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Floral traits and their connection with pollinators and climate

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- **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 geographical 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 might drive variation in floral traits can serve to protect plant–pollinator interactions in globally 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 on 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, whereas 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 Himalayas 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 in interaction with each other.

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

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 been attributed primarily 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 and 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 have shown that the optimal nectar concentration for active or capillary suction feeders (e.g. birds and butterflies) is 30–40 %, whereas that for viscous dippers (bees and flies) is 50–60 % (Kim *et al.*, 2011), as has been observed in flowers visited mainly by these pollinator groups. Furthermore, the hypothesis also predicts that shape and colour will also evolve as a response to pollinator pressure, because different pollinators have different morphologies and visual acuities. For instance, ornithophilous flowers display warm-coloured, long and tubular flowers, whereas bee-visited flowers are open and mostly blue and/or yellow in colour (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 explain floral traits in many plant groups accurately, 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). Furthermore, in many cases, other non-pollinator variables might be contributing to floral traits (e.g. herbivores, parasites and 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 and 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 might 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, given that nectar consists of 50–90 % water, temperature and water availability have been shown to affect nectar production both directly and indirectly (Pacini *et al.*, 2003; Nicolson and Thornburg, 2007). Furthermore, 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 higher 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 attraction of floral visitors (Scaven and Rafferty, 2013; Parachnowitsch *et al.*, 2018; Miladin *et al.*, 2022), which could further reinforce these floral divergences. Additionally, climate can also affect the presence of pollinator fauna (Espíndola and Pliscoff, 2019; Shah *et al.*, 2020) in 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 explained mostly 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), whereas insects such as bumblebees can survive these conditions owing 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, 2010), because closely related species can be evolutionarily constrained and thus share traits (Harvey and Pagel, 1991). In 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 species-rich 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 and Ganesan, 2022), whereas 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.*, 2019*b*). However, given their mountain ecology and the widespread global distribution of the genus, it is also possible that floral traits are explained, at least in part, by climatic variables. In this respect, the genus *Rhododendron* appears to be an ideal system in which to evaluate the potential contributions and interactions of climatic variables and pollinator identity in explaining floral trait variation, allowing us to investigate the pollination syndrome hypothesis further, 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, then quantifying their correlations in species from two continents (North America, Appalachians 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 (Sikkim Himalaya, India, 27°N, 88°E) and North America (Appalachian Mountains, USA, 35°N, 83°W) (Fig. 1). In India, fieldwork was carried out from April to July 2013–2015, 2021 and 2022, in Kyongnosla Alpine Sanctuary, Barsey Rhododendron Sanctuary and Lachen

