Floral traits and their connection with Pollinators and climate

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Abstract

Background and Aims

Floral characteristics vary significantly among plant species, and multiple underlying factors govern this diversity. Although it is widely known that spatial variation in pollinator groups can exert selection on floral traits, the relative contribution of pollinators and climate to the variation of floral traits across large geographic areas remains a little-studied area. Besides furthering our conceptual understanding of these processes, gaining insight into the topic is also of conservation relevance: understanding how climate may drive floral traits variation can serve to protect plant-pollinator interactions under global change conditions.

Methods

We used *Rhododendron* as a model system and collected floral traits (corolla length, nectar volume and concentrations), floral visitors, and climatic data on 21 *Rhododendron* species across two continents (North America-Appalachians and Asia-Himalaya). Based on this we quantified the influence of climate and pollinators to floral traits using phylogeny-informed analyses.

Key results

Our results indicate that there is substantial variation in pollinators and morphological traits across *Rhododendron* species and continents. We came across four pollinator groups: birds, bees, butterflies, and flies. Asian species were commonly visited by birds, bees, and flies, while bees and butterflies were the most common visitors of North American species. The visitor identity explained nectar trait variation, with flowers visited by birds presenting higher volumes of dilute nectar and those visited by insects producing concentrated nectar. Nectar concentration and corolla length exhibited a strong phylogenetic signal across the analysed set of species. We also found that nectar trait variation in the Himalaya could also be explained by climate, which presented significant interactions with pollinator identity.

Conclusions

Our results indicate that both pollinators and climate contribute and interact to drive nectar trait variation, suggesting that both can affect pollination interactions and floral (and plant) evolution individually and interacting with each other.

Keywords: Appalachian, climate, corolla, elevation gradient, floral trait, Himalaya, nectar, pollinator, pollination syndrome, *Rhododendron*

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INTRODUCTION

The exceptional diversity in floral traits of animal-pollinated flowering plants has intrigued many evolutionary biologists, including Darwin (Darwin, 1859; 1862). The factors underpinning their evolution and diversity have primarily been attributed to the selection imposed by pollinating agents (Fenster *et al*., 2004). Pollinators that most frequently visit and efficiently pollinate flowers select for (or against) specific floral traits, which eventually become dominant in the population (Armbruster, 2014). This idea is at the foundation of the pollination syndrome hypothesis, a central concept in plant evolution. This hypothesis predicts that different plant species pollinated by similar pollinator groups are expected to display converging flower traits (e.g., colour, morphology, display of floral rewards) (Faegri and van der Pijl, 1979; Fenster *et al*., 2004; Rosas-Guerrero *et al*., 2014) because each pollinator group differs in their morphological, physiological and behavioural characteristics. For instance, the energetic requirements and physiological restraints of different pollinator groups will promote selection by these pollinators for varying nectar volumes (Kevan and Baker, 1983), with flowers visited by large animals (e.g., birds) usually producing more nectar than flowers visited mainly by smaller insects (Baker and Baker, 1983). Likewise, pollinator identity is also expected to lead to variations in nectar concentration and viscosity. Biophysical models showed that the optimal nectar concentration for active or capillary suction feeders (e.g., birds, butterflies) is 30-40 %, while that for viscous dippers (bees and flies) is 50-60 % (Kim *et al*., 2011), what has been observed in flowers visited mainly by these pollinator groups. Further, the hypothesis also predicts that shape and colour would also evolve as a response to pollinator pressure, since different pollinators have different morphologies and visual acuities. For instance, ornithophilous flowers display warm coloured, long and tubular flowers, while bee-visited flowers are open, and mostly blue and/or yellow-coloured (Faegri and van der Pijl, 1979; Baker and Baker, 1983; Johnson and Nicolson, 2008; Fenster *et al*., 2015).

Although the idea of pollination syndromes has been shown to accurately explain floral traits in many plant groups, there are many cases where it has been hard to reconcile it with biological observations, stimulating much debate (Ollerton *et al*., 2009; Rosas-Guerrero *et al*., 2014). Some of the debate was founded on the observation that most plant species are generalist in their pollination, making it challenging to predict primary pollinators based on floral characteristics (Ollerton *et al*., 2009; Dellinger, 2020). Further, in many cases, other non-pollinator variables may be contributing to floral traits (e.g., herbivores, parasites, climate) (Galen, 2000; Strauss *et al*., 2004; Koski *et al*., 2020; Watson *et al*., 2022). Today, it appears that exploration of these additional variables is necessary to gain a better understanding of the different drivers of floral trait evolution. Indeed, a combination of detailed empirical pollinator observations, measurements of reliable traits (e.g., corolla length, width, nectar rewards), and the consideration of traits other than pollinators could provide a path to advance and test the limitations of the pollination syndromes hypothesis (Dellinger, 2020).

In this context, gaining a deeper understanding of floral evolution in an integrated and realistic manner should involve the investigation and testing of the effects of both biotic and abiotic agents (Galen, 2000). Among the latter, an essential one may be climate (Sullivan and Koski, 2021), which is particularly relevant in the current context of climate change and its potential effect on biodiversity. Climate has been shown to relate to nectar rewards and floral morphology in several ways (Campbell and Powers, 2015; Takkis *et al*., 2015; Weber *et al*., 2020). For example, as nectar consists of 50% - 90% water, temperature and water availability have been shown to directly and indirectly affect nectar production (Pacini *et al*., 2003; Nicolson and Thornburg, 2007). Further, floral nectar volume is unimodally and negatively related to temperature (Takkis *et al*., 2015) and positively related to precipitation (Kuppler and Kotowska, 2021). Floral shape and size have also been shown to be affected by precipitation. The larger water requirements and maintenance costs of large flowers (Teixido and Valladares, 2014) were suggested as drivers for small flower sizes and corolla lengths in areas with low precipitation (Caruso, 2006; Halpern *et al*., 2010; Gallagher and Campbell, 2017; Powers *et al*., 2022).

Besides these direct effects, climate could also affect floral morphology indirectly. These climate-driven morphological changes could lead to differential floral visitor attraction (Scaven and Rafferty, 2013; Parachnowitsch *et al*., 2018; Jenan *et al*., 2022), which could further reinforce these floral divergences. Additionally, climate can also affect pollinator fauna presence (Espíndola and Pliscoff, 2019; Shah *et al*., 2020) under given environmental conditions, which can be particularly relevant for plants occupying elevation gradients (Arroyo *et al*., 1982; Lefebvre *et al*., 2018; Minachilis *et al*., 2020). In such conditions, the variation in pollinator species could be mostly explained by climatic variables (Klomberg *et al*., 2022) and lead to different floral selection regimes and diverging floral shapes. For example, the lower temperatures prevalent at high elevations are not conducive to some groups of pollinators (e.g., reptilian pollinators), while insect such as bumblebees can survive these conditions due to their physiological abilities (Inouye, 2020). Likewise, large-bodied pollinators (e.g., birds) appear to be positively associated with precipitation, because their flight is less affected by rainfall (Maicher *et al*., 2018) than that of small insects.

