

Diurnal variation in plant-pollinator interactions in Bumblebee-pollinated *Impatiens oxyanthera* at two altitudes

Chunmei He

hechunmei_06@163.com

China West Normal University <https://orcid.org/0009-0005-9942-8772>

Li Wang

China West Normal University

Lan Yang

China West Normal University

Qiong Wang

China West Normal University <https://orcid.org/0000-0002-3465-4657>

Research Article

Keywords: *Impatiens oxyanthera*, plant-pollinator interaction, plant reproductive success, altitude, daily variation

Posted Date: August 25th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7301999/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Spatio-temporal matching between plants and pollinators is critical for their interactions, yet small-scale patterns are understudied. We explored these using the endemic *Impatiens oxyanthera* (Balsaminaceae) and its bumblebee pollinators, comparing diurnal variations in floral traits, pollinator size, visit frequency, and reproductive success across two altitudes. High-altitude flowers exhibited greater corolla tube temperature gradients (warmer mornings, cooler afternoons), more nectar, and were visited more frequently by longer-tongued bumblebees than low-altitude counterparts. Morning size matching (lower height differences between corolla tubes and pollinator bodies) was better at high altitudes, alongside more ovules and seeds. Diurnally, corolla temperature gradients peaked at 8:00, with stable nectar volume and sugar composition (except sucrose) throughout the day. High-altitude flowers showed stronger diurnal shifts in corolla-pollinator height matching (better in mornings) than low-altitude ones. At high altitudes, bumblebee visits to female-phase flowers were lowest at 8:00 but increased by 10:00 with rising temperatures. Pollen deposition peaked at 18:00 (with more unfertilized ovules) and reached a trough at 16:00 or 14:00 (fewer unfertilized ovules) across altitudes. Redundancy analysis highlighted corolla temperature and pollination frequency as key drivers of seed number and unfertilized ovules, particularly at high altitudes. Altitude and time significantly influenced these interactions, with more pronounced diurnal variations in interactions and reproductive success at high altitudes, suggesting plant sexual reproduction adapts to temporal environmental heterogeneity.

1 Introduction

Plant sexual reproduction is dependent on specific pollinators due to sessile growth habit (Sun et al. 2014). Of the 350,000 extant angiosperm species, about 78% of temperate species and 94% of tropical species require animal pollination, with the most common and efficient pollinating insects being bees (Ollerton et al. 2011; Tong et al. 2023). Whereas, reward characteristics (heat, nectar quantity and quality) and mechanical fit characteristics (corolla tube depth or nectar spur length) among plant floral characteristics are the key factors influencing pollinator visiting behaviour and pollination efficiency (Hoover et al. 2012; Mu et al. 2015), as they determine foraging efficiency of pollinators and pollination success of plants (Meng et al. 2007; Zhang et al. 2004). Thus, investigating plant reward characteristics and plant-pollinator size matching is crucial for uncovering the mechanisms of plant-pollinator interactions and their reproductive ecological consequences (Ballarin et al. 2022; Ferrero et al. 2011; Wang 2013).

Drivers of the temporal dynamics of plant-pollinator interactions include species internal rhythms and external environmental conditions (Schwarz et al. 2021). It has been shown that the tentacles of *Manduca sexta* exhibit a 24-hour rhythm in response to *Petunia hybrida* under continuous light and dark conditions (Fenske et al. 2018), suggesting that the biological clocks are an important internal driver of plant-pollinator interactions. In addition, environmental factors can further influence plant-pollinator interactions. At low altitudes, temperature is one of the main drivers of pollinator number and species composition during the day (Knop et al. 2018), whereas at high altitudes, solar radiation is the main

factor influencing the daily activity rhythms of insects (Birrell et al. 2020; Xu et al. 2021). It has been found that atmospheric temperature and humidity mainly affected the nectar volume of gummy sage (nectar mainly sucrose), soil moisture mainly affected the nectar volume of meadow sage (nectar mainly glucose and fructose), whereas solar radiation had a significant effect on the level of sucrose and no effect on nectar volume (Malovrh et al. 2024). Such daily variations in plants and pollinators are mutually adaptive, so that there is generally some temporal overlap between plant floral characteristics and pollinator activity (Ballarin et al. 2022; Leiss and Klinkhamer 2005; Owayss et al. 2020). Previously, temporal or phenological matches between plants and pollinators were usually large-scale seasonal matches (Chacoff et al. 2018; Dupont et al. 2009). In contrast, daily variations in biotic and abiotic factors throughout the day lead to rhythmic changes in floral characteristics (e.g., nectar secretion), and thus studying small time-scale plant-pollinator matches can provide an accurate understanding of the subtle mechanisms of plant-pollinator interactions.

Altitude is an important geographic factor influencing the co-evolution of plants and pollinators, and altitudinal gradients lead to changes in a variety of abiotic factors, such as temperature, light, humidity, precipitation, etc. (Dong et al. 2021), which in turn have a significant impact on the composition and structure of plants and animals in the ecosystem (Zhao et al. 2023). Some studies have shown that the different pollinators of plants in different places lead to the differentiation of plant floral characteristics and the formation of different pollinator ecotypes, and that there is a geographic mosaic pattern of co-evolution between plants and pollinators (Wang et al. 2019). In the Hengduan Mountain Range, with the increase in altitude, the nectar volume of *Elaeagnus umbellata* became larger, sugar content decreased, and the frequency of visits by bees decreased while that of sunbirds increased (Pi et al. 2020), suggesting that the nectar characteristics are related to the type of pollinator. Plant-pollinator mutual adaptation at altitude also involves the mutual matching of plant corolla tube length, or nectar spur length, with the tongue length of pollinators (Ferrero et al. 2011). At altitudes where corolla tube length is longer, pollinator tongue are also longer, as a result of mutual selection and co-evolution between the two (Paudel et al. 2016). Corolla tube or nectar spur length affects pollinator activity behaviour and pollen dispersal mechanisms, which in turn affects plant reproductive success in terms of the quantity and quality of pollen carried by pollinators (Huang and Guo 2000). It has been shown that with increasing altitude, the main pollinator of *Primula nivalis* Pall. changes from the long-nosed gadfly to the short-tongued bumblebee of Tianshan Mountain, and in order to adapt to the change of pollinators, the plant has evolved shorter corolla tubes to facilitate pollination by short-tongued bumblebees (Abdusalam et al. 2022). Therefore, it is important to study the matching of floral features of plants at different altitudes with their pollinators to understand the spatial variation in plant-pollinator interactions.

Impatiens oxyanthera Hook. f. (Balsaminaceae), a Chinese endemic bumblebee-pollinated plant, was selected as a research material to test the following hypotheses: (1) There were obvious diurnal variations in floral traits and plant-pollinator interactions due to the changes in abiotic factors and pollinator activity throughout the day. (2) Diurnal variations in floral traits, plant-pollinator interactions and plant fitness at high altitude were more significant than those at low altitude, because there were more significant differences in abiotic factors among times of day.

2 Materials and methods

2.1 Study site

The experimental site was situated on Emei Mountain, China—a transition zone between the eastern Tibetan Plateau and southwestern Sichuan Basin (Li and Shi 2007)—with two sampling plots: a low-altitude site (29°36'13.54" N, 103°21'33.78" E; 932 m a.s.l.) and a high-altitude site (29°34'39.64" N, 103°16'59.29" E; 1241 m a.s.l.). Both plots experience a humid subtropical monsoon climate, support evergreen broadleaf forests, and lie in a region characterized by frequent cloudy and rainy weather (annual rainfall: 1593–1990 mm; annual temperature: 10–17 °C), with yellow loam as the dominant soil type (Zhu et al. 1997). Emei Mountain is part of the East Asia-Himalayas, one of the five major distribution zones for *Impatiens* spp. (Huang and Guo 2000), and ranks among China's key centers for the concentration and differentiation of this genus. It hosts 12 endemic Chinese *Impatiens* species, including *I. oxyanthera* (Yu 2007).

2.2 Study species

Impatiens oxyanthera blooms in late summer and early autumn, flowering from August to November, with pale purplish-red flowers and a nectar spur of 27 - 30 mm, which has a high ornamental value (Li 2018). This species favors habitats with shade and moisture at forest edges and roadsides at an altitude of 560 - 3000 m (Li and Shi 2007). The breeding system is autogamous and heterogamous and requires pollinating insects, the main pollinator being the long-tongued, *Bombus trifasciatus* Smith (Wang 2013).

