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Geographic isolation, pollination syndromes, and pollinator generalization in Himalayan *Roscoea* spp. (Zingiberaceae)

Babu Ram Paudel,[1,2,3] André Kessler,[4] Mani Shrestha,[5,6] Jian Li Zhao,[1,2] and Qing-Jun Li[1,2,†]

[1]*Yunnan Key Laboratory of Plant Reproductive Adaption and Evolutionary Ecology, Yunnan University, Kunming, Yunnan 650091 China*
[2]*Laboratory of Ecology and Evolutionary Biology, State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, China*
[3]*Department of Botany, Prithvi Narayan Campus, Tribhuvan University, Pokhara, Nepal*
[4]*Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853 USA*
[5]*School of Media and Communications, RMIT University, Melbourne, Victoria 3001 Australia*
[6]*Faculty of Information Technology, Monash University, Clayton, Victoria 3800 Australia*

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Abstract. The pollination syndromes have been widely used to predict effective pollinators of plant species and provide the mechanistic explanation of floral diversity. However, in recent years, the predictive applicability of pollination syndromes has been frequently questioned. The accuracy of the syndromes among the closely related plant species may vary temporally and spatially. This suggests the need for the standardized, comprehensive evaluation of factors influencing the matching of a pollination syndrome of a plant species to the predominant pollinator community. We studied the pollination biology of three geographically/phenologically isolated alpine gingers (*Roscoea auriculata*, *Roscoea capitata*, and *Roscoea tumjensis*) exhibiting the correlated suites of floral traits that suggest long-tongued insects as major pollinators, to test for the predictive power of the respective pollination syndrome. We also tested if geographical and temporal isolation affects interspecies breeding system and extent of pollinator generalization. We demonstrate that the three *Roscoea* species are self-compatible but lack autonomous selfing, and thus completely rely on pollinators for pollination success. Five years of observations demonstrate that only diurnal insect visitors foraged on the flowers of the three *Roscoea* species. Estimates of the pollinator importance (pollen deposition) of the observed floral visitors indicate that morphologically mismatched bumblebees contribute more than 90% of pollination service in all the three *Roscoea* species, while long-tongued butterflies and moths function as nectar robbers. We found that geographical isolation and temporal variation in flowering period does not affect the breeding system and pollinator generalization in the three *Roscoea* species. The three geographically and phenologically isolated Himalayan *Roscoea* spp., with long-tongued insect pollination syndrome, exhibit generalized pollination system and primarily rely on the morphologically mismatched *Bombus* species for pollination success. While, for three *Roscoea* spp., our result suggests that pollination syndromes are a weak predictor of efficient contemporary pollinator community, it also substantiates the role of non-pollinator agents for the evolution of floral traits. The evolution of *Bombus* pollination in these alpine gingers suggests how the mismatch between plant and pollinator phenology can lead to the emergence of novel reproductive strategies among the congeners, which ultimately seems to play a key role for the speciation and diversification of the genus *Roscoea* in the Himalayas.

Key words: alpine ginger; evolution; floral traits; Nepal Himalaya; pollination; *Roscoea;*.

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† **E-mail:** qingjun.li@ynu.edu.cn

Introduction

Ecologically functional phenotypes are frequently complex and are dependent on coordinated and correlated expression of multiple individual traits. Such complex phenotypes are usually characterized by high phenotypic integration of the respective component traits (Pigliucci 2003). Such trait correlations can become so characteristic that they have been categorized into ecological syndromes. For example, plant defense syndromes have been conceptualized as suites of covarying defensive traits in plants, whose correlated evolution can result from close association with specific ecological interactions (e.g., certain specific types of herbivores; Agrawal and Fishbein 2006). Probably the most widely used syndrome characterization is that of pollination syndromes, which describe highly correlated floral traits, whose coordinated expression is associated with specific groups of pollinators (e.g., insects, birds, mammals; Fenster et al. 2004), and which underlie the complexity and diversity of floral traits.

The remarkable diversity in floral traits of animal pollinated plants is one of the intriguing phenomena studied by evolutionary biologists since Darwin's pioneering studies (Karron et al. 2012). Although the evolution of floral traits in angiosperms may also be influenced by selection exerted by antagonists, abiotic factors, and random processes, pollinator-mediated selection has been considered as one of the key evolutionary forces that promotes tremendous diversities in floral forms among flowering plants (Stebbins 1970, Johnson and Steiner 2000, Fenster et al. 2004, Parachnowitsch and Kessler 2010). Thus, floral traits of angiosperms such as phenology, display size, length of corolla tube, reward, color signal, and odor perception are evolved in accordance with the preference of most frequent and effective pollinators forming an array of pollination syndromes (Darwin 1862, Faegri and van der Pijl 1979, Fenster et al. 2004). Since Darwin's (1862) prediction of long-nectar-spur "Malagasy star orchid (*Angraecum sesquipedale*)" pollinated by the long-proboscid moth, pollination syndrome hypothesis has been widely used to predict the potential pollinator of many plant species (Ollerton et al. 2009, Armbruster et al. 2011, Johnson 2013). Indeed, several studies have shown that in many plant species, the floral syndromes indicate their specific pollinators (Goldblatt and Manning 2000, Fenster et al. 2004, Pauw 2006, Danieli-Silva et al. 2012, Paudel et al. 2015). However, the plant–pollinator interactions at the community level frequently indicate generalized pollination systems in a majority of plants. Thus, the central theme of pollination syndrome hypothesis, the evolution of floral traits primarily through the interaction with the specialized pollinators, has been questioned by several authors (Waser et al. 1996, Gomez and Zamora 1999, Ollerton et al. 2009, Rosas-Guerrero et al. 2014, Kuriakose et al. 2018, 2019). Some authors have suggested that pollination syndromes are not reliable to predict the potential pollinators (Hingston and McQuillan 2000, Hargreaves et al. 2004, Zhang et al. 2005, Paudel et al. 2017, Kuriakose et al. 2018, 2019). The mounting evidence suggests that plant species with specific pollination syndromes are often pollinated by several types of floral visitors, and in some cases, the contribution of generalized pollinators outweighs the contribution of pollinators predicted by the floral syndromes (Waser et al. 1996, Fleming et al. 2001, Mayfield et al. 2001). For example, flowers of *Ipomopsis aggregata* exhibit hummingbird syndrome, but queens of the bumblebee *Bombus appositus* are the most common visitors to the flowers and deposit pollen more efficiently (Mayfield et al. 2001). Similarly, various species of cacti in the Sonoran Desert display bat pollination syndrome, but hummingbirds, woodpeckers, and finches are found as the effective pollinators (Fleming et al. 2001).

A general consensus that arose from this debate suggests a need of comprehensive study on the pollination biology of related plant species, to evaluate the reliability of pollination syndrome concept (Herrera 1987, Li and Huang 2009, Reynolds et al. 2009, Kuriakose et al. 2019). The selection pressure exerted by a floral visitor primarily depends on its behavior and frequency of visit (Sinu and Shivanna 2007a, b). Thus, each potential visitor affects fitness differently (Fumero-Caban and Melendez-Ackerman 2007). Estimation of pollinator importance is a proxy to determine an effective pollinator of a plant species, as it denotes the overall contribution made by a visitor to a plant for its reproductive success (Fumero-Caban and Melendez-Ackerman 2007,

Nèeman et al. 2010, Liu and Huang 2013). This index of pollination effectiveness indicates the rate of pollen grains deposited by a pollinator on a virgin stigma. Thus, a statistical comparison of the pollinator importance among the different floral visitors enables to identify the effective pollinator of a plant species and to test the reliability of pollination syndromes.