Besides abiotic and biotic factors, the evolutionary history of the species is another important factor that can influence floral morphology (Fenster *et al.*, 2004; Smith SD, 2010), because closely related species may be evolutionarily constrained and thus share traits (Harvey and Pagel, 1991). From this respect, and although pollinators and climate can play direct and indirect roles in the modulation of floral evolution, the number of studies investigating this at the interspecific level and large spatial scales (but see Campbell and Powers, 2015; Weber *et al*., 2020) in a phylogenetic context is still small. In this work, we aim to take these three aspects into account and evaluate the relationships of floral morphology, pollinators and climate in a phylogenetic context, using an exceptional plant-pollinator system: the genus *Rhododendron* (Ericaceae) and its pollinators.

Rhododendron is one of the most speciose flowering plant genera (>1000 species), widely distributed in mountainous regions of North America and Eurasia. Within the genus, there is strong diversity in floral traits and pollinator groups across continents. The fact that birds and bumblebees appear to be common pollinators in Asia (Huang *et al*., 2017; Basnett *et al*., 2022), while butterflies have been described as the main pollinator in at least one North American species (Epps *et al*., 2015), could well explain the diversity of floral traits (Huang *et al*., 2017; Basnett *et al*., 2019b). However, given their mountain ecology and widespread global distribution of the genus, it is also possible that floral traits are at least partially explained by climatic variables. From this respect, the genus *Rhododendron* appears as an ideal system to evaluate the potential contributions and interactions of climatic variables and pollinator identity in explaining floral trait variation, allowing us to further investigate the pollination syndrome hypothesis when the abiotic environment is taken into account (Ollerton *et al*., 2009). Here, we aim to test this idea at a global scale by measuring floral traits, pollinators, and climatic preferences of 21 *Rhododendron* species, and then quantifying their correlations in species from two continents (North America-Appalachian and Asia-Himalaya). Specifically, we ask the following questions: 1) what is the floral trait (corolla length, nectar volume, and concentrations) and pollinator variation within the genus?; 2) do floral traits display phylogenetic signal and do they vary across the two regions?; 3) can the identity of pollinators explain floral trait variation in the genus?; 4) what is the role of climate in explaining floral morphology in *Rhododendron*?

MATERIALS AND METHODS

Study sites

Fieldwork was conducted in Asia (India, Sikkim Himalaya, 27˚N, 88˚E) and North America (USA, Appalachian Mountains, 35˚N, 83˚W) (Fig. 1). In India, fieldwork was carried out from April to July 2013-2015 and 2021-2022, in Kyongnosla Alpine Sanctuary, Barsey Rhododendron Sanctuary, and Lachen valley (2300-4230 meters above sea level; m). In Sikkim Himalaya, the temperate forests between 2300-3000 m are formed by tall *Rhododendron* trees co-occurring with oaks and other broad-leaf trees. Between 3000-3800 m, *Rhododendron* dominates the understory of tall conifer subalpine forests, while it occurs as dwarf shrubs with herbaceous plants and dwarf conifers between 3900-4200 m. The largest number of *Rhododendron* species in North America are found in the Appalachian Mountains, where the focus of our fieldwork was in 2022. During the peak flowering months (June and July), we conducted fieldwork in the Southern Appalachian Mountains inside Pisgah and Nantahala National Forests (North Carolina; 1008-1820 m), and Patuxent Research Refuge, Swallow Falls, and Run State Park (Maryland; 39-692 m). In the Appalachians, all *Rhododendron* occurred as understory of native spruce, fir, chestnut, and oak forests.

Selected species

Species selection was made following two strategies. Fieldwork in Sikkim was carried out in locations with high *Rhododendron* diversity: Barsey Rhododendron Sanctuary, Lachen Valley, and Kyongnosla Alpine Sanctuary (Singh *et al*., 2009). Because of the very large number of species and the logistic limitations of studying them all, in Barsey and Lachen, species were selected based on published data from the region (Hooker, 1949-51; Subba *et al*., 2018). Further, in these two locations, rare and less abundant species such as *R. trifloum, R. edgeworthii* and *R. dalhousiae* were not included in the study. Due to a lack of previous data for the Kyongnosla Alpine Sanctuary, at that site we randomly laid 27 50 × 20 m vegetation plots, with all species present within them becoming the focal species (see Basnett *et al*., 2019a for more details). For North American species, we referred to the Flora of North America (https://www.efloras.org), and the plant databases of the American Rhododendron Society (https://www.rhododendron.org/search_intro.asp) and the Azalea Society of America (https://www.azaleas.org/azaleas/). Using this, we focused on the states of North Carolina and Maryland as our two sampling regions because both regions have a relatively high diversity of *Rhododendron* with high overlapping flowering phenologies. Therefore, taking advantage of the peak flowering months, we sampled all species in bloom between June and July. In summary, we studied 15 in Asia (*R. anthopogon, R. arboreum, R. barbatum, R. campanulatum, R. cinnabarinum, R. campylocarpum, R. falconeri, R. grande, R. hodgsonii, R. lanatum, R. lepidotum, R. setosum, R. thomsonii, R. wallichi,* and *R. wightii*), and six *Rhododendron* species in North America (*R. arborescens, R. calendulaceum, R. catawbiense, R. cumberlandense, R. maximum,* and *R. viscosum*).

Floral traits

We measured all floral traits directly in the field for a total of 25 to 30 flowers on up to ten individual plants per species. These ten individuals were randomly selected across localities, and we measured two to five flowers per individual plant. The traits measured were corolla length (vertical length), nectar volume and concentration. We focused on these traits because they had been previously identified as relevant to *Rhododendron* pollinator attraction (Huang *et al*., 2017; Basnett *et al*., 2019b). For corolla measurements, we used a calibrated digital Vernier calliper (Mitutoyo) to an accuracy of 0.01 mm. To measure nectar volume and concentration, flower buds that were likely to open the next day were enclosed in mesh bags that allow air circulation but prevent visits by floral visitors and nectar robbers. The following day, between 8:00 am and 11:00 am, nectar volume and concentration were measured using a micropipette (50 μL) and a pocket refractometer (Vee Gee Scientific), respectively.