2.3 Research methods

On 29 March 2020, experimental plots were established on open, flat agricultural land at both altitudes on Mount Emei. At each altitude, four plots were randomly assigned, each containing 15 pots (32 cm outer diameter, 20 cm height) arranged in 3 rows × 5 columns; plots were spaced 1 m apart, with 50 cm between pots. One seedling was planted per pot, totaling 60 plants per altitude.

To simulate the natural light environment, a shade net (transmittance: $26.83 \pm 0.66\%$) was installed 2 m above the plots. All seedlings and soil were sourced from low-altitude agricultural land. Pots were prepped with a layer of tiles at the base for drainage and aeration, then filled with equal amounts of agricultural soil. Routine maintenance included weeding, watering, fertilization, and pesticide application as needed.

2.3.1 Monitoring of environmental factors and determination of air temperature in corolla tube

From 28 May to 23 November 2020, three temperature and humidity loggers (DS1923G, Maxim/Dallas Semiconductor Inc., USA) were randomly installed in the experimental plots at each altitudes to monitor the atmospheric temperature and humidity. Soil temperature at a depth of 10 cm was recorded with a temperature sensor (DS1921G-F5, Maxim/Dallas Semiconductor Inc., USA). All data are recorded hourly

(Table S1, Fig. S1). Corolla tube temperatures were measured during peak flowering (>50% of experimental plants in bloom) using a K-type thermocouple thermometer (HB6801, Wenzhou Haibao Instrument Co., Ltd.): at high altitude from 21 to 26 August, and at low altitude from 7 to 13 October 2020, focusing on first-day flowers in both male and female phases for each altitude. For a detailed description see the Supporting Information.

2.3.2 Determination of nectar volume and sugar content

Nectar, a key nutritional reward provided by *I. oxyanthera* to pollinators, was studied for its spatiotemporal patterns in quantity and quality. To exclude pollinator disturbance, pre-opening flower buds were bagged with 160-mesh (10 cm × 12 cm) white nylon gauze: at low altitude (15 - 20 September) and high altitude (4 - 16 October 2020). Nectar volume was measured during the first day of male or female phases at 2-h intervals (8:00–10:00 to 18:00–20:00) using a 10 µL flat-tip micro-sampler (701N, Hamilton, USA), with 26 randomly selected flowers (across four plots) per time period. Nectar samples (10 µL per tube) were absorbed onto qualitative filter paper strips, stored in 1.5 mL capped centrifuge tubes, and collected in five replicates per altitude, time period, and floral phase. Samples were preserved at -80 °C in an ultra-low temperature freezer. Details of nectar concentration measurement are provided in the Supporting Information.

2.3.3 Plant-pollinator size matching studies

Pollinators were collected using ethyl acetate-filled poison vials during their foraging activity at two altitudes, across six 2-h intervals (8:00–10:00 to 18:00–20:00), from 17 August–21 September and 22 October–29 October 2020. Digital vernier calipers (CD-15AX, 0.01 mm accuracy, Mitutoyo) were used to measure pollinator traits (body length/width/height, tongue length) and floral traits of visited flowers (corolla tube length/width/height, nectar spur length, spur curvature). Plant-pollinator size matching was calculated by subtracting corresponding pollinator dimensions (body/tongue length) from floral traits (corolla tube/nectar spur length). Total flower length = corolla tube length + nectar spur length; total bumblebee length = body length + tongue length. Pollinators were stored in specimen boxes post-measurement and weighed (to 0.0001 g) using an electronic balance on 1 December 2020.

2.3.4 Observation of pollinators and measurement of pollination efficiency

Pollinator visiting behavior was observed during peak bloom: 31 August–2 September (high altitude) and 17–20 October 2020 (low altitude). Mature flower buds were bagged with gauze 12 h pre-flowering; bags were removed on the first day of the male or female phase. Over 3 days, 30 flowers (20 male-phase, 10 female-phase, randomly selected) were observed daily across nine 1-h intervals (8:00–9:00 to 18:00–19:00). Pollination frequency was calculated as total visits/(total observation hours × number of observed flowers). Details of pollination efficiency assessment are provided in the Supporting Information.

2.3.5 Measurement of plant reproductive success

Female reproductive success was assessed via seed set per fruit, seeds per fruit, and fruit set. At high altitude (26 August–21 September) and low altitude (30 September–24 October 2020), mature flower buds were bagged during six 1-h intervals on the first day of the female phase (8:00–9:00, 10:00–11:00, 12:00–13:00, 14:00–15:00, 16:00–17:00, 18:00–19:00) and re-bagged after a single pollinator visit. Gauze bags were removed post-flowering to avoid impeding fruit development, with 30 female-phase flowers treated per time period at both altitudes. After three weeks, fruit set (1 = set, 0 = not set) was recorded for marked flowers. Seeds and unfertilized ovules (on the capsule axis) per fruit were counted under a light microscope. Seed set was calculated as $[\text{seeds}/(\text{seeds} + \text{unfertilized ovules})] \times 100\%$; fruit set as (number of harvested fruits/total flowers).

2.4 Data analysis

All data were statistically analyzed using R Studio software (version 4.4.0). A general linear model was applied to data that followed a normal distribution, whereas a generalized linear model was used for non-normally distributed data. See Support information for details.

3 Results

3.1 Air temperature difference between inside and outside the corolla tube

Altitude, time of day, and their interaction significantly affected the air temperature difference between the inside and outside of the corolla tube. Compared to low altitude, high altitude significantly increased the internal corolla temperature, external corolla temperature, and their difference by 52.43%, 55.39%, and 21.57%, respectively (Figs. 1, S2). At both altitudes, the temperature difference showed a significant trend of decreasing then increasing throughout the day: it peaked at 8:00 and reached its minimum at 16:00. At high altitude, the difference at 16:00 was negative, indicating lower internal than external corolla temperatures at this time (Fig. 1). Temporal variations in the temperature difference were consistent between male and female floral phases. In the male phase, the difference at 16:00 decreased significantly by 118.48% (high altitude) and 79.12% (low altitude) compared to 8:00 (Fig. 1a). Similarly, in the female phase, the difference at 16:00 decreased by 119.30% (high altitude) and 79.89% (low altitude) relative to 8:00 (Fig. 1b). No significant differences between male and female phases were observed for these three temperature indicators (Table 2).

3.2 Nectar volume and sugar content

Altitude significantly affected female-phase nectar volume but not male-phase volume: at 8:00, low-altitude female-phase nectar volume was 49.32% higher than that at high altitude (Fig. 2A). No diurnal variations in nectar volume were observed at either altitude (Fig. 2B). Sucrose concentration showed significant diurnal variation at high altitude but was unaffected by altitude or floral phase (Figs. 3a, b). In the male phase at high altitude, sucrose concentration at 14:00 was 43.59–43.70% lower than at 10:00,

12:00, and 18:00; in the female phase at high altitude, it was 44.28% lower at 12:00 than at 8:00. Fructose concentration was significantly affected by altitude in the female phase: at 8:00, low-altitude female-phase fructose concentration was 66.62% lower than at high altitude (Fig. 3d). No significant effects of altitude or time of day on male-phase fructose concentration were detected (Fig. 3c). Nectar glucose concentration and its ratio showed no significant differences across altitudes, times of day, or floral phases (Figs. 3e–h).

3.3 Flower and pollinator size matching

Altitude, time of day, and their interaction significantly affected the height difference between the corolla tube and pollinators. Compared to low altitude, high altitude significantly reduced this height difference by 31.72% (8:00) and 42.01% (10:00) but increased it by 39.53% (16:00). At high altitude, the height difference at 14:00, 16:00, and 18:00 was 92.75%, 98.00%, and 95.55% higher than at 10:00, respectively; no significant diurnal variations were observed at low altitude (Fig. 4c). Altitude and time of day had no significant effects on other morphological matching indices, including the length and width differences between the corolla tube and pollinators, the length difference between the nectar spur and pollinator tongue, and the total length difference between the flower and pollinators (Figs. 4a, b, d, e).