The little-studied genus *Roscoea* is a Himalayan endemic alpine member of the primarily tropical family Zingiberaceae (Cowley 2007). Plants of this genus grow between 1200 m and 4880 m a.s.l. from Kashmir in the west through the Himalayas in Nepal, India, Bhutan, and southwest China (Cowley 1982, 2007). It has two disjunct lineages (Himalayan and North-Indo-Chinese) whereby the Brahmaputra River likely acts as a topographic barrier between the two groups (Cowley 2007, Zhao et al. 2016). Most of the *Roscoea* species exhibit floral traits commonly associated with the long-tongued fly pollination syndromes such as absence of discernible fragrance, presence of nectar as a reward, elongated reproductive organs situated away from the nectar source, and long corolla tube that shows compatible match with the tongue length of long-tongued insects (Goldblatt and Manning 2000, Cowley 2007). In accordance with the pollination syndrome, one of the Himalayan *Roscoea* species (*R. purpurea*) exhibits obligate specialized pollination with a long-tongued fly (*Philoliche longirostris*; Paudel et al. 2015). However, studies on the reproductive biology of other *Roscoea* species either in the Himalayas or in the Indo-Chinese region did not find any long-tongued insects as the effective pollinators (Zhang and Li 2008, Zhang et al. 2010, Fan and Li 2012, Paudel et al. 2017). These contrasting findings necessitate additional studies to address the question of whether the Himalayan *Roscoea* species exhibit specialized pollination system as reflected by their floral morphology or undergo adaptive evolution toward the local guilds of pollinator communities.

In this study, we evaluate the predictive power of pollination syndrome in three Himalayan *Roscoea* species (*R. auriculata*, *R. capitata*, and *R. tumjensis*). These three *Roscoea* species have an entirely non-overlapping distribution but have partially overlapped flowering phenology. Nonetheless, they exhibit the suite of floral traits that is consistent with that of *R. purpurea*. In geographically isolated populations, due to limited gene flow, plant species are likely to experience either divergence in floral traits associated with shift in pollination system (Grant and Grant 1965, Grant 1992) or convergence in floral traits driven by an adaptation to a common pollinator type (Fenster et al. 2004, Anderson and Johnson 2009). Thus, the three *Roscoea* species offer an ideal setting to assess if geographical isolation acts as a driving force for the convergent/divergent evolution of floral traits and pollination system in closely related plant species. Here, we focused (1) to study the breeding system of three allopatric Himalayan *Roscoea* species (*R. auriculata*, *R. capitata*, and *R. tumjensis*); (2) to test if the suites of floral traits in these alpine gingers correspond to the predicted specialization toward long-tongued insects; and (3) to test if geographic isolation and temporal variation in flowering period affect the interspecies breeding system, among the three alpine gingers.

Materials and Methods

Study species

Three Himalayan *Roscoea* spp. (*R. auriculata*, *R. capitata*, and *R. tumjensis*) that have non-overlapping geographical distribution (Fig. 1; Appendix S1: Table S1) are considered under the current study. All of these species are perennial herbs and flower sequentially from May to September (Fig. 2). Among them, *R. auriculata* has a wide distribution range in the eastern Himalaya (east of Nepal to India and Bhutan) in between the elevations of 2130–4880 m and grows on open rocky ground with deep purple or occasionally white flowers. *Roscoea capitata* and *R. tumjensis* are endemic to Nepal. Among *Roscoea, R. capitata* is the species growing at the lowest elevations in the Himalayan Mountains, between 1200 and 2600 m. It has purple flowers, whereas *R. tumjensis* has pale lilac, lilac-blue, or bright purple flowers and mostly grows on the open meadows and under the canopy of *Pinus* and *Rhododendron* forests, between the elevations of 2014 and 3050 m (Cowley 1982, 2007). Detailed floral phenology of the three *Roscoea* species is provided in Fig. 2.

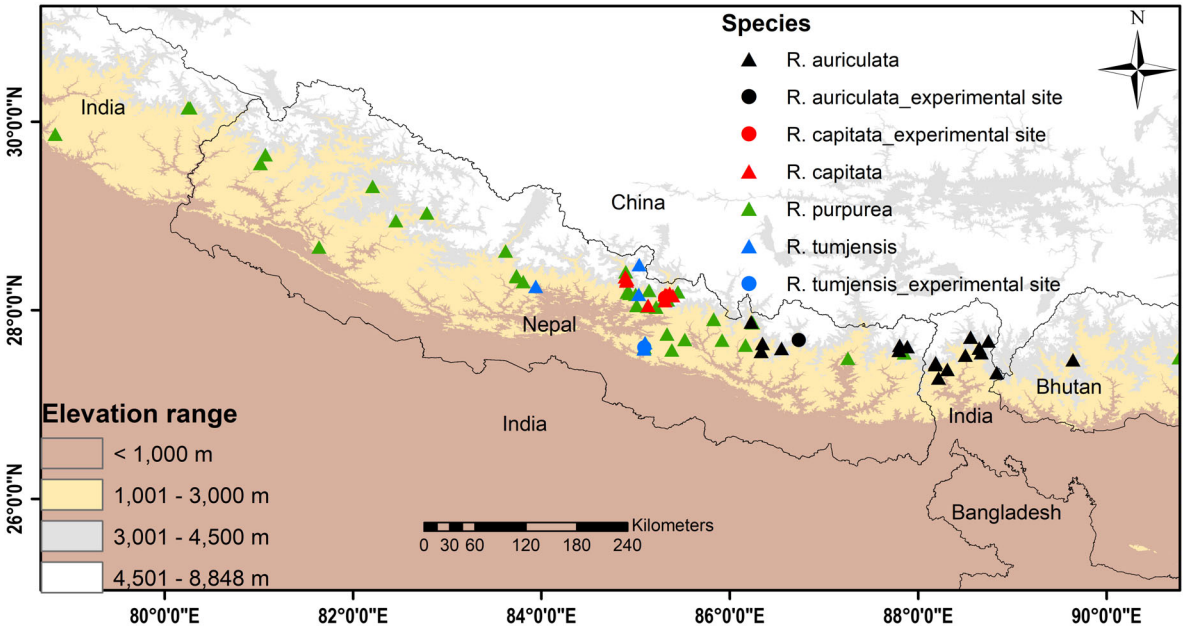


Fig. 1. The entire distribution range of three Himalayan *Roscoea* species (*R. auriculata, R. capitata*, and *R. tumjensis*) and the entire distribution of *R. purpurea*.

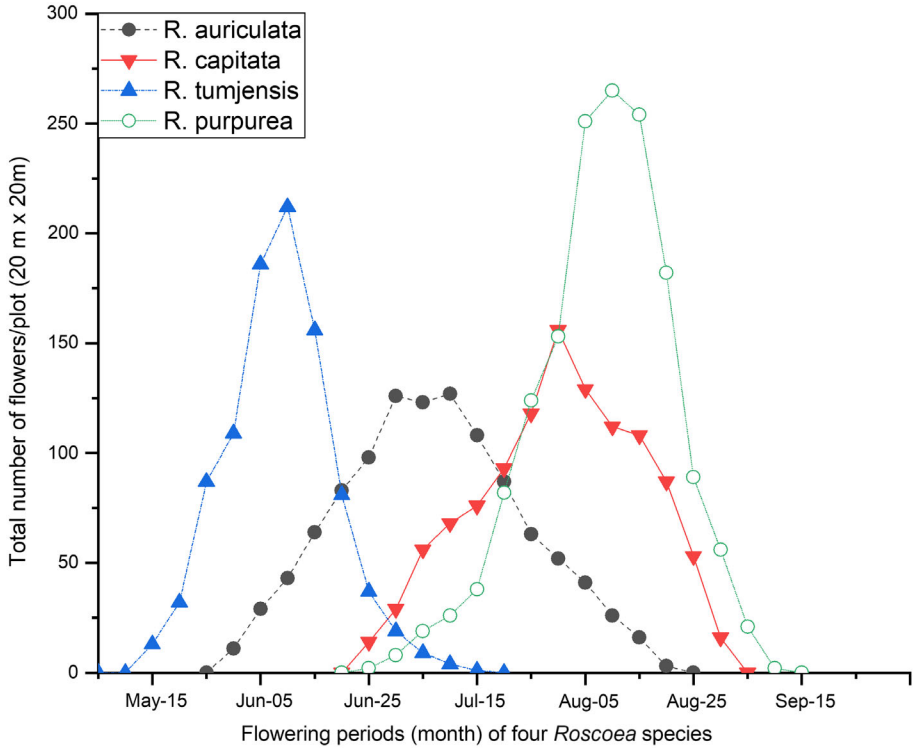


Fig. 2. Flowering phenology of three *Roscoea* species (*R. auriculata, R. capitata*, and *R. tumjensis*) and *R. purpurea*.