Pollinator groups

Pollinator visitation study per species was carried out at each location, and depending on species distribution, the total number of locations varied across species (Table 1). At each locality and for each species, we randomly selected five individuals growing within 15 m to 30 m of each other. For each of the five individuals, we tagged one flowering branch, and performed rotating 10-minute-long observations of the five tagged branches (i.e., we observed the first branch for 10 minutes, then moved to the next one, observed that one for 10 minutes, and continued with this pattern until the observation time for that locality was over). All localities were observed for several hours (starting between 6:00-7:30 AM and ending between 12:30-3:00 PM), and on clear sunny days. Following Stout (2007), only floral visitors seen collecting pollen and depositing it on the stigma were recorded and considered pollinators. All pollinator identities were noted, and visits tallied.

Although we did not directly evaluate the efficiency of each pollinator group, we recorded the frequency of visits, which we used to approximate their importance for pollination. This information has been proposed as a good surrogate for a pollinator's relative importance to the plant species in the absence of data on pollinator efficiency (Fenster *et al*., 2004). Pollinators were classified as birds, bees, butterflies, or flies. All pollinators were photographed using a Digital Single-Lens Reflex Camera (Lumix GH5, lens 50-150 mm). These images were used to carry out online identification to main families (flies) and species (butterflies), using tools present in the Insect Identification (https://www.insectidentification.org/) and Global Biotic Interactions (GloBI, https://www.globalbioticinteractions.org/) services. Further, all observed bees were captured and preserved in 70 % alcohol and transported to the laboratory. In the lab, they were processed, pinned, and sent to an insect taxonomist for identification. Asian bee specimens were identified by Dr. Saini Mallikat and Dr. Yeshwanth H, while Sam Droege identified North American bee specimens. Birds were photographed and identified with the aid of the field guide Birds of India (Kazmierczak and Perlo, 2000) and ebird (https://ebird.org/).

Variation in floral traits and pollinator visits

Variations in floral traits and pollinator visits were studied at two levels. We used a one-way analysis of variance (ANOVA) with an $\alpha=0.05$ to compare mean differences in floral traits and pollinator groups across *Rhododendron* species. Species mean for all individual plants per sampling location were considered for the analysis and all data that did not display normality were log-transformed to meet the test's assumptions. If the ANOVA tests were significant, we performed a non-parametric post-hoc Tukey-Honest Significance Test (Tukey HSD) for floral traits and pollinator groups. Here, significant pairwise comparison values between two plant species would indicate that they display significantly different floral traits and pollinator visits counts.

Because floral traits and pollinators can be phylogenetically structured within the genus, we also conducted phylogenetic comparative analyses to account for the possible influence of the evolutionary history of the genus on floral trait variations. To do this, we used a recent published phylogeny of the genus (Ding *et al*., 2020) and pruned it to retain the species studied here. Using this phylogeny, the phylogenetic signal for the three floral traits studied here was evaluated using two metrics: Pagel's λ (Pagel 1999) and Blomberg's K (Blomberg *et al*., 2003). Pagel's λ can range between 0 (no phylogenetic signal) and 1 (high phylogenetic signal) and is calculated using maximum likelihood approaches. Intermediate values of Pagel's λ indicate that the trait evolution is phylogenetically correlated but does not follow a Brownian motion model of trait evolution (Pagel 1999). Blomberg's K explains the observed degree of similarity among closely-related species compared with expectations obtained from Brownian motion (Kembel *et al.,* 2010). Blomberg's K values close to one indicate a trait evolution consistent with a Brownian motion model, while values close to zero indicate a random distribution of trait values along the phylogeny (Blomberg *et al.,* 2003). Blomberg's K and Pagels λ significance was tested by comparing the observed K and λ value to a null distribution generated by comparing 1000 randomizations of trait values across the phylogenetic tree tips, using the *phylosig* function in the R package phytools (2012).

To determine whether floral traits and pollinator visits vary across the two regions, we considered region (Appalachians and Himalaya) as an independent categorical variable and conducted a phylogenetic ANOVA (pANOVA) using the function *phylANOVA* in the R package phytools (Revell, 2012). For each of these regression models we performed 1,000 simulations with post-hoc t-tests.

Relationship between floral traits and pollinators

For this analysis, we considered the mean of pollinator visits per flower per species as an explanatory variable and the mean of floral traits per species averaged across different localities as the response variable. The response variable was first log10-transformed to reach normality and using these data a Phylogenetic Generalised Least Square regression model (PGLS) was used to test for relationships between floral traits and pollinator groups. PGLS considers phylogenetic non-independence in the data (Freckleton *et al.,* 2002), and the analysis was performed using the functions *corPagel* in the APE R package (Paradis *et al*., 2004), and *gls* of the nlme R package (Pinheiro, Bates, DebRoy and Sarkar; R Core Team, 2018). PGLS was carried out using a correlation structure that accounts for phylogenetic dependencies between species based on Pagel λ index, with $\lambda = 0$ indicating phylogenetic independence, $\lambda = 1$ indicating species covariation following a Brownian motion model of evolution, and $\lambda > 1$ indicating more covariation than expected under a Brownian model. The best-fit models were identified using a stepwise model selection procedure using the Akaike's information criterion (AIC).

Relationships between floral traits, climate, and pollinators

For each sampled locality, we extracted bioclimatic variables from the WorldClim database (https://www.worldclim.org) using a grid resolution of 30 arc-seconds (~ 1 km) (Fick *et al*., 2017). The R package corrplot (Wang *et al*., 2013) was used to determine correlation coefficients between all climatic variables for the Asian or the North American species separately. Variables with a Pearson's correlation coefficient $|r^2|> 0.8$ were removed. Based on this, we retained mean annual air temperature (bio1), temperature seasonality (bio4), and precipitation seasonality (bio15) for the Asian dataset, and mean annual air temperature (bio1), mean diurnal air temperature range 2 (bio2), and precipitation amount of the driest month (bio14) for the North American dataset (Supplementary data Fig. S1) for further analyses.

To understand variation in the climatic preferences of the species, we performed a parallel phylogenetic principal component analysis (pPCA) with the phytools *phyl.pca* function (Revell, 2012) for the Asian and North American species. Once the pPCAs done, the PC scores for the first two PC axes of each species were extracted and used in the following results.

Because the climatic data were obtained separately for each of the two regions (Asia and North America), to understand the relationship between floral traits, pollinators and climatic data, we fitted a PGLS using the function *corPagel* in the APE R package (Paradis *et al*., 2004). For each region, we used pPCA scores of the first two PC axes (PC1, PC2) as climatic predictors. Each species' mean pollinators visits per flower averaged across localities was considered as a second predictor variable, and each species' mean floral trait averaged across localities was used as a response variable. A best-fit model was selected for each floral trait based on AIC (Akaike, 1981). In this analysis, predictor variables significantly contributing to the model and displaying significant interactions were considered important in explaining floral trait variation.