3.4 Frequency of pollinator visits to flowers and pollination efficiency

Gender and time of day significantly affected pollinator visit frequency. At high altitude, male and female phases exhibited two daily visit peaks (10:00–11:00 and 15:00–17:00), whereas no significant diurnal rhythm was observed at low altitude (Fig. 5). At high altitude, female-phase visit frequency was 27.83% lower than that of the male phase. For high-altitude male phases, visit frequency at 8:00–9:00, 12:00–13:00, and 18:00–19:00 was 46.51%, 47.67%, and 54.65% lower than at 15:00–16:00, respectively. For high-altitude female phases, visit frequency at 8:00–9:00, 12:00–13:00, 13:00–14:00, and 18:00–19:00 was 75.00%, 70.83%, 62.50%, and 66.67% lower than at 10:00–11:00, respectively (Fig. 5). The interaction of altitude, gender, and time of day had no significant effect on visit frequency.

Time of day strongly affected pollen deposition in *I. oxyanthera*: at both altitudes, pollen deposition at 18:00 was significantly higher than at 10:00, 14:00, and 16:00 (Fig. 6). At high altitude, 18:00 deposition was 12.84% (vs. 14:00), 26.26% (vs. 10:00), and 51.75% (vs. 16:00) higher; at low altitude, it was 52.91% (vs. 10:00), 174.55% (vs. 16:00), and 226.49% (vs. 14:00) higher. No significant differences in pollen deposition were found between altitudes at the same time of day (Fig. 6).

3.5 Female reproductive success

Altitude significantly affected the number of seeds per fruit in *I. oxyanthera*: both seeds per fruit and ovule number were higher at high altitude than at low altitude, with significant increases observed at most time points. Specifically, seeds per fruit at high altitude were 30.42–52.72% higher than at low altitude at 8:00, 12:00, 14:00, 16:00, and 18:00 (Fig. 7a), while ovule number was 14.01–20.10% higher at

8:00, 12:00, and 18:00 (Fig. 7c). Time of day significantly affected unfertilized ovule number at high altitude, with 30.32–30.84% fewer unfertilized ovules at 14:00 and 16:00 than at 18:00; no diurnal variation was detected at low altitude (Fig. 7b). Altitude, time of day, and their interaction had no significant effects on single-fruit set or overall fruit set (Figs. 7d, e).

Redundancy analysis (RDA) explored associations between sample distribution, plant traits, and pollination frequency (Fig. 8). The combined variables significantly explained sample variation (Fig. 8a, $P = 0.038$), with corolla tube temperature (Corotemp) and pollination frequency (frequency) showing the strongest explanatory power (long arrows, small angles with RDA1). Samples with high unfertilized ovule counts (unfertilized), fruit number (fruit), and total seed number (seeds) clustered in the high-RDA1 region, correlating positively with corolla tube temperature and pollination frequency.

Group comparisons revealed clear separation between high- and low-altitude samples in RDA space (Fig. 8b, $P = 0.015$): high-altitude samples dominated the high-RDA1 region, correlating positively with corolla tube temperature and pollination frequency, while low-altitude samples concentrated in the low-RDA1 region with weaker associations.

4. Discussion

4.1 Effects of altitude on floral characteristics, pollinator visitation and plant reproductive success

Altitudinal changes result in the changes in environmental factors such as temperature, light and humidity, with temperature being an important factor influencing plant nectar secretion (Descamps et al. 2021a) and pollinator foraging behaviour (Descamps et al. 2021b). In this study, the temperature inside and outside the corolla tube were significantly higher at high altitude than at low altitude. The possible mechanism might be attributed to the following factors: At high-altitude regions, solar radiation is intensified while ambient temperatures are lower. The tubular corolla structure of *I. oxyanthera* likely reduces convective air exchange between the corolla interior and exterior, thereby providing thermal insulation (Koski et al. 2024). However, their temperature difference in the morning at high altitude were significantly higher than at low altitude, and the opposite was true for temperature difference in the afternoon. That is, the corolla tube was warmer in the morning and cooler in the afternoon at high altitude. This result is similar to other studies, where lower environmental temperatures in the morning cause plants to increase their respiration rate and energy release, and the respiratory chain decouples to release energy in the form of heat to warm the air (Herrera and Medrano 2017). In the afternoon, when environmental temperatures are higher, the lower temperature inside the corolla tube attracts bumblebees, which prefer cooler nectar, to pollinate the flowers (Sherer et al. 2024).

Nectar is the most important and common reward provided by flowers to pollinators (Heil 2011; Pansarin and Estanislau Do Amaral 2008). The quantity and quality of nectar determines the energy gain of pollinators, which regulates pollinator visiting behaviour and pollination efficiency, thus influencing the

reproductive success of plants (Dreisig 2012). Many studies have shown that nectar secretion is mainly influenced by various environmental factors such as temperature, light, and moisture (Descamps et al. 2021a). In our study, nectar volume per flower at high altitude were significantly more than that at low altitude, especially for female-phase flowers. This may be attributed to the higher temperature inside the corolla tube at high altitude compared to low altitudes. Under well-watered conditions, higher flower temperatures promote nectar secretion (Malovrh and Ravnjak 2024). The abundance of nectar at high altitude results in increased pollinator visitation.

For long corolla tube or long nectar spur plants, plant-pollinator size match is another key functional factor shaping the interaction and co-evolution between plants and pollinators (Klumpers et al. 2019; Naghiloo et al. 2021). In contrast, nectar-feeding flower visitors are mainly determined by three factors: the length of the insect's mouthparts, net energy gain, and the minimum temperature during flight (Corbet et al. 1995). In this study, the tongue length of high-altitude pollinators was significantly higher than that of low-altitude ones. Pollinating bumblebees can reach as deep as possible into the nectar spurs by extending their legs and thus pushing their head and thorax into the labellum (Wilson 1995). In particular, the height difference between the two partners was significantly lower at high altitude than at low altitude in the morning. Therefore, high-altitude plants and pollinators are more compatible, resulting in significantly higher pollination frequencies at high altitude compared to low altitude.

Altitudinal gradients ultimately affect reproductive success by directly influencing plant reproductive output and by indirectly influencing pollinator visits (Arroyo et al. 2013; Nocentini et al. 2013; Pacini et al. 2003). On the one hand, each flower had more ovules at high altitude in this study. On the other hand, more nectar volume of female-phase flowers, closer matching of plants and pollinators and optimal corolla tube temperatures resulted in higher visit frequency of pollinators at high altitude. These were ultimately responsible for a significantly more seeds of single fruit at high altitude than at low altitude.

4.2 Effect of time on floral characteristics, pollinator visits and plant reproductive success

Nectar sugar composition is associated with flower-visiting insect species. For example, hummingbirds, moths and bumblebees prefer to visit sucrose-rich nectar, while rich hexoses (glucose, fructose) are associated with non-specific insect flower-visiting behaviour (Nicolson 2007; Perret et al. 2001). In this study, nectar sucrose concentrations were significantly higher than glucose and fructose concentrations at both altitudes, thus attracting bumblebee-like pollinators such as the *B. trifasciatus* to visit the flowers. The study further revealed that *B. trifasciatus* exhibited the highest pollination activity at 10:00 and the lowest at 8:00 during the female phase of flowers. The reason for this may be that at 10:00 the ambient temperature gradually increases and the bumblebees start to come out and move around, and the temperature inside the corolla tube at 10:00 is warmer than the ambient temperature, which enables the bumblebees to reduce the amount of energy needed to raise their bodies to flight temperature before leaving the flower (Rands and Whitney 2008). Moreover, the height difference between plants and pollinators is the smallest and pollination frequency was the highest at 10:00. Although nectar sucrose

concentration and air temperature inside corolla tubes was higher at 8:00 than other daytime periods for female-phase flowers, pollination frequency was the lowest at 8:00 due to lower ambient temperatures .

Our study found that high-altitude flowers had the lowest pollen deposition and number of unfertilized ovules at 16:00, but the highest ones at 18:00. Our study also revealed that the number of pollen grain deposited on the stigma by bumblebees was substantially greater than the number of ovules in the ovary, indicating that the elevated count of unfertilized ovules at 18:00 was attributable not to pollen quantity but to pollen quality. At 16:00, a larger height difference between high-altitude plants and their pollinators maybe lead to reduced pollen grain deposited on the stigma. Moreover, the relatively high external environmental temperature at this time, coupled with a lower temperature within the corolla tube, may enhance pollen viability (Sherer et al. 2024), thereby resulting in a decreased number of unfertilized ovules at 16:00.

