacant niche (Schwartz-Tzachor *et al.* 2006). Our study suggests that generalised pollen-foraging bees now occupy the pollinator position for the two *Roscoea* species investigated. Our results also suggest that pollinator changes have resulted in pollen limitation of seed production in

R. cautleoides and *R. humeana* (Figs 2 and 3). Pollinator changes, including shifts in species composition of pollinators and losses of pollinators, can potentially have demographic and evolutionary consequences for plant populations (Campbell 2008). Such a reproductive failure may have negative effects on the abundance and viability of populations, which may be at risk of extirpation due to the changes in pollinators, as well as changes in land use and global climate change. Plant species that are less dependent on pollinators and those that have compensatory mechanisms should be less pollen-limited and thereby could avoid extinction.

In the present study, our results indicate that reproduction in *R. cautleoides* and *R. humeana* is dependent on animal pollinators. Compared to most tropical gingers, *R. cautleoides* and *R. humeana* have several unique floral traits, such as long-lived flowers, small inflorescence size and few inflorescences per plant. The longevity of a single flower may be the most unique character distinguishing *Roscoea* from other gingers. Most members of the ginger family have flowers that usually last 1 day or less (Larsen *et al.* 1998; but see Gao *et al.* 2004; Wang *et al.* 2004), but flowers of the two *Roscoea* species last from 6 to 8 days (Table 1). As far as we know, these flowers have the longest floral duration in the Zingiberaceae. Our observations indicate that pollinators visited both species of *Roscoea* with very low frequency and were absent for part of the flowering season. It is reasonable to expect that the longevity of a flower will at least in part determine the probability and the frequency that a flower will be visited at a given level of pollinator activity (Primack 1985; Ashman & Schoen 1994). Some studies have documented that long-lived flowers increase fruit or seed set in the alpine habitat where few and unpredictable pollinators dominate (Bingham & Orthner 1998; Rathcke 2003). Our manipulation experiments indicate that reducing functional floral longevities decreased fruit set in both studied populations (Fig. 4; Table 2). Additional evidence comes from a sympatric congeneric species, *R. schneideriana*, which is a self-pollinating species. In this plant the longevity of a single flower is 4 days (Zhang & Li 2008), implying that pollinator-dependent species require increased floral longevity to ensure pollination success. Whether or not the prolongation of flowering time is the result of changes in pollinators, we suggest that the long flowering duration has nevertheless allowed the plants to survive in a much reduced pollinator environment.

Bond (1994) demonstrated that plants might rely on compensatory mechanisms to continue to persist even without their mutualistic pollinating partners. Our results demonstrate that long floral duration helps to increase pollination success (Fig. 4; Table 2). The pollination experiments showed that both species of *Roscoea* that were studied are self-compatible (Figs 2 and 3). Because of the close arrangement of pollen and stigma in the flower and the presence of self-compatibility, pollination by pollen-collecting bees has been successful. Pollen:ovule ratios (P/O), which are also used to determine breeding system (Cruden 1977), suggested that the two *Roscoea* species are facultatively autogamous. We therefore infer that high floral longevity, coupled with facultative selfing and generalist pollinators, provide a compensatory floral mechanism in *R. cautleoides* and *R. humeana* that has allowed these species to persist in habitats where the suspected mutualistic pollinators are no longer present.

Conclusions and further studies

Our study presents a striking example that compensatory floral mechanisms help to ensure reproductive success in light of the apparent loss of specialised pollinators. Alternatively, contemporary pollinators may not reflect the historical conditions in which various pollination syndromes evolved (Li & Huang 2009). Some *Roscoea* species may have adapted to other types of pollinator. *Roscoea cautleoides*, for example, has a shortened floral tube and its nectar may be available to some short-tongued insect visitors. Such radiations of pollination systems have been reported in other taxa (Manning & Goldblatt 2005; Ley & Classen-Bockhoff 2009). Spatial and temporal variation in pollinator service has been widely reported in plants (reviewed in Price *et al.* 2005). Thus, detailed investigations of the species at additional study sites in the Chinese Himalayas are required in order to understand the full scope of the plant–pollinator interaction in these species of *Roscoea*. Furthermore, since anthropogenic change has caused much concern regarding the fate of biodiversity, it would be profitable to more closely focus on plant–pollinator interactions in such threatened biodiversity hotspots.

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