Study sites

We observed the natural history of the three *Roscoea* species at six populations across the Nepalese Himalayas, with two populations for each species (Fig. 1; Appendix S1: Table S1). We performed the detailed study on pollinator efficiencies at three populations, one population for each species (Fig. 1; Appendix S1: Table S1). The natural population of *R. auriculata* was studied at Lukla (86°43′59.7″, 27°41′12.8″, 2871 m), eastern Nepal, where it grows in *Rhododendron* forest habitats. This site experiences subalpine climate with cool winter and heavy snowfall from November to February. The population of *R. capitata* was studied at Thulo Bharkhu (28°7′49.3″, 85°18′53.3″, 1887 m), near Dhunche (Rasuwa). The vegetation at this site mainly comprises *Pinus–Alnus* forest. This site experiences temperate climate with warm summer and cool winter. The population of *R. tumjensis* was studied at Daman (27°36′32.8″, 85°5′36.6″, 2334 m), central Nepal. Vegetation at this site is dominated by mixed forests of *Pinus*, *Quercus*, and *Rhododendron*. This site experiences temperate-to-subalpine climate with warm summer and cool winter.

Floral biology

We studied the floral biology of the three *Roscoea* species, from May to August, for five consecutive years (2013–2017) at their respective sites (Fig. 1). We recorded the time of anthesis, anther dehiscence, and stigma receptivity by direct observation. Floral longevity, the time interval from the day of anthesis until wilting of the flower, was also recorded by direct observation. We measured a total of 21 floral traits (Table 1) from the freshly opened flowers of the three *Roscoea* species following the method of Paudel et al. (2015, 2017), with 20 replicates for each species and study year. Floral traits within a species did not differ among the years (one-way ANOVA, $P > 0.05$). Thus, floral traits of a species from all the studied years were pooled together for further analysis. We used a one-way ANOVA followed by Tukey honestly significant difference procedure (HSD) to analyze the difference in floral traits among the three species (Table 1).

The evolution of floral traits in related plant species may be structured by gene flow or common descendent so that populations with geographic proximity will have similar trait values (Pauw et al. 2009, Newman et al. 2014). Thus, the floral traits of the three *Roscoea* species were further analyzed using a Mantel test with 9999 permutations in the *ade4* package of version R3.4.3 (R Core Team 2017). The non-significant or negative correlation between pairwise geographical distance or altitudinal distance and pairwise trait variation signifies that traits values are the outcome of independent evolution rather than common descendent or gene flow across sites (Pauw et al. 2009, Newman et al. 2014, Paudel et al. 2016).

Breeding system

We conducted six types of pollination treatments, to understand the natural breeding system of the three Himalayan *Roscoea* species (detailed experimental procedure in Appendix S1: Method S1). Fruits were individually collected after about 30 d, and the percentage of fruit set and seed number per fruit of each treatment were recorded as a function of the above treatments.

To test the effect of self-crossing vs. outcrossing and to estimate the self-compatibility in the three Himalayan *Roscoea* species, fruit set percentage and seed number per fruit between hand-self-pollinated and hand-cross-pollinated flowers were analyzed using generalized linear models (GLMs) with binomial (for fruit set) and Poisson distribution (for seed set) of errors. To test if fruit and seed set in three Himalayan *Roscoea* species are affected by pollen limitation, differences in fruit set and seed set between natural and supplemental pollination were analyzed using GLMs with the binomial and Poisson distribution of errors, respectively.

Observation of floral visitors

To characterize the pollinator communities of the three Himalayan *Roscoea* species, two observation plots (10 × 10 m) were laid down at the respective sites when plants were densely flowering. The observations were made for five consecutive years (2013–2017) and repeated after the gap of two years (2019). At each plot, we observed all types of floral visitors for a total of 24 h for diurnal visitors and 4 h for nocturnal visitors, so that, in a population, we made a total of 48 h of observation for diurnal visitors and

Table 1. Mean (± standard error) values of 21 floral traits of three Himalayan *Roscoea* species.

Floral traits	*R. auriculata*	*R. capitata*	*R. tumjensis*	*F*	*P*
Advertising traits					
Flower per inflorescence (No.)	4.3 ± 0.26	45.3 ± 2.09	4.9 ± 0.29	*365.561*	0.000
Time of anthesis	07:00–08:00 hours	07:00–08:00 hours	07:00–08:00 hours	2.799	**0.069**
Floral longevity (d)	4.15 ± 0.19	4.65 ± 0.18	4.2 ± 0.22	1.867	**0.164**
Labellum width (mm)	29.3 ± 0.92	19.25 ± 0.41	23.75 ± 0.36	64.82	0.000
Labellum length (mm)	44.05 ± 1.13	33.4 ± 0.67	38.35 ± 0.57	41.074	0.000
Floral diameter (mm)	55.85 ± 1.56	41.1 ± 0.57	43.2 ± 0.5	63.137	0.000
Floral display area (mm^2)	1657.9 ± 96.63	791.3 ± 20.7	1026.4 ± 20.88	59.052	0.000
Lateral petal length (mm)	31.4 ± 0.59	25.45 ± 0.47	31.8 ± 0.59	40.903	0.000
Dorsal petal length (mm)	33.05 ± 0.63	23.45 ± 0.46	22.15 ± 0.44	129.318	0.000
Dorsal petal breadth (mm)	15.4 ± 0.73	6.15 ± 0.19	6.65 ± 0.18	133.037	0.000
Staminodes length (mm)	19.65 ± 0.43	30.15 ± 0.56	33 ± 0.57	175.5	0.000
Staminodes breadth (mm)	7.4 ± 0.18	5.85 ± 0.16	12.15 ± 0.49	105.992	0.000
Mechanical traits					
Corolla tube length (mm)	85.05 ± 2	39.85 ± 0.65	77.2 ± 1.59	251.424	0.000
Nectar level in corolla tube (mm)	73.5 ± 1.92	30 ± 0.75	58.95 ± 1.63	211.422	0.000
Nectar accessible distance (mm)	11.5 ± 0.23	9.85 ± 0.46	18.25 ± 0.37	142.986	0.000
Length of staminal appendages (mm)	6.35 ± 0.18	6.0 ± 0.21	5.40 ± 0.15	6.687	0.002
Functional traits					
Anther length (mm)	7.05 ± 0.21	6.65 ± 0.2	8.55 ± 0.21	22.672	0.000
Ovary length (mm)	19.65 ± 0.64	6.6 ± 0.18	17.35 ± 0.56	188.225	0.000
Anther–stigma distance (mm)	2.05 ± 0.16	2.3 ± 0.1	2.95 ± 0.13	11.133	0.000
Pollen number/flower (No.)	12086.25 ± 651.2	13090.25 ± 680.41	17372 ± 821.03	15.143	0.000
Ovule/flower (No.)	43.35 ± 1.07	35.2 ± 1.38	45.95 ± 2.11	12.492	0.000
Pollen–ovule ratio	281.96 ± 17.54	392.16 ± 33.04	390.09 ± 23.45	6.113	0.004

Notes: Differences in floral traits among the three *Roscoea* species were analyzed by a one-way ANOVA ($N = 20$). All the floral traits, except time of anthesis and floral longevity (shown in boldface), differed at $P < 0.01$, among the three species.