RESULTS

Floral traits

When comparing all species, we found significant differences in nectar concentration (pANOVA, $F_{20, 68}= 47.25$, $P < 0.0001$), nectar volume (pANOVA, $F_{20, 68}= 24.12$, $P < 0.0001$), and corolla length (pANOVA, $F_{20, 68} = 91.53$, $P < 0.0001$). *Rhododendron maximum* had the highest nectar concentrations, and generally nectar concentration in North American species (13.05% to 33.86 %) was higher than in Asian species (3.02 % to 28.22 %) (Table 1). Significant pairwise differences in nectar concentration were observed between many species, but the largest difference was observed between North American species *R. maximum* and all Asian species (Supplementary data Table S1).

In contrast, nectar volume was generally higher in the Asian species (*R. thomsonii, R. arboreum, R. barbatum*, and *R. lanatum* had the highest nectar volume) than in their North American counterparts. Further, the former also showed larger interspecific variations in nectar volume (1.13 to 81.78 µL/flower) than the latter. The largest pairwise difference in nectar concentration was observed between *R. thomsonii* and other Asian and North American species. Himalayan species also showed high interspecific variation in corolla length, ranging from 13.65 to 59.80 mm, with *R. grande*, *R. falconeri* and *R. thomsonii* having the longest corollas. The corolla of *R. griffithianum* and *R. grande* was also significantly longer than that of other Himalayan and North American species (Supplementary data Table S1).

Floral visitors

Birds were the most common pollinators of low elevation Asian species, followed by bees and flies at high elevations. In contrast, primarily bees and butterflies pollinated North American species (Table 1, Supplementary data Table S2). Among Himalayan species, fire-tailed sunbirds (*Aethopyga gouldiae*), Mrs. Gould's sunbirds (*Aethopyga gouldiae*), fire-tailed Myzornis (*Myzornis pyrrhoura*), Tickell's leaf-warblers (*Phylloscopus affinis*), black-faced laughing thrushes (*Trochalopteron affine*), Himalayan white-browed rosefinches *(Carpodacus thura)*, stripe-throated yuhinas (*Yuhina gularis*), rufous-vented yuhinas (*Yuhina occipitalis*), rufous sibias (*Heterophasia capistrata*), hoary-throated Barwings (*Actinodura nipalensis*) and white-collared blackbirds (*Turdus albocintus*) were the most commonly observed bird visitors (Supplementary data Fig. 2). In North America, in contrast, we only observed the migratory ruby-throated hummingbird (*Archilochus colubris*) pollinating *Rhododendron*. Among insect pollinators, in Asia, we encountered *Bombus tunicatus, B. pyrosoma, B. rufofaciatus and B. festivus* at high elevations, and *Apis dorsata* and *A. cerana* at low elevations, while in North America we encountered *Andrena cornelli*, *A. rufosignata* and *Lasioglossum sp.* mostly at low elevations and *Bombus impatiens*, *B. vagans*, *B. sandersoni* and *B. bimaculatus* at all elevations (Supplementary data Fig. 2). In both regions, most of the pollinating flies belonged to the families Muscidae and Syrphidae. Finally, we observed butterflies only in North America, where three butterfly species visited flowers: eastern tiger swallowtails (*Papilio glaucus*), silver-spotted skippers (*Epargyreus clarus*), and great spangled fritillaries (*Speyeria cybele*) (Fig. 2).

At the inter-specific level (and excluding butterflies, present only in North America), pollination by birds (ANOVA, $F_{20, 68} = 22.79$, $P < 0.0001$), bees (ANOVA, $F_{20, 68} =18.72$, $P < 0.0001$), and flies (ANOVA, $F_{20, 68} =3.20$, $P < 0.0001$) varied significantly across species. North American *R. arborescens* and *R. catawbiense* appeared to be strongly preferred by bees and thus showed the largest difference in bee visitation, when compared to the Asian *R. hodgsonii, R. campanulatum* and *R. barbatum*, which were less preferred by bees. High elevation Asian species such as *R. anthopogon* and *R. aeruginosum* were significantly preferred by fly visitors, when compared to all other plant species. In the case of bird pollination, low elevation Asian species *R. arboreum, R. thomsonii, R. barbatum,* and *R. grande* showed significantly more bird visits than the remainder Asian and all North American species (Table 1).

Influence of phylogeny on floral traits and relationship with pollinators

Both Blomberg's K and Pagel's λ showed a significant phylogenetic signal for nectar concentration, while corolla length showed a significant signal only for Pagel's λ. No apparent phylogenetic signal existed in nectar volume across species (Table 2). Similarly, when taking into account the evolutionary relationships, we did not observe significant differences in corolla length (pANOVA, $F = 0.19$, $P = 0.84$), or nectar volume (pANOVA, $F = 2.87$, $P = 0.43$) and concentration (pANOVA, $F =12.3$, $P = 0.12$) in species means between the two regions.

PGLS results indicated that nectar concentration and volume significantly correlate with pollinator identity. Nectar volume showed a significant positive relationship with bird visitation (slope = 0.20, AIC = 35.08, $\lambda = 0.41$, $P = 0.03$; Fig. 3A). In general, birds were more strongly associated with flowers with higher nectar volume and relatively longer corollas. In contrast, nectar concentration showed a significant negative relationship with birds (slope = -0.11, AIC = 11.79, $\lambda = 0.48$, $P < 0.015$; Fig. 3B) and a positive relationship with bees (slope = 0.16, AIC = 11.79, $\lambda = 0.48$, $P < 0.025$; Fig. 3C). No significant relationship between corolla length and pollinator groups were observed. For all the significant PGLS models, the estimated Pagel's λ was typically much greater than zero (Supplementary Table 3).

Climatic preferences

The pPCA results of the Himalayan species demonstrate that 99% of the total variance was explained by the first principal component (pPC1-99.83 % and pPC2-00.15 %) (Supplementary data Fig. 3A, B). The largest contribution to pPC1 was that of temperature seasonality (bio4), while mean annual air temperature (bio1) and precipitation seasonality (bio15) were the most important in pPC2 (Supplementary data Fig. 3B). The results indicates that *R. arboreum, R. grande, R. barbatum* and *R. falconeri* occupy a different climatic niche from the rest of the

Himalayan species, with larger pPC1 scores (Table 1), which indicates that they occur in regions with higher temperature seasonality, while the opposite is the case for *R. lepidotum, R. wightii* and *R. anthopogon* (Supplementary data Fig. 3B). Similarly, 99% of the total variance was explained by the first two pPCA axes for the Appalachian species (pPC1-61.35% and pPC2-38.59%; Supplementary data Fig. 3C, D). The strongest contributor to pPC1 was mean annual air temperature (bio01), while those of pPC2 were mean precipitation of the driest month (bio14) and mean diurnal air temperature range (bio02) (Supplementary data Fig. 3D). *Rhododendron maximum* was shown to occupy a climatic space different from the remainder of the Appalachian species, with lower pPC1 scores (Table 1) and a negative association with mean annual air temperatures. On the other hand, *R. calendulaceum* and *R. cumberlandence* shared similar climatic spaces, positively associated with higher precipitation during the driest month (Supplementary data Fig. 3D).