4.3 Effects of altitude and time interactions on floral characteristics, pollinator visitation and plant reproductive success

The interaction between altitude and time of day had a highly significant effect on the temperature difference between the inside and outside of the corolla tube, as well as on the width and height differences between plants and pollinators. In the morning, the distribution of heat inside and outside the corolla tube changed more rapidly due to the thin air at high altitude, which weakly attenuates solar radiation (Zhou et al. 2011). After the rapid cooling of the atmosphere at high altitude in the afternoon, the heat inside the corolla tube was relatively well maintained, which led to a more significant recovery of the temperature difference. In the morning, the width and height differences between plants and pollinators at high altitude were smaller than those at low altitude, indicating the two partners were better matched (Naghiloo et al. 2021), resulting in a higher frequency of pollination for high-altitude plants.

Although the independent effect of altitude on pollen deposition was not significant, the interaction of altitude and time significantly affected pollen deposition. This interaction led to differences in the trend and magnitude of pollen deposition at different altitudes during the day. The possible reason for this is that micro-meteorological conditions (e.g., wind speed, temperature, humidity, etc.) (Herrera and Medrano 2017), flower-pollinator matching degree and handling time of pollinator on a flower vary by time of day at different altitudes, and these factors affect pollen dispersal and deposition. Although the interaction of time of day and altitude significantly affected the above-mentioned floral characteristics and visit frequency of pollinators, it did not significantly affect plant reproductive success. The findings indicate that sexual reproduction in plants can, to a certain degree, buffer external environmental fluctuations, thereby ensuring the stability of sexual reproduction.

In conclusion, high-altitude plants exhibit more pronounced temperature differences between the inside and outside of corolla tube over time, produce more nectar, and have pollinators with longer tongues that

are better matched to corolla tube, especially in the morning, resulting in higher pollination frequencies. With more ovules per flower, high-altitude plants had higher reproductive success at the single-fruit level compared to low-altitude ones. Corolla tube temperature and pollination frequency were responsible for plant reproductive success. Throughout the time of day, the temperature difference in the corolla tube reaches its maximum at 8:00, while nectar volume and sugar components other than sucrose remain stable throughout the day. The height difference between high-altitude flowers and pollinators is smaller in the morning than in the afternoon, and pollination frequency varies significantly throughout the day at high altitudes. Pollen deposition peaks at 18:00 and reaches a low point at 14:00 or 16:00 at both high and low altitudes. Diurnal variations in floral traits and flower-pollinator interactions were significantly stronger at high altitude, indicating adaptation to environmental variability (Fig. S6).

However, our study only consider two altitudes on Mount Emei, and other flower traits such as petal light reflectance intensity and floral scent which mediate plant-pollinator interactions may also vary diurnally. Future studies should investigate these traits to better explain pollinator behavioral changes and further explore the fine-scale spatio-temporal patterns of plant-pollinator interactions.

Declarations

Conflict of Interest

The authors declare no conflicts of interest

Acknowledgements

This study was supported by Sichuan Science and Technology Program (2023NSFSC0136), Scientific research innovation team project of China West Normal University (CXTD2020-4), and National Key R&D Program of China (2022YFE0115200).

References

1. Abdusalam A, Liao WJ, Zhang ZQ, Li QJ (2022) Pollinator shifts along an elevation gradient mediate different response in self-pollination in heterostylous *Primula nivalis*. *Journal of Systematics and Evolution* 60:186-195 doi: 10.1111/jse.12639
2. Arroyo MTK, Dudley LS, Jespersen G, Pacheco DA, Cavieres LA (2013) Temperature-driven flower longevity in a high-alpine species of *Oxalis* influences reproductive assurance. *New Phytologist* 200:1260-1268 doi: 10.1111/nph.12443
3. Ballarin CS, Filho LH, Amorim MJWD (2022) Intra-seasonal and daily variations in nectar availability affect bee assemblage in a monodominant afforested Brazilian Cerrado. *Austral Ecology* 47:1315-1328 doi: 10.1111/aec.13218

4. Birrell JH et al. (2020) Insects in high-elevation streams: Life in extreme environments imperiled by climate change. *Global Change Biology* 26:6667-6684 doi: 10.1111/gcb.15356
5. Chacoff NP, Resasco J, Vázquez DP (2018) Interaction frequency, network position, and the temporal persistence of interactions in a plant–pollinator network. *Ecology* 99:21-28 doi: 10.1002/ecy.2063
6. Corbet et al. (1995) The competition box: a graphical aid to forecasting pollinator performance. *Journal of Applied Ecology* 32:707-719 doi: 10.2307/2404810
7. Descamps C, Boubnan N, Jacquemart A, Quinet M (2021a) Growing and Flowering in a Changing Climate: Effects of Higher Temperatures and Drought Stress on the Bee-Pollinated Species *Impatiens glandulifera* Royle. *Plants* 10:988 doi: 10.3390/plants10050988
8. Descamps C, Jambrek A, Quinet M, Jacquemart A (2021b) Warm Temperatures Reduce Flower Attractiveness and Bumblebee Foraging. *Insects* 12:493 doi: 10.3390/insects12060493
9. Dong X et al. (2021) Patterns of Altitudinal Distribution of Species Diversity of Desert Gobi Shrub Communities in West Hexi Corridor of China. *Scientia Silvae Sinicae* 57:168-178 doi: 10.11707/j.1001-7488.20210217
10. Dreisig H (2012) How long to stay on a plant: the response of bumblebees to encountered nectar levels. *Arthropod-Plant Interactions* 6:315-325 doi: 10.1007/s11829-011-9169-9
11. Dupont YL, Padrón B, Olesen JM, Petanidou T (2009) Spatio-temporal variation in the structure of pollination networks. *Oikos* 118:1261-1269 doi: 10.1111/j.1600-0706.2009.17594.x
12. Fenske MP, Nguyen LP, Horn EK, Riffell JA, Imaizumi T (2018) Circadian clocks of both plants and pollinators influence flower seeking behavior of the pollinator hawkmoth *Manduca sexta*. *Scientific Reports* 8:2842 doi: 10.1038/s41598-018-21251-x
13. Ferrero V, Castro S, Sánchez JM, Navarro L (2011) Stigma–anther reciprocity, pollinators, and pollen transfer efficiency in populations of heterostylous species of *Lithodora* and *Glandora* (Boraginaceae). *Plant Systematics and Evolution* 291:267-276 doi: 10.1007/s00606-010-0387-x
14. Heil M (2011) Nectar: generation, regulation and ecological functions. *Trends in Plant Science* 16:191-200 doi: 10.1016/j.tplants.2011.01.003
15. Herrera CM, Medrano M (2017) Pollination consequences of simulated intrafloral microbial warming in an early-blooming herb. *Urban & Fischer* 232:142-149 doi: 10.1016/j.flora.2016.10.003
16. Hoover et al. (2012) Warming, CO₂, and nitrogen deposition interactively affect a plant-pollinator mutualism. *ECOL LETT* 15:227-234 doi: 10.1111/j.1461-0248.2011.01729.x
17. Huang S, Guo Y (2000) Advances in Pollination Biology. *Chinese Science Bulletin* 45:225-237 doi: 10.3321/j.issn:0023-074X.2000.03.001
18. Klumpers SGT, Stang M, Klinkhamer PGL (2019) Foraging efficiency and size matching in a plant–pollinator community: the importance of sugar content and tongue length. *Ecology Letters* 22:469-479 doi: 10.1111/ele.13204