8 h of observation for nocturnal visitors. For diurnal visitors, observations were made continuously for 4 h with three observation periods (7:00–11:00, 11:00–15:00, and 15:00–19:00 hours) per day. For nocturnal visitors, observations were made from 20:00 to 22:00 hours. Foraging behavior (legitimate or illegitimate) of all the visitors was recorded by direct observation. A visit was considered legitimate if a visitor's body had come in contact with the reproductive parts and ultimately transferred pollen grains to the stigma. To estimate the visitation frequency, we counted the number of flowers within the observational plot and total numbers of visit/hour, to a flower, by a visitor type. Visitation frequency of each type of visitor was calculated in terms of the number of visit per flower per hour. To analyze the variation in visitation frequencies of all the floral visitors within a plant species, we used a two-way ANOVA, with floral visitor types and years as fixed factors, followed by Tukey HSD. We also measured the tongue length of the respective floral visitor, and the tongue length compatibility was compared with the corolla tube length. The assessment of the match between the floral traits and pollinator traits provides an important insight in evaluating the hypothesis of pollination syndromes (Anderson et al. 2010).

Pollination efficiency

We calculated the pollination efficiency of a visitor type by counting the number of pollen grains deposited on a virgin stigma during a single foraging bout, following the method of Inouye et al. (1994), Nèeman et al. (2010), and Paudel et al. (2015; detailed experimental procedure in Appendix S1: Method S2). We conducted the experiments, at the same site, for two consecutive years to test if the pollination efficiency of a visitor to a plant species varies by year. We used a two-way ANOVA (with visitor types and years as fixed factors) followed by Tukey HSD to examine the differences in

pollinator importance of the visitor types within a plant species.

Results

Floral biology

Blooming times between *R. tumjensis* (early flowering) and *R. capitata* (late flowering) do not overlap, whereas *R. auriculata* blooms intermediate from May to August, overlapping with the other two species at the beginning and the end of the flowering period, respectively (Fig. 2). Anthesis in the three *Roscoea* species occurred in the early morning (before 08:00 hours), and synchronously anthers became ready to dehisce and stigma became receptive. The flowers were receptive from the day of anthesis until wilting. Thus, all three *Roscoea* species showed blooming patterns consistent with diurnal pollination. All the floral traits, except anthesis time and floral longevity, differed among the three *Roscoea* species (one-way ANOVA, $P < 0.001$; Table 1). The Mantel test indicates a strong correlation of floral traits, except floral longevity (Table 2), either with the altitude or with the geographical position.

Breeding system

Neither bagged emasculated flowers nor bagged control flowers of all three *Roscoea* species set fruit. These results suggest the absence of apomixis and autonomous selfing. We found that natural (control flowers exposed to the pollinators) and artificially pollinated flowers (hand-cross-pollinated, hand-self-pollinated, and supplemented) of the three *Roscoea* species set fruit. Thus, our results suggest that *R. auriculata*, *R. capitata*, and *R. tumjensis* are self-compatible but dependent on pollinators for reproductive success.

Table 2. Relationship of floral traits of three *Roscoea* species with elevation and geographic distance.

	Relation with elevational distance		Relation with geographic distance	
Floral traits	Observed R^2	*P* value	Observed R^2	*P* value
Advertising traits				
Flower per inflorescence (No.)	0.6073	<0.001	0.1896	<0.001
Floral longevity (d)	**−0.0096**	**0.5982**	**−0.0087**	**0.5593**
Labellum width (mm)	0.5947	<0.001	0.4929	<0.001
Labellum length (mm)	0.4807	<0.001	0.3959	<0.001
Floral diameter (mm)	0.5268	<0.001	0.5954	<0.001
Floral display area (mm^2)	0.5496	<0.001	0.5546	<0.001
Lateral petal length (mm)	0.3406	<0.001	0.1227	<0.001
Dorsal petal length (mm)	0.5973	<0.001	0.7759	<0.001
Dorsal petal breadth (mm)	0.6349	<0.001	0.7593	<0.001
Staminodes length (mm)	0.6161	<0.001	0.8295	<0.001
Staminodes breadth (mm)	0.1042	0.003	0.1857	<0.001
Mechanical traits				
Corolla tube length (mm)	0.7118	<0.001	0.3453	<0.001
Nectar level in corolla tube (mm)	0.7817	<0.001	0.4858	<0.001
Nectar accessible distance (mm)	0.1177	0.0023	0.2252	0.0031
Length of staminal appendages (mm)	0.0589	0.0416	0.8757	0.0127
Functional traits				
Anther length (mm)	0.3041	0.0146	0.0701	0.0206
Ovary length (mm)	0.6946	<0.001	0.3438	<0.001
Anther–stigma distance (mm)	0.054	0.045	0.1453	<0.001
Pollen number/flower (No.)	0.1694	0.0386	0.1004	0.0112
Ovule number/flower (No.)	0.0745	0.0144	0.1334	0.0294
Pollen–ovule ratio	0.0787	0.0167	0.0553	0.0076

Notes: The relationships were non-significant only for floral longevity (shown in boldface), while for the rest of the traits, the relationships were significant, indicating that the evolution of the floral traits in the three Himalayan *Roscoea* species (except floral longevity) is primarily related to several abiotic factors including altitude and geographic proximity among the study sites.

The percentage of fruit set in hand-self-pollinated and hand-cross-pollinated flowers of the three *Roscoea* species did not differ between the pollination treatments, years, and year–treatment interactions ($P > 0.05$; Appendix S1: Table S2). Similarly, seed number per fruit, in all the three *Roscoea* species, did not differ for pollination treatment, years, and year–treatment interaction in hand-self-pollinated and hand-cross-pollinated flowers ($P > 0.05$; Appendix S1: Table S2). These results further support that the three *Roscoea* species are fully self-compatible (Appendix S1: Fig. S1).

The fruit set resulting from either natural or supplementary pollination in the three *Roscoea* species did not differ between years ($P > 0.05$; Appendix S1: Table S3). However, the two pollination treatments resulted in different fruit set ($P < 0.001$; Appendix S1: Table S3). We found that in all the three *Roscoea* species, the percentage of fruit set in naturally pollinated flowers was substantially lower than that of fruit set in supplemental pollinated flowers (Appendix S1: Fig. S2). Similarly, the seed number per fruit did not vary with the year ($P > 0.05$; Appendix S1: Table S3) but differed between the treatments (natural vs. supplementary pollination; $P < 0.05$; Appendix S1: Table S3). In all the three *Roscoea* species, the seed number per fruit in supplemented pollination is substantially higher than in natural pollination. These results indicate that natural fruit and seed set in all three species are affected by pollen limitation (Appendix S1: Fig. S2).

Observation of floral visitors

In our observations, we did not find any spatial variation in the pollinator composition of the three *Roscoea* species. Our five years of field observations during the peak blooming period of the three *Roscoea* species across their respective distribution sites in the Himalayas (Fig. 1) demonstrate that two *Bombus* species (*B. haemorrhoidalis* and *B. tanguticus*) and a butterfly (*Pachliopta aristolochiae*) actively visited the flowers of *R. auriculata* (Fig. 3d–f). The flowers of *R. capitata* were visited by a bumblebee (*B. haemorrhoidalis*) and a moth (*Macroglossum nycteris*; Fig. 3g, h). Similarly, two bumblebee species (*Bombus flavescens* and *B. haemorrhoidalis*) and a moth (*M. nycteris*) were found actively visiting the flowers of *R. tumjensis* (Fig. 3h–j). However, in our observation, we did not find any nocturnal visitors to forage on the flowers of the three *Roscoea* species.