Floral traits, pollinators, and climate

Among the Himalayan species, nectar concentration appeared to be negatively associated with bird pollination (slope = - 0.74, AIC = 33.73, $P = 0.002$, $\lambda = 0.22$) and positively associated with the interaction between pPC1 and bird pollination (slope = 0.0018, AIC = 33.73, $P = 0.005$, $\lambda = 0.22$). For nectar volume, we observed the best two models to identify significant positive relationships with bird pollination (slope = 0.189, AIC = 29.37, $P = 0.03$, $\lambda = 0.99$) and pPC1 (slope = 0.001, AIC = 39.48, $P = 0.03$, $\lambda = 0.71$), but no significant interactions between these variables. The estimated Pagel's λ was typically higher for nectar volume than for nectar concentration. Corolla length did not show any apparent relationship with neither pollinator identity or climate (Table 3, Supplementary Table 4; Supplementary data Fig. 2).

In North American species, nectar volume showed a significant positive relationship with bee pollination (slope = 0.314, AIC = 11.66, $P < 0.042$, $\lambda = 1.82$). Similarly, nectar concentration appeared to be positively associated with bee visitation (slope = 0.08, AIC = 5.90, $\lambda < 0$, $P < 0.002$). We did not observe any significant relationship between nectar concentration or volume with climatic variables, although we did observe a marginally significant strong positive trend of nectar concentration with butterfly pollination (slope = 2.25, AIC = 0.18, $P = 0.06$, $\lambda = 2.9$). Our PGLS did not identify any significant relationship between corolla length and pollinator identity or climate (Table 3, Supplementary Table 4).

Generally, our results found that pollinators explain the variation in floral traits in the two regions. Overall, our tests identified positive relationships between bird pollination and nectar volume, and bee pollination and nectar concentration. While climatic patterns and their contributions were different across the two regions, significant relationships between floral traits, pollinators and climate were only observed among Himalayan species.

DISCUSSION

This study aimed to understand the extent to which pollinators and climatic conditions affect traits important to pollination using the highly diverse plant genus *Rhododendron* as a model. A second aim of this study was to identify regional and local trends associated with the pollination biology of genus *Rhododendron*. For this, we investigated the intergeneric variation and associations of floral traits and pollinators of *Rhododendron* species from two continents, which allowed us to gain substantial insights into the pollination natural history and biology of the group. Generally, we observed strong interspecific variation in pollinator identity and floral traits within the genus. We found that Asian species are commonly pollinated by birds, bees and flies, while butterflies and bees were the most common pollinators of North American species, indicating biogeographic patterns of variation in the identity of pollinators and thus on potential selective pressures in the genus. Our PGLS supported the presence of pollination syndromes in the genus, but also showed an effect of climate on nectar traits and pollinators. Species pollinated by birds displayed higher nectar volume and lower concentration, than those pollinated by bees or butterflies. Finally, climatic variables such as temperature seasonality and mean annual air temperature also appeared to affect floral traits and interact with pollinator identity to explain floral trait variation, building on the evidence that floral trait variation is driven by contributions (and interactions) of both biotic and abiotic variables. The latter results suggest that changes in climate have the power to affect floral traits important for pollination interactions in this plant group, with potential yet unknown effects on the plant's abilities to sustain fitness under changing climatic conditions.

Pollinators across continents

One of the goals of this study was to characterize the pollinator guild of several *Rhododendron* species from two regions (Asia and North America) to then evaluate whether floral traits relate to different pollinator groups. Prior to our study, our knowledge on *Rhododendron* pollinators was restricted and strongly spatially biased. For example, birds had been reported as common pollinators in several Eastern Himalaya and Asian high-elevation species, with specialist nectarivores (e.g., fire-tailed sunbirds, *Aethopyga gouldiae, A. ignicauda*) and non-specialist nectarivores (e.g., warbling white-eye, *Zosterops japonicus*; leaf-warbler, *Phylloscopus affinis*; black-faced laughing thrush, *Trochalopteron affine*) described as common pollinators of several *Rhododendron* in Sikkim-India, China and Nepal (Georgian *et al*., 2015; Huang *et al*., 2017; Ollerton, 2020; Basnett *et al*., 2019b). Among insects, bumblebees and flies had been reported as common floral visitors of Eastern Himalayan *Rhododendron* (e.g., China and Sikkim-India, Huang *et al*., 2017; Basnett *et al*., 2019b). While the pollinators of Himalayan *Rhododendron* were known to some extent, our knowledge of the reproductive biology of North American species was extremely restricted. Among the thirty North American species, and until the present study, knowledge of floral visitors was available only for the Flame Azalea (*R. calendulaceum*), for which large butterflies (especially *P. glaucum*) had been recognized as performing nearly all pollination (Epps *et al*., 2015). In this context, our study presents a clear advance in our understanding of the reproductive natural history of *Rhododendron* across large spatial scales and, through the implementation of similar protocols, allows for a comparison across continents. Here, we identified some similarities and differences across regions. While we found that bumblebees and nectarivorous birds are floral visitors in both regions, we also found pollination specificities in each continent: bird pollinators are clearly more abundant in the Himalayan species than in the Appalachian, while butterfly pollination is restricted to North American species.

In the Himalayas, we found bumblebee pollination to be common in high-elevations species, and bird pollination to be common among low- and mid-elevation taxa. Our results recovered pollination patterns that agree and expand those found in other works. For instance, previous work had identified birds as important contributors to *Rhododendron* reproduction in China (Huang *et al*., 2017), and our study expands this information to more species. Further, we recovered a relationship between general biodiversity patterns and floral visitation in the Himalayas. Indeed, the Eastern Himalayan Mountain system supports a hyper-diverse bird community, with maximum diversity recorded mainly at mid-elevations (Price *et al*., 2014), where the highest *Rhododendron* diversity is also found (Basnett *et al*., 2022). Coincidentally, this is where we identified *Rhododendron* to be most commonly visited by birds. Building on this, an interesting hypothesis worth testing in future works is whether this high bird diversity could explain the high diversity of *Rhododendron* in this area. From a conservation perspective, the elevational pattern we observed (i.e., bird pollination at mid- and low elevations and bumblebee pollination at high elevations) should be considered when evaluating the ability of *Rhododendron* to reproduce under climate change.