19. Knop E et al. (2018) Rush hours in flower visitors over a day–night cycle. *Insect Conservation and Diversity* 11:267-275 doi: 10.1111/icad.12277
20. Koski M, Heiling J, Aplan J (2024) Behavioural Thermoregulation of Flowers via Petal Movement. *Ecology letters* 27:e14524 doi: 10.1111/ele.14524
21. Leiss KA, Klinkhamer PGL (2005) Spatial distribution of nectar production in a natural *Echium vulgare* population: Implications for pollinator behaviour. *Basic & Applied Ecology* 6:317-324 doi: 10.1016/j.baae.2005.02.006
22. Li D (2018) Impact of Nectar Robbing on Female Reproductive Success of *Impatiens Oxyanthera* (Balsaminaceae) Under Simulated Climate Warming. In, vol. Master thesis. China West Normal University, Sichuan province, china
23. Li Z, Shi L (2007) Plants of Mount Emei. Beijing Science and Technology Publishing, Beijing
24. Malovrh K, Ravnjak B (2024) Nectar Production and Three Main Sugars in Nectar of *Salvia pratensis* and *Salvia glutinosa* in Correlation with Abiotic Factors. *Agriculture-Basel* 14:668 doi: 10.3390/agriculture14050668
25. Malovrh K, Ravnjak B, Bavcon J, Križman M (2024) Nectar Production and Three Main Sugars in Nectar of *Salvia pratensis* and *Salvia glutinosa* in Correlation with Abiotic Factors. *Agriculture* 14:668 doi: 10.3390/agriculture14050668
26. Meng Y, Xu H, Chen X, Cai Q (2007) Pollination efficiency of the main bee pollinators of *Hedysarum leava*, a legume in Mu Us Sandland, Inner Mongolia. *Biodiversity Science* 15:633-638 doi: 10.1360/biodiv.070151
27. Mu J et al. (2015) Artificial asymmetric warming reduces nectar yield in a Tibetan alpine species of Asteraceae. *Annals of Botany* 116:899-906 doi: 10.1093/aob/mcv042
28. Naghiloo S, Nikzat Siahkolaee S, Esmailou Z (2021) Size-matching as an important driver of plant–pollinator interactions. *Plant Biology* 23:583-591 doi: 10.1111/plb.13248
29. Nicolson SW (2007) Nectar consumers. *Nectaries & Nectar* 7:289-342 doi: 10.1007/978-1-4020-5937-7_7
30. Nocentini D, Pacini E, Guarnieri M, Martelli D, Nepi M (2013) Intrapopulation heterogeneity in floral nectar attributes and foraging insects of an ecotonal Mediterranean species. *Plant Ecology* 214:799-809 doi: 10.1007/s11258-013-0204-z
31. Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321-326 doi: 10.1111/j.1600-0706.2010.18644.x
32. Owayss AA, Shebl MA, Iqbal J, Awad AM, Raweh HS, Alqarni AS (2020) *Phacelia tanacetifolia* can enhance conservation of honey bees and wild bees in the drastic hot-arid subtropical Central Arabia. *Journal of Apicultural Research* 59:569-582 doi: 10.1080/00218839.2020.1735731
33. Pacini E, Nepi M, Vesprini JL (2003) Nectar biodiversity: a short review. *Plant Systematics & Evolution* 238:7-21 doi: 10.1007/s00606-002-0277-y

34. Pansarin ER, Estanislau Do Amaral MDC (2008) Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): A study of floral morphology, reproductive biology and pollination strategy. *Flora - Morphology, Distribution, Functional Ecology of Plants* 203:474-483 doi: 10.1016/j.flora.2007.07.004
35. Paudel BR, Shrestha M, Burd M, Adhikari S, Sun YS, Li QJ (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 doi: 10.1111/nph.13974
36. Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V (2001) Nectar Sugar Composition in Relation to Pollination Syndromes in Sinningieae (Gesneriaceae). *Annals of Botany* 87:267-273 doi: 10.1006/anbo.2000.1331
37. Pi HQ, Quan QM, Wu B, Lv XW, Shen LM, Huang SQ (2020) An altitude-related shift of relative abundance from insect to sunbird pollination in *Eleagnus umbellata* (Elaeagnaceae). *Journal of Systematics and Evolution* 59:1266-1275 doi: 10.1111/jse.12685
38. Rands SA, Whitney HM (2008) Floral Temperature and Optimal Foraging: Is Heat a Feasible Floral Reward for Pollinators? *PloS one* 3:e2007 doi: 10.1371/journal.pone.0002007
39. Schwarz B, Dormann CF, Vázquez DP, Fründ J (2021) Within-day dynamics of plant–pollinator networks are dominated by early flower closure: an experimental test of network plasticity. *Oecologia* 196:781-794 doi: 10.1007/s00442-021-04952-5
40. Sherer TN, Heiling JM, Koski MH (2024) Floral thermal biology in relation to pollen thermal performance in an early spring flowering plant. *Plant biology* 26:811-820 doi: 10.1111/plb.13660
41. Sun M, Karin G, Schiestl FP (2014) Floral adaptation to local pollinator guilds in a terrestrial orchid. *Annals of Botany* 113:289-300 doi: 10.1093/aob/mct219
42. Tong Z et al. (2023) New calculations indicate that 90% of flowering plant species are animal-pollinated. *National Science Review* 10:nwad219 doi: 10.1093/nsr/nwad219
43. Wang Q (2013) Biological Effects of Experimental Warming on Polliantion in *Impatiens oxyanthera* (Balsaminaceae). In, vol. Ph.D. The University of Chinese Academy of Science, Beijing, China
44. Wang X, Tang X, Tong Z, Huang S (2019) Geographic mosaic theory of coevolution in plant and pollinator interaction: Process, evidence, prospect. *Chinese Science Bulletin* 64:1702-1710 doi: 10.1360/N972018-01213
45. Wilson P (1995) Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumblebee bodies. *Blackwell Publishing Ltd* 55:355-383 doi: 10.1016/s0024-4066(05)80003-3
46. Xu X et al. (2021) Bimodal activity of diurnal flower visitation at high elevation. *Ecology and Evolution* 11:13487-13500 doi: 10.1002/ece3.8074
47. Yu S (2007) *Impatiens macrovexilla* var. *yaoshanensis* S. X. Yu, Y. L. Chen H. N. Qin, a new variety of Balsaminaceae from Guangxi, China. *Acta Phytotaxonomica Sinica* 45:708-712 doi: 10.1360/aps06037

48. Zhang M, Lu S, Zhao G, Liu L (2004) Pollinators of plants. Bulletin of Biology 39:19-20 doi: 10.3969/j.issn.0006-3193.2004.05.008
49. Zhao L et al. (2023) Effects of Environmental Factors on the Spatial Distribution Pattern and Diversity of Insect Communities along Altitude Gradients in Guandi Mountain, China. Insects 14:224 doi: 10.3390/insects14030224
50. Zhou Y et al. (2011) Factors influencing altitudinal patterns of C 3 plant foliar carbon isotope composition of grasslands on the Qinghai-Tibet Plateau, China. Alpine Botany 121:79-90 doi: 10.1007/s00035-011-0093-5
51. Zhu X, Jiang W, Zhu L, Jin Y (1997) Study on Present Environmental Situation of the Emei Mountain. Sichuan Environment 16:10-18 doi: CNKI:SUN:SCHJ.0.1997-02-001

Figures

Fig. 1

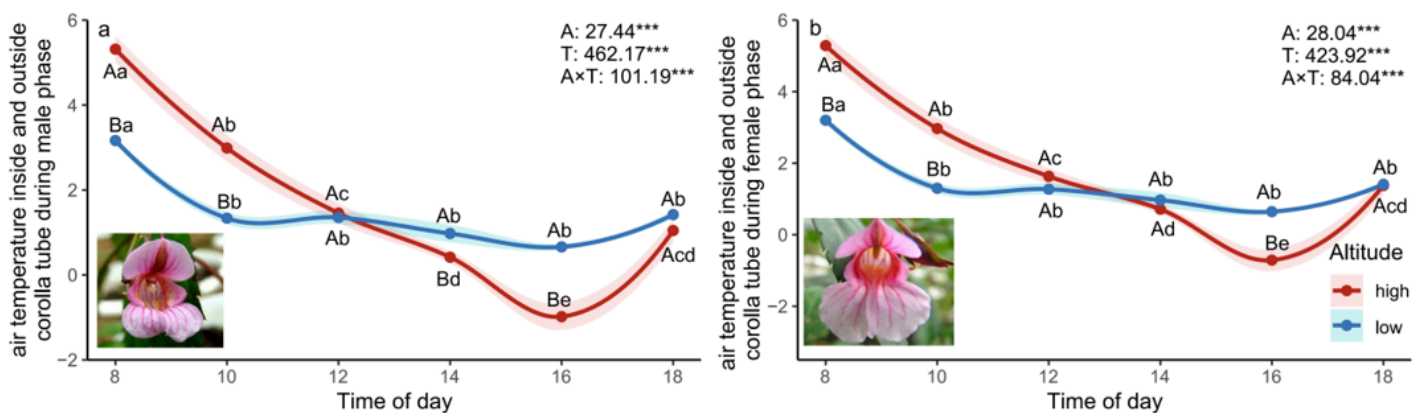


Figure 1

Temperature difference between inside and outside the corolla tube during male (a) and female (b) phases. The two coloured lines represent two altitudes, and the two semi-transparent coloured bands represent the error bands (standard error) for the two altitudes in each time period. A, the effect of altitude; T, the effect of time of day; A × T, the interaction effect of altitude and time of day. Different uppercase letters indicate significant differences in means between two altitudes at the same time, and different lowercase letters indicate significant differences in means among different times of the day at the same altitude. ***, $p < 0.001$