We found that the tongue length of the floral visitors did not match with the corolla tube length of the three *Roscoea* spp. The average tongue length of all the visitors was shorter than the average corolla tube length of the three *Roscoea* spp. (Appendix S1: Fig. S3). The tongue length of a moth (*M. nycteris*) was long enough to reach the nectar, while the tongue length of *Bombus* species did not seem to be long enough to reach the nectar (Table 1; Appendix S1: Fig. S3).

Visitation frequency

Visitation frequencies of floral visitors of *R. auriculata* (*B. haemorrhoidalis*, *B. tanguticus*, and *P. aristolochiae*) did not differ between the years but differed for the visitor species ($P < 0.005$; Appendix S1: Table S4). Visitations by the butterfly species were very low, compared to the two bumblebee species (Fig. 4a), while the visitation frequency between the two bumblebee species did not differ (Tukey HSD test, $P > 0.05$). Visitation frequencies of the floral visitors of *R. capitata* (*B. haemorrhoidalis* and *M. nycteris*) did not differ between the years ($P > 0.05$; Appendix S1: Table S5) but differed between the visitors ($P < 0.001$; Appendix S1: Table S5), where the visitation frequency of *M. nycteris* was higher than that of *B. haemorrhoidalis* (Fig. 4b). The visitation frequencies of the floral visitors of *R. tumjensis* did not differ between the years ($P > 0.05$; Appendix S1: Table S6) but differed among the three pollinator species (*B. flavescens*, *B. haemorrhoidalis*, and *M. nycteris*, $P < 0.001$; Appendix S1: Table S6). However, visitation frequencies between two bumblebee species (*B. flavescens* and *B. haemorrhoidalis*) did not differ (post hoc test, Tukey HSD, $P > 0.05$). Among the three floral visitors of *R. tumjensis*, *B. flavescens* had least visitation frequency, while *M. nycteris* had the highest visitation frequency (Fig. 4c). Among all the floral visitors of the three *Roscoea* species, visitation frequency of the butterfly species (*P. aristolochiae*) to the flowers of *R. auriculata* was least, while visitation frequency of the moth species (*M. nycteris*) toward the flowers of *R. tumjensis* was highest (Fig. 4a–c).



Fig. 3. Three Himalayan *Roscoea* species and their floral visitors. (a), (b), and (c) respectively represent the flowering individuals of *R. auriculata*, *R. capitata*, and *R. tumjensis* at their natural habitats. (d–f) Floral visitors of *R. auriculata;* (d) a butterfly (*Pachliopta aristolochiae*), (e) *Bombus haemorrhoidalis*, and (f) *Bombus tanguticus*. (g, h) Floral visitors of *R. capitata*; (g) *B. haemorrhoidalis* and (h) a moth (*Macroglossum nycteris*). (h–j) Floral visitors of *R. tumjensis*; (h) *M. nycteris*, (i) *Bombus flavescens*, and (j) *B. haemorrhoidalis. Bombus haemorrhoidalis* (e, g, and j) is the common visitor and most effective pollinator of the three *Roscoea* species. A moth (*M. nycteris;* h) is the common visitor (nectar robber) of *R. capitata* and *R. tumjensis*.

Pollination efficiency

The number of pollen grains deposited on a virgin stigma of *R. auriculata* differed among the three major visitors (*B. haemorrhoidalis*, *B. tanguticus*, and *P. aristolochiae*, $P < 0.001$; Appendix S1: Table S4) but did not vary with the year ($P > 0.05$; Appendix S1: Table S4). We found *B. haemorrhoidalis* as the most efficient pollinator as it deposited the highest number of pollen grains on virgin stigmas. *Bombus tanguticus*, although this bumblebee species mainly forages the flower through the edge of the corolla tube for nectar robbing and also contributes to pollen deposition during its occasional attempts to sip nectar through the mouth of the corolla tube, while the butterfly did not deposit any pollen grains on *R. auriculata* (Fig. 4d).

As in *R. auriculata*, the number of pollen grains deposited on *R. capitata* and *R. tumjensis* stigmata varied with the floral visitors ($P < 0.001$; Appendix S1: Tables S5, S6) but did not differ between the years ($P > 0.05$; Appendix S1: Tables S5, S6). For *R. capitata*, pollen deposition by the bumblebee, *B. haemorrhoidalis,* was far greater than that by a moth (*M. nycteris*; Fig. 4e). Among the three visitors of *R. tumjensis*, *B. haemorrhoidalis* deposited the highest number of pollen grains followed by *B. flavescens* (the number of pollen grains deposited by two bumblebees also differed, post hoc Tukey HSD, $P < 0.05$), while *M. nycteris* deposited extremely low numbers of pollen grains during individual foraging bouts (Fig. 4f). Among all the floral visitors of the three *Roscoea* species, *B. haemorrhoidalis* deposited the highest number of pollen grains on the flowers of *R. tumjensis* followed by *B. haemorrhoidalis* on the flowers of *R. auriculata*. The moth species (*M. nycteris*) deposited the least numbers of pollen grains in the flowers of *R. capitata*, while the butterfly (*P. aristolochiae*) did not deposit any pollen grains to the flowers of *R. auriculata* (Fig. 4d–f). Our data revealed that in all the three *Roscoea* species, *Bombus* species contribute more than 90% of the pollination service. This suggests that the three *Roscoea* species primarily rely on *Bombus* species for effective pollination, with *B. haemorrhoidalis* as the common and most efficient pollinator.