Contrasting with the Himalayas, (bumble)bees and butterflies were the most common pollinators of North American species, while bird pollination was uncommon (the seasonal and migratory ruby-throated hummingbird was the only bird occasionally seen pollinating two *Rhododendron* species). It appears that a unique feature of floral visitation in North America was the strong presence of butterflies, a pollinator never observed in the Himalayan species studied here. Our observations indicate that although not always the most frequent, butterfly pollinators are the most efficient, visiting the largest number of flowers per plant visit. Further, their wing-flapping behaviour allowed contacting both the male and female reproductive organs, contributing to pollen transfer (pers. obs.). This result agrees with previous observations that butterflies appear to be efficient pollinators of one North American *Rhododendron* (*R. calendulaceum*; Epps *et al*., 2015), morphologically similar to other butterfly-visited American species studied here (i.e., *R. arborescens*, *R. viscosum*, and *R. cumberlandense*). This stark difference in main floral visitation groups between the Appalachians (butterflies) and the Himalayas (birds) was surprising at first. In fact, although North America's east coast is comparatively poorer in bird richness than the Himalayas (Quintero and Jetz, 2018), the Himalaya has in fact a high diversity of butterflies. However, our results may also be reflective of the evolutionary and biogeographic history of this pollinator group. In fact, butterflies have been shown to have originated in North and Central

America, colonizing Asia only later on in their evolution (Kawahara AY. *et al*., 2023). Such a scenario would have provided more (co)evolutionary opportunities to North American than Asian plant species to establish mutualistic interactions with this group of pollinators, which could also explain the intercontinental differences we observe. Beyond historical factors, the phenology of the plant species may also explain this observation: the *Rhododendron* flowering season in the Himalayas (May-August) overlaps strongly with that of Primulaceae and Rosaceae, which are commonly pollinated by butterflies (Huang *et al*., 2015; Gurung *et al*., 2018; Paudel *et al*., 2019). It is thus possible that in this region butterflies prefer non-*Rhododendron* flowers, avoiding competition with bird pollinators, which could also explain in part the biogeographic pattern we observe.

Along with butterflies, (bumble)bees were the most common pollinators of North American *Rhododendron*, visiting flowers for both nectar and pollen, and likely contributing to pollen deposition through buzz pollination and their constant movement from the male reproductive parts to the nectar pouches (pers. obs.). The case was slightly different for smaller *Andrena* bees, which were abundant below 1000 m, and which likely contribute less than bumblebees to pollination, given that they rarely touch the female reproductive organs (pers. obs.). Finally, we also observed occasional hummingbird visits in North America. These birds are common and efficient pollinators in the region (Wessinger *et al*., 2019; Kay and Grossenbache, 2022), even at low visitation rates (Janeček *et al*., 2011; Song *et al*., 2019). It is thus likely that these birds can contribute to the pollination of the two plant species they were observed on, although this will have to be explicitly tested in future studies.

Pollinators and floral traits

Considerable evidence indicates that pollinators can drive the evolution of plants and flowers (Van der Niet and Johnson, 2014). Floral morphology has been shown to evolve in response to the selection induced by suites of pollinators. As a result, over time, it may lead to morphological convergence in floral morphology in association with a pollinator group (Fenster *et al*., 2004). The convergence in floral traits has been commonly shown to occur at the family and genus level in several plant groups (Newman *et al*., 2014; Murúa and Espíndola, 2015; Lagomarsino *et al*., 2017), and recently, there has been strong evidence to support intraspecific associations between floral traits and floral visitors at large spatial scales (Weber *et al*., 2020; Neves *et al*., 2020). Our transcontinental field-based study suggests that *Rhododendron*'s large floral and species diversity is likely driven at least partially by their pollinators, suggesting that pollinators may be playing and/or may have played a key role in the extreme diversity of the genus. These results are analogous to those observed in other plant systems and indicate a connection between floral traits important to pollination attraction and flower-pollinator fitting, and pollinator group (Smith, 2008; Muchhala, 2014; Garcia *et al*., 2020). Thus, our results agree with predictions made by the pollinator syndromes hypothesis.

Specifically, our PGLS identified nectar volume to be significantly and positively correlated with bird pollination in most Himalayan species. Interestingly, and although we did not consider floral colour in the present study, most of these bird-preferred species with higher nectar volume are red, crimson, or orange (Basnett *et al*., 2019a; Georgian *et al*., 2015; Huang *et al*., 2017), colours usually related to bird pollination syndromes (Faegri and van der Pijl,1979; Fenster *et al*., 2004). Further, among bird-visited species, such taxa as *R. thomsonii* and *R. arborescens* commonly visited by obligatory nectarivorous birds (hummingbirds and sunbirds) had significantly higher nectar volumes (Table 1) than those visited by facultative nectarivorous birds (e.g., laughing thrush, warblers), which could further indicate that it is not just the identity but also the level of specialization of the floral visitor that can be driving floral evolution in the group (Chmel *et al*., 2021).

Another general trend we recovered in our analysis was the positive relationship between nectar concentration and bee pollination. Insects, especially (bumble)bees and flies, are known to prefer flowers with concentrated nectar (Baker and Baker, 1990; Hill *et al*., 2001; Pamminger *et al*., 2019). Although this trend is shared across the two continents, the North American species generally displayed significantly higher nectar concentrations than their Himalayan counterparts, likely due to their higher insect visitation rates. We also recorded abundant bumblebee visits among North American *Rhododendron* species; bumblebees are usually known to prefer more concentrated nectar because they produce substantial quantities of metabolic water during flight, which counteracts water loss through evaporation and excretion, making them less reliant on dilute nectars for rehydration (Nicolson, 2009). In contrast to bumblebees, honeybees prefer warmer and less concentrated nectar (Nicolson *et al*., 2013). Interestingly, we observed a trend that matches this evidence: in our sampling, *Apis* bees were common floral visitors of low-elevation Himalayan species (e.g., *R. arboreum, R. barbatum*), which coincidentally display diluted and high-volume nectar (Table 1).

Finally, we also found that plant species pollinated mostly by butterflies (e.g., *R. arborescens* and *R. viscosum*) displayed lower nectar concentrations than those pollinated by bumblebees (e.g., *R. catawbiense, R. maximum*), a trend also found in other plant groups (Baker and Baker, 1983). Although we did not measure reproductive organ arrangements and flexion, butterfly visitation was common among species that displayed extended, loosely bundled, and upwardreflexed stamens and pistils. Interestingly, such floral characteristics were common among North American *Rhododendron* species.