Fig. 2

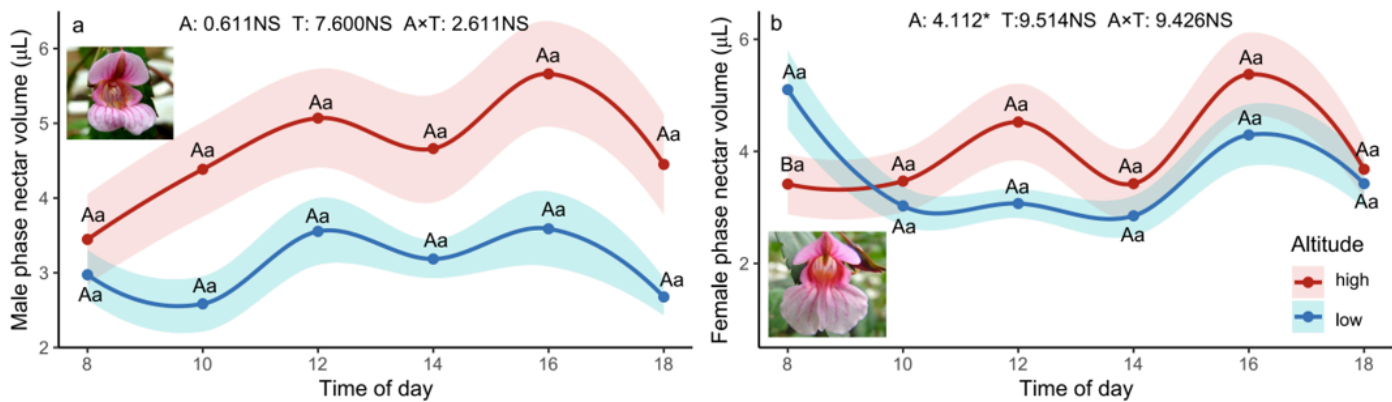


Figure 2

Effects of altitude and time of day on nectar volume per flower of *I. oxyanthera* in the male (a) and female (b) phases . NS, no significance; *, $p < 0.05$

Fig. 3

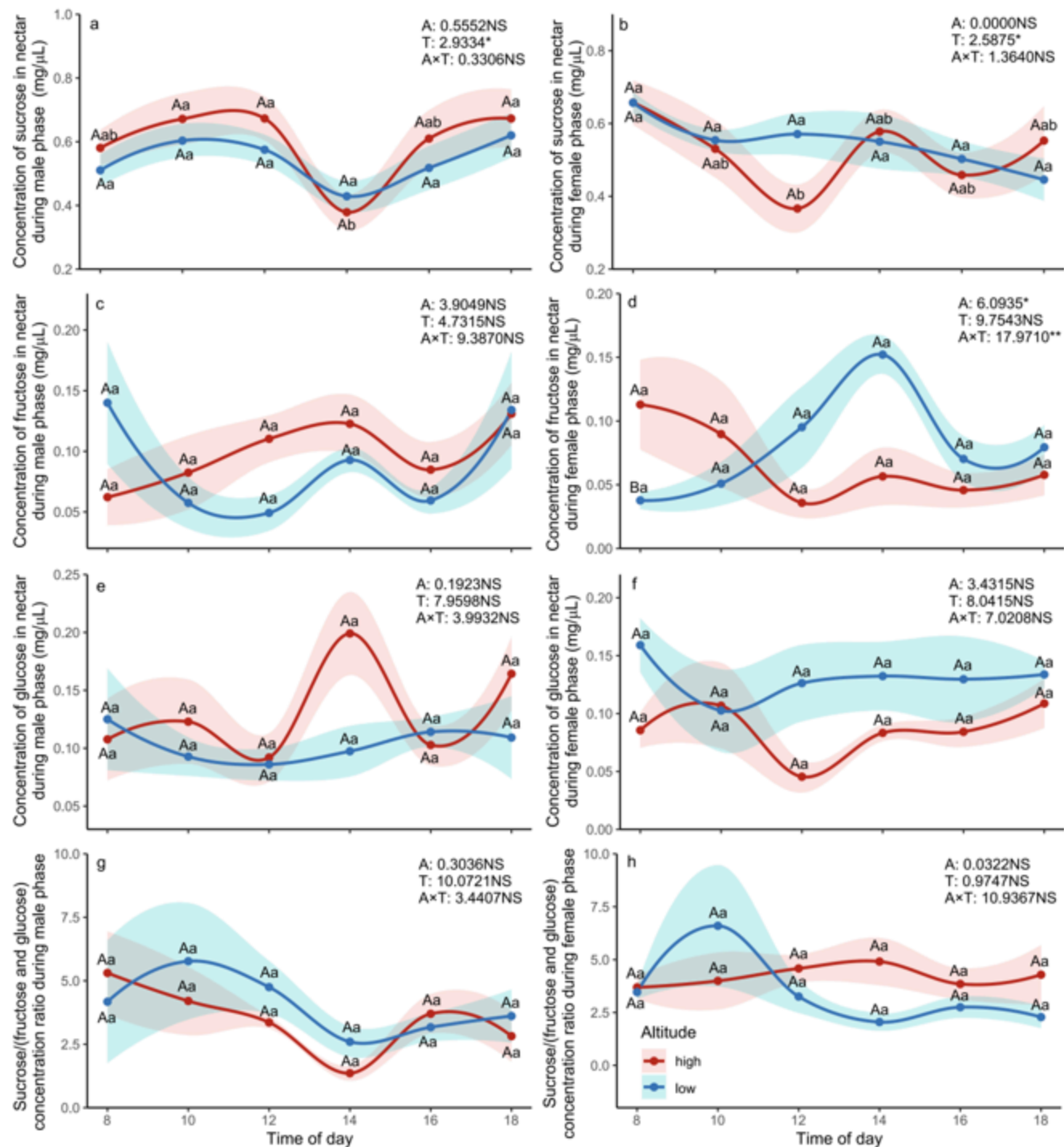


Figure 3

Effects of altitude and time of day on nectar sugar concentration in male (a, c, e, g) and female (b, d, f, h) phases of *I. oxyanthera*. (a)(b). Sucrose concentration; (c)(d). Fructose concentration; (e)(f). Glucose concentration; (g)(h). Sucrose / (fructose and glucose) concentration. NS, no significance; *, $p < 0.05$; **, $p < 0.01$