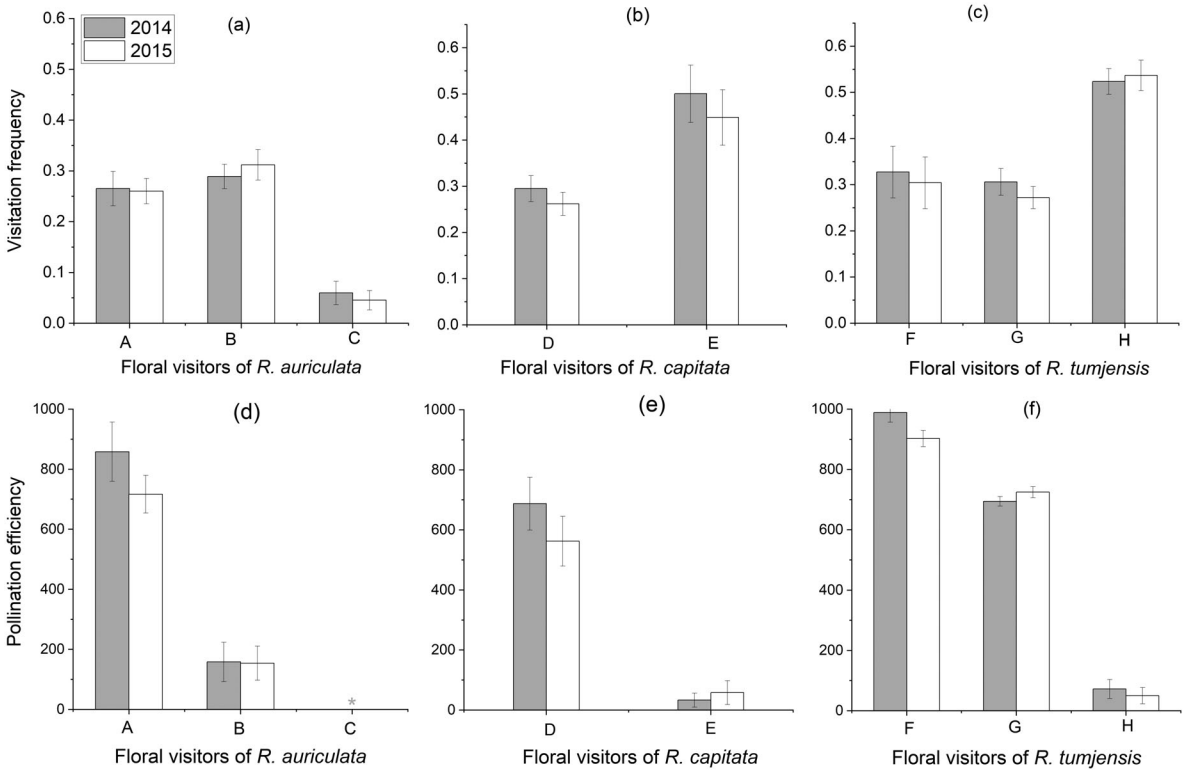


Fig. 4. Visitation frequencies and pollinator importance of floral visitors of three Himalayan *Roscoea* species. (a–c) Variation in visitation frequencies (No. of visits/flower/hour) and (d–e) variation in pollinator importance (no. of pollen grains deposited on a virgin stigma during a single visit of a visitor), among the floral visitors of the three Himalayan *Roscoea* species. Pollinator importance of a pollinator estimates the expected number of pollen grains a visitor would deposit on a virgin stigma during its single foraging bout and is considered a proxy to define pollinator specialization. (a–c) Visitation frequencies of floral visitors of *Roscoea auriculata*, *Roscoea capitata*, and *Roscoea tumjensis*, respectively. (d–f) Pollinator importance of floral visitors of *R. auriculata*, *R. capitata*, and *R. tumjensis*, respectively. An asterisk mark in the figure indicates zero value. Symbols (A–H) in *x*-axis represent the floral visitors of three *Roscoea* spp. (A–C) Floral visitors of *R. auriculata*. (A) *Bombus haemorrhoidalis*; (B) *Bombus tanguticus*; and (C) *Pachliopta aristolochiae*. (D and E) Floral visitors of *R. capitata*. (D) *B. haemorrhoidalis*; (E) *Macroglossum nycteris*. (F–H) Floral visitors of *R. tumjensis*. (F) *B. haemorrhoidalis*; (G) *Bombus flavescens*; and (H) *M. nycteris*.

Discussion

Pollination syndromes and generalization

Roscoea species show typical long-tongued fly pollination syndrome such as zygomorphic flowers with wide labellum as a landing platform for its pollinators, long corolla tube with nectar, absence of discernible fragrance, elongated anther, and stigma situated away from the source of nectar (Zhang et al. 2010, Fan and Li 2012, Paudel et al. 2015, 2017). Indeed, evidences such as historical observations (Fletcher and Son 1931, Dierl 1968) and recent findings (Paudel et al. 2015, 2016) from *R. purpurea* coupled with phylogenetic data (Ngamriabsakul et al. 2000, Zhao et al. 2016) suggest that a long-tongued fly (*P. longirostris*) could be the principal pollinator of *Roscoea* species, at least in the Nepalese Himalayas. This fly is the only species of long-tongued flies existing in the Himalayas (Goldblatt and Manning 2000) and is an exclusive specialized pollinator of *R. purpurea*, one of the widespread Himalayan *Roscoea* species (Cowley 2007, Paudel et al. 2015, Zhao et al.

2016). Thus, we anticipated that the three Himalayan *Roscoea* species (*R. auriculata*, *R. capitata*, and *R. tumjensis*), having overlapped distribution and/or flowering phenology with *R. purpurea*, also exhibit specialized pollination mutualism with long-tongued flies, at least within the overlapped populations. However, in our extensive observation during the entire blooming period (Fig. 2), over the last five years (2013–2017), we never witnessed the fly (*P. longirostris*) visiting the flowers of these three *Roscoea* species. Consistent with our current finding, another Himalayan *Roscoea* species (*R. alpina*; Paudel et al. 2017) and all the currently studied North-Indo-Chinese *Roscoea* spp. lack long-tongued flies as the pollinators (Zhang and Li 2008, Zhang et al. 2010, Fan and Li 2012), despite expressing the long-tongued fly pollination syndrome.

In accordance with the apparent (long-tongue) pollination syndrome, other long-tongued insects such as butterflies and moths visit the flowers of the three *Roscoea* species in this study. However, our quantifications of pollen deposition and relative pollinator importance suggest that these two groups of insects (butterfly and moth) function as nectar robbers rather than pollinators. They make no or very negligible contribution to the pollination of the three Himalayan *Roscoea* species. On the other hand, *Bombus* species, despite an apparent mismatch between the corolla tube length and tongue length (Appendix S1: Fig. S3), offer more than 90% of the pollination services to the three *Roscoea* species. Moreover, our observations indicate that almost all the foraging bouts of *Bombus* species occurring through the entrance of the corolla tube (i.e., except the visits of *B. tanguticus* occurring through the edge of the corolla tube) comprised successful pollination events. Thus, based on these results, we conclude that the three *Roscoea* species, despite apparent long-tongued fly pollination syndromes, exhibit generalized pollination system and primarily rely on *Bombus* species for successful pollination. Our results reveal that the three *Roscoea* species, despite the fact that they are not geographically overlapping (Fig. 1) and their peak blooming times are clearly separated (Fig. 2), are effectively pollinated by *B. haemorrhoidalis* across all the sites and years. Hence, breeding system and pollinator generalization in the three Himalayan *Roscoea* species are neither affected by geographical disjunction nor by phenological isolation. This result, thus, reiterates that suites of floral traits matching apparent pollination syndromes, in our case long-tongued fly pollination syndromes of *Roscoea* species as discussed above, are not always reliable to predict the effective pollinator of a plant species, and is consistent with the previous findings (Paudel et al. 2017, Kuriakose et al. 2018, 2019).

The implication of the absence of original pollinator

We found that the peak blooming period of *R. auriculata* and *R. tumjensis* does not overlap with the winged stage of *P. longirostris*. *Philoliche longirostris* attains its winged (active) stage from the end of July through August, but peak blooming period of *R. auriculata* and *R. tumjensis* occurs during May–June. Thus, the temporal mismatch between the blooming period of plant and winged stage of a long-tongued fly could be the major factor that has forced *R. auriculata* and *R. tumjensis* to rely on an alternative pollinator community (to rely on bumblebees rather than long-tongued flies) for effective pollination. On the other hand, although the blooming period of *R. capitata* overlaps with the active (winged) stage of *P. longirostris*, the fly never visits the flowers of *R. capitata*. Similarly, bumblebees never visit the flowers of *R. purpurea*. It is reasonable to hypothesize that to avoid interspecies reproductive interference associated with pollinators competition and to conserve species integrity, *R. purpurea* and *R. capitata,* at the sympatric populations, may have developed the suites of floral traits that would enable these two *Roscoea* species to attract different pollinators. The current findings suggest variation in a number of ecological factors driving the emergence of new pollination systems, in the closely related plant species. Indeed, temporal and spatial mismatch and pressure from competitive plants/pollinators could disturb the mutualism between plants and their original pollinators (Miller-struttmann et al. 2015), which may lead to the evolution of different pollination systems, under different ecological conditions (Hegland et al. 2009).

According to Bond (1994), when original pollinators either become unavailable or somehow lose their attraction to the flowers of a plant

species, the original mutualism may break down and unspecialized pollinator may occupy the vacant niche (Schwartz-Tzachor et al. 2006). Our results suggest that bumblebees may have occupied such a vacant niche in the absence of a specialized pollinator in the three Himalayan *Roscoea* species. Consistent with our result, *Roscoea debilis* also exhibits mutualism with a yellow carpenter bee (*Xylocopa confusa*) in the absence of its specialized pollinator (Fan and Li 2012) in the Chinese Himalaya. The shift of mutualism in the absence of the principal pollinator also occurs in a long-corolla-tubed *Pedicularis* of the Chinese Himalayas (Huang and Fenster 2007) and in a tropical plant *Tacca chantrieri* (Zhang et al. 2005).