Climate, pollinators, and the floral morphology of *Rhododendron*

Many controlled and field-based experiments have shown that climate can affect floral morphology (Weber *et al*., 2020), pigmentation (Koski *et al*., 2020; Sullivan and Koski, 2021) and floral rewards (Takkis *et al*., 2015; McCombs *et al*., 2022). These changes in floral traits in response to abiotic conditions suggest that these variables have the potential to impact floral attractiveness to visitors. In this context, our study identifies a trend in the relationship between floral traits and climatic conditions, indicating that climate explains some of the variations in floral traits in the species studied here. Specifically, we found temperature seasonality and mean annual air temperature to be significantly correlated with nectar traits and pollinator identity in the Himalayas and North America, respectively. Further, we also found that Himalayan species with high nectar volumes and long corollas (*R. arboreum, R. grande, R. falconeri, R. barbatum*) were present at localities with higher temperature seasonality, mean annual air temperature and precipitation seasonality, and that birds were the most common pollinators of these plant taxa. In a context of climate change, this result suggests that climate has the power to affect pollination-relevant traits in *Rhododendron*, with unknown effects on the species' survival.

Both nectar traits and flower size have been suggested to be correlated with temperature and water availability (Carroll *et al*., 2001; Gallagher and Campbell, 2017; Takkis *et al*., 2017; Kuppler and Kotowska, 2021). First, although the literature is not conclusive, the relative content of sugars in nectar can vary in response to temperature: while some studies describe no correlation between nectar concentration and this environmental factor (Clearwater *et al*., 2018), others suggests that increases in temperature can decrease nectar secretion (Takkis *et al*., 2018), and yet others find that moderately elevated temperatures can increase it (Pacini and Nepi, 2007; Nocentini *et al*., 2013). Further, precipitation has been shown to be associated with the presence of dilute nectar (Eisikowitch and Woodell, 1975), which could make these flowers more attractive to birds than to other types of pollinators (Baker and Baker, 1983). Further, at least one study suggests that dry environmental conditions can be associated with low amounts of concentrated nectar (Devoto *et al*., 2006), especially in temperate regions. Furthermore, high rainfall has been associated with reduced insect visits (Lawson and Rands, 2019), but does not generally affect bird visitation (Ortega-Jimenez and Dudley, 2012; Gonzalez *et al*., 2009). Our results recovered trends that agree with many of these studies. On the one hand, in the Himalayas, species experiencing the highest temperature seasonality, mean annual air temperature and precipitation seasonality were those which displayed the longest corollas and largest nectar volumes, and which were most commonly pollinated by birds. Interestingly, these same species also experience the highest rainfall and temperature during the onset and peak flowering season (Basnett *et al*., 2019a). Our results support the idea that in this region both climatic conditions and pollinator identity can interact, leading to the trends we observed: increased bird pollination in high precipitation conditions.

In contrast to the Himalayan species, we observed generalized high nectar concentration among the North American species, which were commonly preferred by bees. In this region, however, we did not recover any significant interaction between climate and pollinators on floral traits, and this could due to our species sample size in this region. However, it is of note that we recovered a marginally-significant positive interaction between butterfly visitation and mean annual air temperature. The flowering of *Rhododendron* in Maryland and North Carolina starts in late April and peaks between June and July, and during this time the region also experiences high temperature. Warm climatic condition and clear days are known to have a positive influence on butterfly visitation, as butterflies are ectothermic, deriving their internal heat almost exclusively through basking (Heinrich,1993). Bumblebees, on the other hand can tolerate both warm and cold temperatures (Heinrich, 1993), which could provide another line of evidence for increased bumblebee visitation and decreased butterfly visitation among high elevation North American *Rhododendron* species.

CONCLUSIONS

Pollinator visits and climate have the potential to explain variations in floral traits in plants. Here, we studied the genus *Rhododendron* and confirmed the presence of a relationship between pollinator identity and floral traits in the genus. Generally, our results showed that nectar traits appear to be correlated with pollinator groups. We show that bird-visited plants display large nectar volumes, while bee visited flowers have flowers with high nectar concentration. We also found substantial evidence of a role played by climatic variables on explaining floral traits, especially in the Himalayas. While we observed interactions between bird visitation with temperature seasonality in most Himalayan species, we found that butterfly and bee visitation explained floral trait variation in North American species. We thus demonstrate, using transcontinental comparisons, that both floral visitors and climatic conditions can drive floral trait variation in one of the most globally diverse groups of plants.

Despite these results representing clear progress in the characterization of the pollinator cohorts, trait variation and presence of pollination syndromes in *Rhododendron*, our work also opens new research avenues for the field. For example, future analyses should directly quantify pollen deposition by each pollinator group, and test for nocturnal pollination. Further, while here we focused on main pollinator groups, future studies could expand their sampling to investigate species-level pollinator abundance, behaviour and level of interactions with *Rhododendron* species. Moreover, because we identify traits that appear to be important to pollinator choice and that can respond to climatic conditions, future works should investigate short-term effects in the context of climate change, and long-term evolutionary trends in these floral traits using phylogenetic tools. Such work would allow, on the one hand, understanding their roles in the diversification of the genus and, on the other, provide a predictive framework to evaluate impact and develop conservation strategies for these species in the face of climate change.

SUPPLEMENTARY DATA

Supplementary data consist of the following.

Table S1: Non-parametric post-hoc Tukey-Honest Significance Test result for floral traits.

Table S2: Non-parametric post-hoc Tukey-Honest Significance Test result for pollinator groups.

Table S3: Summary of Phylogenetic Generalized Least Squares Model fitting for the global floral traits to pollinator visitation frequency.

Table S4: Summary of Phylogenetic Generalized Least Squares Model fitting for the Asian and North American floral traits to pollinator visitation frequency and climate.

Fig. S1. Correlation plot of bioclimatic variable for Asia (A) and North America (B).

Fig. S2. Relationship between floral traits and pollinator groups. Different symbols represent within-group pollinator compositions; empty bee symbol: only bumblebees observed; filled bee symbol: bumblebees and other bees observed; filled bird symbol: high obligatory nectarivores observed; empty bird symbol: high facultative nectarivores observed.

Fig. S3. Phylomorphospace of PC1 and PC2 from a phylogenetic PCA using climatic data of Asian (A) and North American (C) species. Tip colours indicate different species. Phylogenetic PCA of bioclimatic variables for the Himalayan (B) and the Appalachian (D). Only pPC1 and pPC2 are shown.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Elevational range, number of sampled localities, total number of flowers measured, mean floral trait values (± SE), pollinator visits/flower, and climatic PC scores for all species studied in this work. Species are sorted by continent and then by nectar concentration.