Fig. 4

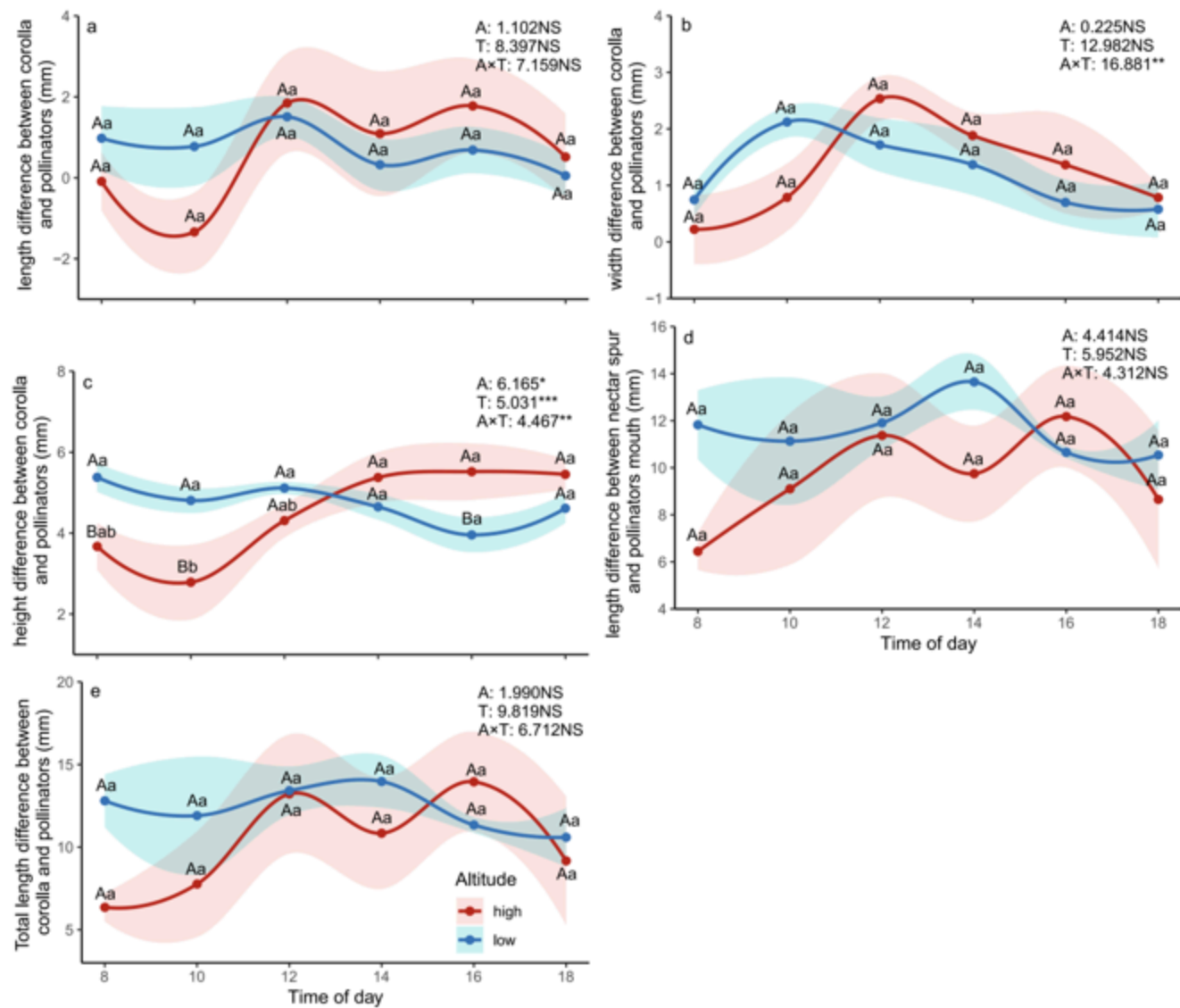


Figure 4

Effects of altitude and time of day on size match between flowers and pollinators. (a). Length difference between corolla tube and pollinator body; (b). Width difference between corolla tube and pollinator body; (c). Height difference between corolla tube and pollinator body; (d). Length difference between nectar spur and pollinator tongue; (e). Total length difference between flower and pollinator. NS, no significance; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Fig. 5

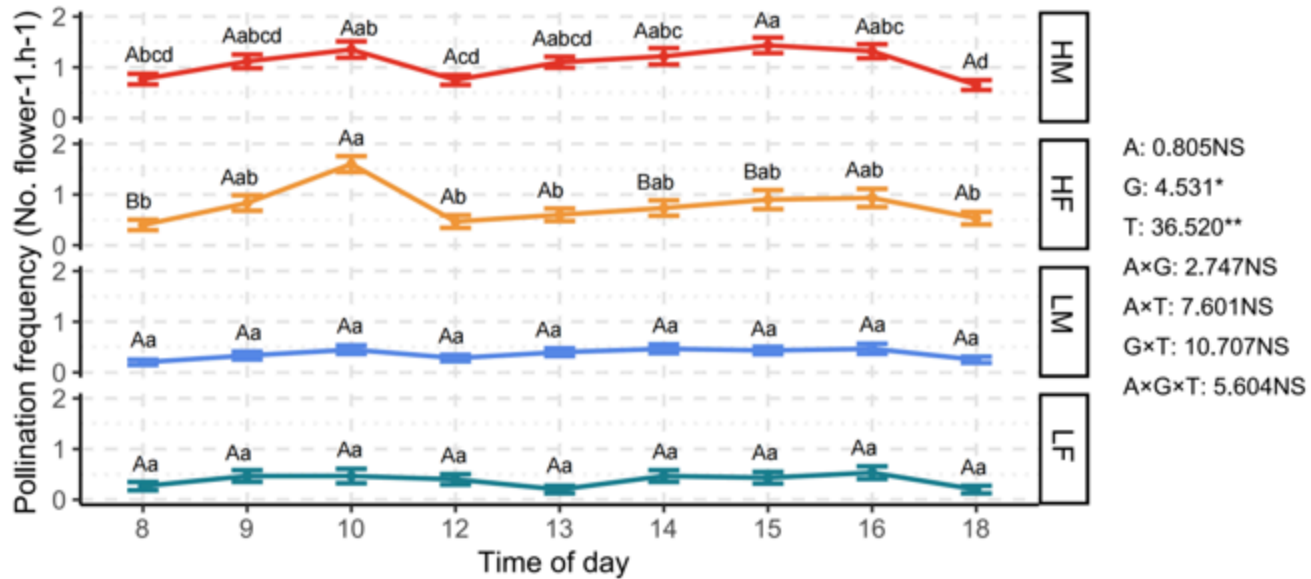


Figure 5

Effects of altitude and time of day on the pollination frequency of pollinators of *I. oxyanthera*. HM, the male-phase flowers of *I. oxyanthera* at high altitude, HF, the female-phase flowers of *I. oxyanthera* at high altitude, LM, the male-phase flowers of *I. oxyanthera* at low altitude, and LF, the female-phase flowers of *I. oxyanthera* at low altitude. Different uppercase letters in the figure indicate significant differences in the average values between the same altitude and time period (HM and HF, LM and LF), and different lowercase letters indicate significant differences in mean values among different times of the day for the same case. A, the effect of altitude; G, the effect of phase; T, the effect of time of day; A × G, the interaction effect of altitude and phase; A × T, the interaction effect of altitude and time of day; G × T, the interaction effect of phase and time of day; A × G × T, the interaction effect of altitude, phase and time of day. NS, no significance; *, $p < 0.05$; **, $p < 0.01$

Fig. 6

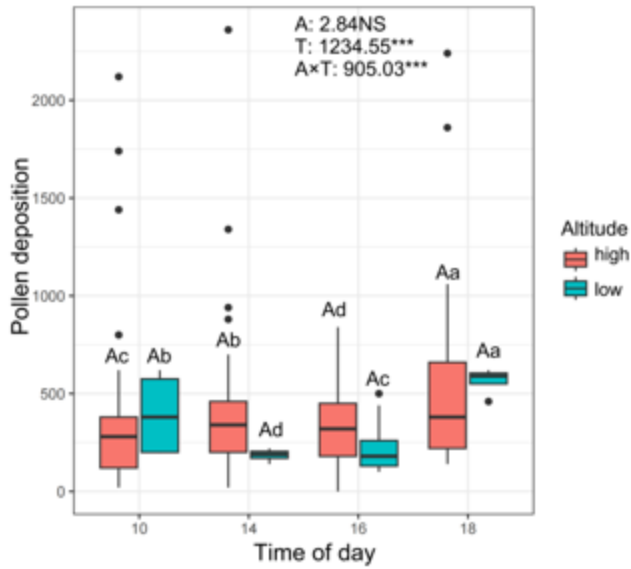


Figure 6

Effects of altitude on pollen deposition in *I. oxyanthera*. A, the effect of altitude; T, the effect of time of day; A × T, the interaction effect of altitude and time of day. Different uppercase letters indicate significant differences in means between two altitudes at the same time, and different lowercase letters indicate significant differences in means among different times of the day at the same altitude. NS, no significance;***, $p < 0.001$