The current result indicates that bumblebees are the generalized pollinators of the three Himalayan *Roscoea* species (the narrowly distributed and recently diverged species), while they do not visit *R. alpina* and *R. purpurea* (the ancestral *Roscoea* species with widespread distribution; Cowley 2007, Paudel et al. 2015, 2017, Zhao et al. 2016). The phylogenetic data suggest that *R. purpurea* seems to be a sister to *R. auriculata*, a lineage that underwent localized diversification (Ngamriabsakul et al. 2000, Zhao et al. 2016). Thus, based on the phylogeny and the current finding, we suggest that the absence of a long-tongued fly as the pollinator led to the evolution of *Bombus* pollination in the three Himalayan *Roscoea* species. The retention of long corolla tube in the Himalayan *Roscoea* species represents the holdover traits from its history of long-tongued fly pollination. Consistent with our findings, large cardamom (Zingiberaceae) in the central Himalayas, India, exhibit *Bombus* pollination system (Sinu et al. 2011). A recent finding suggests that the evolution of different pollination system in two sympatric *Roscoea* species plays a key role in reproductive isolation and to conserve species integrity (Paudel et al. 2018). Thus, the evolution of *Bombus* pollination in the three Himalayan *Roscoea* species could be a major driver of speciation and diversification of Himalayan *Roscoea* species. Further phylogenetic work including all *Roscoea* species with better resolution will shed light on speciation and diversification of genus *Roscoea* in the Himalayas.

Potential drivers of natural selection

Closely related plant species that share common functional groups of pollinators are thought to have convergent floral traits, largely driven by pollinator-mediated selection (Schemske 1981, Patterson and Givnish 2004, Anderson and Johnson 2009, Kuriakose et al. 2019). Reversely, differences in floral trait expression among congeners are thought to have been the consequences of adaptation to different pollinators (Schemske and Bradshaw 1999, Muchhala 2003). Contrasting with these views, our study system shows divergence in floral traits expression; that is, the floral traits of the three *Roscoea* species are clearly divergent among the species, but they primarily rely on the morphologically mismatched *Bombus* species for the effective and successful pollination. The finding of a generalized pollination system in the three *Roscoea* species does not corroborate their floral morphology that indicates specialized pollination mutualism with long-tongued insects. It, thus, suggests that the floral features of the three *Roscoea* species, which seem to be the evolutionary outcomes of selection by long-tongued insects, may, in fact, reflect the outcomes of diverse drivers of selection. Our study identified butterflies, moths, and even some *Bombus* spp. as functional nectar robbers of the three *Roscoea* species and thus potential drivers of selection on floral traits. Similarly, we observed that the flowers of the three *Roscoea* species are often damaged by herbivory, especially by beetles. Thus, herbivory can be another potential agent of natural selection on the floral traits of these alpine gingers. It is also likely that non-pollinator agents such as biotic and abiotic variables of community composition could play a major role in the evolution of floral traits in these alpine gingers. The positive correlation of the floral traits either with the altitude or with the abiotic environment derived from latitude and longitude (as revealed by the Mantel test; Table 2) and the incongruent relationship between the floral traits and effective pollinator (bumblebees) support a hypothesis of non-pollinator driven floral evolution in the three *Roscoea* species. Another example from this genus further supports this view. *Roscoea alpina* expresses the longest corolla tubes of all the Himalayan *Roscoea* species but is autonomously selfing (Paudel et al. 2017). However, further natural selection studies with manipulating the pollinators/non-pollinators are

required to explore the key drivers of divergent floral morphologies among the Himalayan *Roscoea* species.

Conclusions

The three geographically and phenologically isolated Himalayan *Roscoea* species, with long-tongued insect pollination syndrome, exhibit generalized pollination system and employ *B. haemorrhoidalis* as the common and most effective pollinator. While our results reiterate an increasing number of recent studies that pollination syndromes are a rather weak predictive concept and thus often fail to align a role of contemporary pollinators in shaping floral traits, they also suggest the role of different non-pollinator agents in the evolution/diversification of flower gestalt. The evolution of *Bombus* pollination system in these alpine gingers suggests how the mismatch between pollinator emerging time and flowering time causes shifting of reproductive strategy among closely related plant species. The evolution of different pollination systems, among the congeners, in sympatric populations ultimately seems to play a key role for the speciation and diversification of the genus *Roscoea* in the Himalayas.

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Literature Cited

Agrawal, A. A., and M. Fishbein. 2006. Plant defense syndromes. Ecology 87:132–149.

Anderson, B., and S. D. Johnson. 2009. Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants. New Phytologist 182:533–540.

Anderson, B., J. S. Terblanche, and A. G. Ellis. 2010. Predictable patterns of trait mismatches between interacting plants and insects. BMC Evolutionary Biology 10:204.

Armbruster, W. S., Y.-B. Gong, and S.-Q. Huang. 2011. Are pollination "syndromes" predictive? Asian *Dalechampia* fit neotropical models. American Naturalist 178:135–143.

Bond, W. J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society B: Biological Sciences 344:83–90.

Cowley, E. J. 1982. A revision of *Roscoea* (Zingiberaceae). Kew Bulletin 36:747–777.

Cowley, E. J. 2007. The genus Roscoea. Royal Botanic Garden, Kew, UK.

Danieli-Silva, A., J. M. T. de Souza, A. J. Donatti, R. P. Campos, J. Vicente-Silva, L. Freitas, and I. G. Varassin. 2012. Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? Oikos 121:35–43.

Darwin, C. 1862. On the various contrivances by which British and foreign orchids are fertilized by insects. John Murray, London, UK.

Dierl, W. 1968. Zur Nahrungsaufnahme von *Corizoneura longirostris* (Hardwicke) (Diptera: Tabanidae). Khumbu Himal 3:76–81.

Faegri, K., and L. van der Pijl. 1979. Principles of pollination ecology. Pergamon Press, Oxford, UK.

Fan, Y. L., and Q.-J. Li. 2012. Stigmatic fluid aids self-pollination in *Roscoea debilis* (Zingiberaceae): a new delayed selfing mechanism. Annals of Botany 110:969–975.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35:375–403.

Fleming, T., C. Sahley, J. Holland, J. Nason, and J. Hamrick. 2001. Sonoran Desert columnar cacti and the evolution of generalised pollination systems. Ecological Monographs 71:511–530.

Fletcher, T., and S. Son. 1931. A veterinary entomology for India, part XIV. Journal of Veterinary Science and Animal Husbandry 1:192–199.

Fumero-Caban, J., and E. Melendez-Ackerman. 2007. Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). American Journal of Botany 94:419–424.

Goldblatt, P., and J. C. Manning. 2000. The long-proboscid fly pollination system in Southern Africa. Annals of the Missouri Botanical Garden 87:146–170.

Gomez, J., and R. Zamora. 1999. Generalization vs. specialization in the pollination system of *Hormatophylla spinosa* (Cruciferae). Ecology 80:796–805.

Grant, V. 1992. Floral isolation between ornithophilous and sphingophilous species of *Ipomopsis* and *Aquilegia*. Proceedings of the National Academy of Sciences USA 89:11828–11831.

Grant, V., and K. A. Grant. 1965. Flower pollination in the Phlox family. Columbia University Press, New York, New York, USA.

Hargreaves, A. L., S. D. Johnson, and E. Nol. 2004. Do floral syndromes predict specialization in plant pollination systems? An experimental test in an "ornithophilous" African *Protea*. Oecologia 140:295–301.

Hegland, S. J., A. Nielsen, A. Lázaro, A. L. Bjerknesand, and Ø. Totland. 2009. How does climate warming affect plant–pollinator interactions? Ecology Letters 12:184–195.

Herrera, C. M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos 50:79–90.

Hingston, A., and P. McQuillan. 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania? Austral Ecology 25:600–609.