Species	Elevation (m.a.s.l.)	Localities (n)	Flowers (n)	Nectar conc. (%)	Nectar vol. (μL)	Corolla length (mm)	Average pollinator visits/flw.				Climate	
							Bird	Bee	Fly	Bfly	PC1	PC2
North America												
R. maximum	450-488	3	25	33.86 +/-0.86	3.49 +/-0.20	34.16 +/-0.55	0	2.6	0.12	0	5.64	-36.8
R. catawbiense	1800-1820	2	25	24.97 +/-0.68	8.33 +/-0.31	42.92 +/-2.06	0	3.57	0.02	0	46.01	12.03
R. arborescens	692-1622	4	25	24.71 +/-0.28	8.82 +/-0.10	40.83 +/-0.75	0.17	0.95	0.06	0.1	-24.07	-0.94
R. viscosum	39-1000	4	25	15.92 +/-0.31	3.39 +/-0.14	32.96 +/-1.35	0.16	0.23	0.42	0.12	19.13	-6.61
R. calendulaceum	1642	2	25	15.54 +/-0.09	2.14 +/-0.11	32.45 +/-0.10	0	0.74	0.14	0.02	-35.04	23.46
R. cumberlandense	1462-1554	2	25	13.05 +/-0.99	3.6 +/-1.00	37.15 +/-0.97	0	0.37	0.01	0.03	-31.04	17.97
Asia												
R. lepidotum	3900-4230	5	30	28.22 +/-0.52	8.70 +/-0.47	13.65 +/-0.23	0	0.21	0.28	0	26.36	-16.3
R. setosum	3900-4000	8	30	15.36 +/-0.35	1.27 +/-0.05	18.66 +/-0.11	0	0.67	0.31	0	-236.81	-16.6
R. wallichi	3800-3900	2	25	13.23 +/-1.41	6.89 +/-0.71	35.5 +/-0.42	0	0.01	0.17	0	-514.68	7.91
R. anthopogon	3800-4230	7	30	13.17 +/-0.55	1.13 +/-0.05	19.02 +/-0.33	0	0.27	0.02	0	31.02	-14.6
R. grande	2600-2900	3	28	11.25 +/-1.95	14.23 +/-2.27	59.8 +/-0.84	0.2	0.04	0.05	0	352.19	38.46
R. wightii	4000-4230	5	30	10.33 +/-0.27	5.93 +/-0.41	43.87 +/-0.54	0.07	0.16	0.29	0	21.91	-17.7
R. campanulatum	3400-3900	9	30	10.15 +/-0.36	9.73 +/-0.35	43.44 +/-0.36	0.25	0.06	0.16	0	75.19	-0.48
R. campylocarpum	3600-3800	4	30	9.71 +/-0.83	4.94 +/-0.41	37.89 +/-0.42	0.13	0.15	0.29	0	-248.49	8.11
R. falconeri	2700-2900	2	28	7.96 +/-0.19	22.08 +/-2.80	53.67 +/-1.44	0.24	0.08	0.01	0	355.01	37.8
R. lanatum	3700-3800	2	25	5.8 +/-0.56	39.16 +/-2.07	49.56 +/-0.44	0.47	0.02	0.36	0	76.56	1.65
R. cinnabarinum	3200-3600	5	30	5.68 +/-0.13	17.75 +/-0.56	38.67 +/-0.46	0.43	0.17	0.05	0	-5.64	10.59
R. barbatum	2800-2900	2	28	5.2 +/-0.07	33.05 +/-4.21	35.36 +/-0.74	0.34	0.02	0.03	0	358.01	37.8
R. hodgsonii	3500-3800	5	30	5.03 +/-0.18	11.92 +/-0.93	36.48 +/-0.49	0.82	0.04	0.06	0	76.57	2.23
R. arboreum	2600-2900	5	28	4.09 +/-0.81	56.07 +/-2.73	43.61 +/-0.17	0.64	0.41	0.05	0	358.55	39.13
R. thomsonii	3400-3800	6	30	3.02 +/-0.10	81.78 +/-5.43	50.74 +/-0.39	0.57	0.05	0.03	0	59.99	-4.93

Table 2. Strength and statistical significance of phylogenetic signal (Blomberg's K and Pagel's λ) of the floral traits. Significant values are bolded.

Traits	Blomberg's K	*P*-value	Pagel's λ	*P*-value
Corolla length	0.42	0.07	6.47	0.01
Nectar concentration	0.84	0.003	8.57	0.003
Nectar volume	0.35	0.28	0.18	0.67

Table 3. Summary of results of the Phylogenetic Generalized Linear Model fitting of climatic variables and pollinator type visitation frequency and their interactions, to floral traits, per continent. Signs indicate direction of the effect (positive or negative) of significant variables. Symbols indicate levels of significance: * $P < 0.05$ **, $P < 0.01$.

Continent	Trait	Fly	Bee	Bird	Butterfly	pPC1	pPC2	Bird pollination ×		Butterfly pollination ×	
								pPC1	pPC2	pPC1	pPC2
Asia	Nectar Volume			+*		-*					
	Nectar Concentration			-**				+**			
	Corolla Length										
North America	Nectar Volume		+*								
	Nectar Concentration		+**		+						
	Corolla Length										

Fig. 1. Sampling localities of the *Rhododendron* species studied here. Asia - Sikkim Himalaya (A), North America - Appalachians (B). Sampling localities are shown with red dots. Background colour indicates elevation (see figure legend for scale). Inset figures highlights region.

Fig. 2. Species observed as pollinators of *Rhododendron* in North America (A-G) and Asia (H-T). A. *Bombus sp*. visiting *R. arborescens*. B. Eastern tiger swallowtail on *R. arborescens*. C. *Bombus sp.* and *R. catawbiense*. D. Ruby-throated hummingbird and *R. arborescens*. E. *Bombus sp.* and *R. maximum*. F. Silver-spotted skipper on *R. arborsecens*. G. *Andrena sp*. on *R. calendulaceum*. H. Male Fire-tailed sunbird and *R. thomsonii*. I. Rufous-vented yuhina and *R. falconeri.* J. Flies and *R. campylocarpum.* K. *Bombus sp.* and *R. campanulatum*. L. Hoverfly and *R. setosum*. M. Hoary-throated Barwing and *R. arboreum*. N. *Bombus sp.* and *R. setosum.* O. *Bombus sp*. and *R. anthopogon*. P. Rufous sibia and *R. arboreum*. Q. *Bombus sp*. and *R. lepidotum*. R. Female Fire-tailed sunbird and *R. cinnabarinum*. S. Female Fire-tailed sunbird and *R. campylocarpum.* T. Black-faced laughing thrush and *R. hodgsonii*.

Fig. **3**. Relationship between floral traits with pollinator groups. Points represent species means with ± SE. Solid lines show trend in floral traits as measured using Phylogenetic Generalized Least-Squares.