Fig. 7

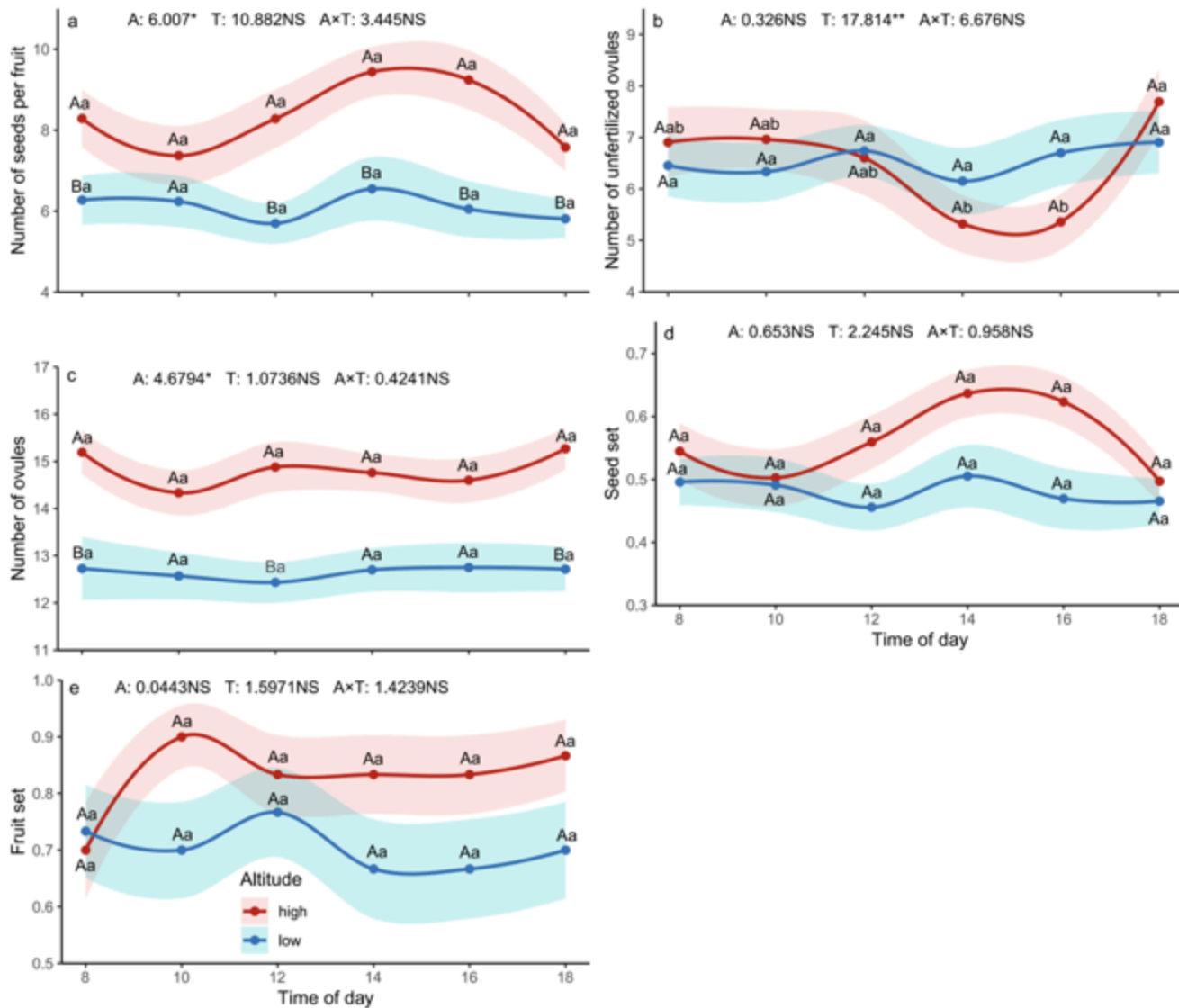


Figure 7

Effects of altitude and time of day on female reproductive success of *I. oxyanthera*. (a). Number of seeds per fruit; (b) Number of unfertilized ovules per fruit; (c). Number of ovules; (d). Seed set; (e). Fruit set. NS, no significance; *, $p < 0.05$; **, $p < 0.01$

Fig. 8

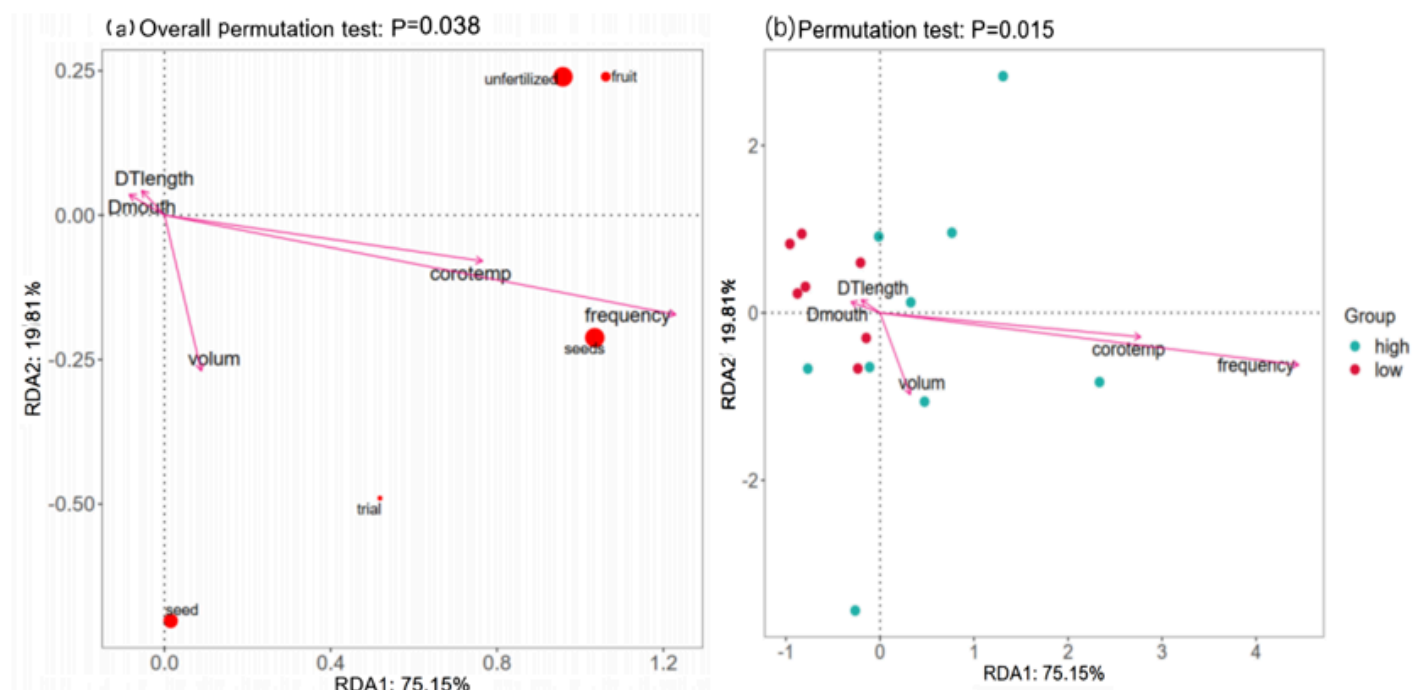


Figure 8

Redundancy Analysis (RDA) Sorting Chart. (a). Demonstrating plant reproductive success in relation to floral characteristics, match and pollination frequency; (b). Demonstrating the relationship between reproductive success and floral characteristics, match and pollination frequency in plants at two altitudes. fruit, number of enlarged fruits; trial, number of flowers produce fruits; seed, average number of seeds; seeds, summarise the number of seeds; unfertilized, number of unfertilized ovules; Dmouth, length difference between nectar spur and pollinators mouth; DTlength, Total length difference between corolla and pollinators; corotemp, corolla tube temperature; frequency, pollination frequency; volum, nectar volum

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformation.pdf](#)
- [data.xlsx](#)