Huang, S., and C. B. Fenster. 2007. Absence of long-proboscid pollinators for long corolla-tubed Himalayan *Pedicularis* species: implications for evolution of corolla length. International Journal of Plant Sciences 168:325–331.

Inouye, D., D. Gill, M. Dudash, and C. Fenster. 1994. A model of lexicon for pollen fate. American Journal of Botany 81:1517–1530.

Johnson, K. A. 2013. Are there pollination syndromes in the Australian epacrids (Ericaceae: Styphelioideae)? A novel statistical method to identify key floral traits per syndrome Annals of Botany 112:141–149.

Johnson, S. D., and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. Trends in Ecology & Evolution 15:140–143.

Karron, J. D., C. T. Ivey, R. J. Mitchell, M. R. Whitehead, R. Peakall, and A. L. Case. 2012. New perspectives on the evolution of plant mating systems. Annals of Botany 109:493–503.

Kuriakose, G., P. Sinu, and K. Shivanna. 2018. Ant pollination of *Syzygium occidentale*, an endemic tree species of tropical rain forests of the Western Ghats, India. Arthropod-Plant Interactions 12:647–655.

Kuriakose, G., P. Sinu, and K. Shivanna. 2019. Floral traits predict pollination syndrome in *Syzygium* species: a study on four endemic species of the Western Ghats, India. Australian Journal of Botany 66:575–582.

Li, J. K., and S. Q. Huang. 2009. Effective pollinators of Asian sacred lotus (*Nelumbo nucifera*): Contemporary pollinators may not reflect the historical pollination syndrome. Annals of Botany 104:845–851.

Liu, C.-Q., and S.-Q. Huang. 2013. Floral divergence, pollinator partitioning and the spatiotemporal pattern of plant–pollinator interactions in three sympatric *Adenophora* species. Oecologia 173:1411–1423.

Mayfield, M., N. Waser, and M. Price. 2001. Exploring the "Most Effective Pollinator Principle" with complex flowers: bumblebees and *Ipomopsis aggregata*. Annals of Botany 88:591–596.

Miller-struttmann, N. E., J. C. Geib, J. D. Franklin, P. G. Kevan, R. M. Holdo, D. Ebert-may, A. M. Lynn, J. A. Kettenbach, E. Hedrick, and C. Galen. 2015. Functional mismatch in a bumble bee pollination mutualism under climate change. Science 349:1541–1544.

Muchhala, N. 2003. Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). Oecologia 134:373–380.

Newman, E., J. Manning, and B. Anderson. 2014. Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. Annals of Botany 113:373–384.

Ngamriabsakul, C., M. F. Newman, and Q. C. B. Cronk. 2000. Phylogeny and disjunction in *Roscoea* (Zingiberaceae). Edinburgh Journal of Botany 57:39–61.

Nèeman, G., A. Jürgens, L. Newstrom-Lloyd, S. G. Potts, and A. Dafni. 2010. A framework for comparing pollinator performance: effectiveness and efficiency. Biological Reviews of the Cambridge Philosophical Society 85:435–451.

Ollerton, J., R. Alarcon, N. M. Waser, M. V. Price, S. Watts, L. Cranmer, A. Hingston, C. I. Peter, and J. Rotenberry. 2009. A global test of the pollination syndrome hypothesis. Annals of Botany 103:1471–1480.

Parachnowitsch, A. L., and A. Kessler. 2010. Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. New Phytologist 188:393–402.

Patterson, T. B., and T. J. Givnish. 2004. Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): evidence from a cpDNA phylogeny. New Phytologist 161:253–264.

Paudel, B. R., M. Burd, M. Shrestha, A. G. Dyer, and Q.-J. Li. 2018. Reproductive isolation in alpine gingers: How do coexisting *Roscoea* (*R. purpurea* and *R. tumjensis*) conserve species integrity? Evolution 72:1840–1850.

Paudel, B. R., M. Shrestha, M. Burd, S. Adhikari, Y. Sun, and Q.-J. Li. 2016. Coevolutionary elaboration of pollination-related traits in an alpine ginger (*Roscoea purpurea*) and a tabanid fly in the Nepalese Himalayas. New Phytologist 211:1402–1411.

Paudel, B. R., M. Shrestha, A. G. Dyer, and Q.-J. Li. 2017. Ginger and the beetle: evidence of primitive pollination system in a Himalayan endemic alpine ginger (*Roscoea alpina*, Zingiberaceae). PLOS ONE 12:e0180460.

Paudel, B. R., M. Shrestha, A. G. Dyer, X. Zhu, A. Abdusalam, and Q.-J. Li. 2015. Out of Africa: evidence of the obligate mutualism between long corolla tubed plant and long tongued fly in the Himalayas. Ecology and Evolution 5:5240–5251.

Pauw, A. 2006. Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). American Journal of Botany 93:917–926.

Pauw, A., J. Stofberg, and R. J. Waterman. 2009. Flies and flowers in Darwin's race. Evolution 63:268–279.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters 6:265–272.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, R. J., M. J. Westbrook, A. S. Rohde, J. M. Cridland, C. B. Fenster, and M. R. Dudash. 2009. Pollinator specialization and pollination syndromes of three related North American *Silene*. Ecology 90:2077–2087.

Rosas-Guerrero, V., R. Aguilar, S. Martén-Rodríguez, L. Ashworth, M. Lopezaraiza-Mikel, J. M. Bastida, and M. Quesada. 2014. A quantitative review of pollination syndromes: Do floral traits predict effective pollinators? Ecology Letters 17:388–400.

Schemske, D. W. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. Ecology 62:946–954.

Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proceedings of the National Academy of Sciences USA 96:11910–11915.

Schwartz-Tzachor, R., A. Dafni, S. G. Potts, and D. Eisikowitch. 2006. An ancient pollinator of a contemporary plant (*Cyclamen persicum*): when pollination syndromes break down. Flora: Morphology, Distribution, Functional Ecology of Plants 201:370–373.

Sinu, P., G. Kuriakose, and K. Shivanna. 2011. Is the bumblebee (*Bombus haemorrhoidalis*) the only pollinator of large cardamom in central Himalayas, India? Apidologie 42:690–695.

Sinu, P., and K. Shivanna. 2007*a*. Pollination biology of large cardamom (*Amomum subulatum*). Current Science 93:548–552.

Sinu, P., and K. Shivanna. 2007*b*. Pollination ecology of cardamom (*Elettaria cardamomum*) in the Western Ghats, India. Journal of Tropical Ecology 23:493–496.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in Angiosperms, I: pollination mechanisms. Annual Review of Ecology and Systematics 1:307–326.

Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043–1060.

Zhang, L., S. Barrett, J. Gao, J. Chen, W. Cole, Y. Liu, Z. Bai, and Q.-J. Li. 2005. Predicting mating pattern from pollination syndromes: the case of "sapromyiophily" in *Tacca chantrieri* (Taccaceae). American Journal of Botany 92:517–524.

Zhang, Z.-Q., W. J. Kress, W.-J. Xie, P.-Y. Ren, J.-Y. Gao, and Q.-J. Li. 2010. Reproductive biology of two Himalayan alpine gingers (*Roscoea* spp., Zingiberaceae) in China: pollination syndrome and compensatory floral mechanisms. Plant Biology 13:582–589.

Zhang, Z.-Q., and Q.-J. Li. 2008. Autonomous selfing provides reproductive assurance in an alpine ginger *Roscoea schneideriana* (Zingiberaceae). Annals of Botany 102:531–538.

Zhao, J. L., Y. M. Xia, C. H. Cannon, W. J. Kress, and Q. J. Li. 2016. Evolutionary diversification of alpine ginger reflects the early uplift of the Himalayan-Tibetan Plateau and rapid extrusion of Indochina. Gondwana Research 32:232–241.

Supporting Information

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