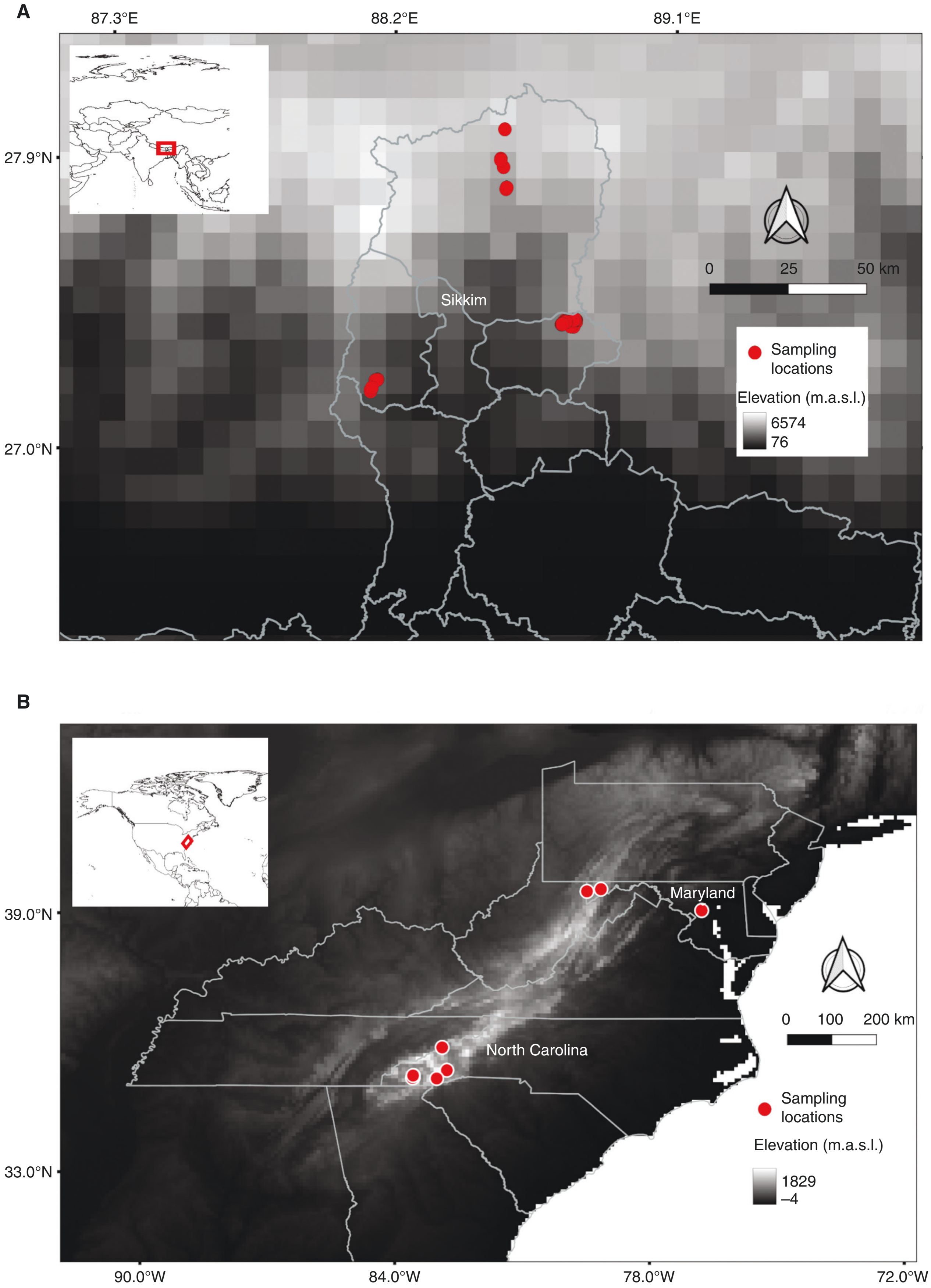

FIG. 1. Sampling localities of the *Rhododendron* species studied here. (A) Sikkim, India. (B) MD and NC, USA. Sampling localities are shown with red dots. Background colour indicates elevation (see key for scale). Insets highlight regions sampled in India and the USA.

valley (2300–4230 m a.s.l.). In Sikkim Himalaya, the temperate forests between 2300 and 3000 m a.s.l. are formed by tall *Rhododendron* trees co-occurring with oaks and other broadleaf trees. Between 3000 and 3800 m a.s.l., *Rhododendron* dominates the understorey of tall conifer subalpine forests, and between 3900–4200 m a.s.l. it occurs as dwarf shrubs with herbaceous plants and dwarf conifers. The largest numbers 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 (NC, USA; 1008–1820 m a.s.l.), and Patuxent Research Refuge, Swallow Falls and Run State Park (MD, USA; 39–692 m a.s.l.). In the Appalachians, all *Rhododendron* occurred as understorey of native spruce, fir, chestnut and oak forests.

Selected species

Species selection was made following two strategies. Fieldwork in Sikkim, India 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). Furthermore, in these two locations, rare and less abundant species, such as *Rhododendron trifloum*, *R. edgeworthii* and *R. dalhousiae*, were not included in the study. Owing to a lack of previous data for the Kyongnosla Alpine Sanctuary, at that site we randomly laid 27 vegetation plots measuring 50 m × 20 m, with all species present within them becoming the focal species (for more details, see Basnett *et al.*, 2019*a*). 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 these, we focused on the states of NC and MD 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 *Rhododendron* species in Asia (*Rhododendron 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 in North America (*Rhododendron 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 25–30 flowers on up to ten individual plants per species. These ten individuals were selected randomly across localities, and we measured two to five flowers per individual plant. The traits measured were corolla length (Supplementary Data Fig. S1), nectar volume and nectar concentration. We focused on these traits because they had been identified previously as relevant to *Rhododendron* pollinator attraction (Huang *et al.*, 2017; Basnett *et al.*, 2019*b*). For corolla measurements, we used calibrated digital Vernier callipers (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 0800 and 1100 h, nectar volume and concentration were measured using a micropipette (50 µL) and a pocket refractometer (Vee Gee Scientific), respectively.

Pollinator groups

A 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 15–30 m of each other. For each of the five individuals, we tagged one flowering branch and performed rotating 10-min-long observations of the five tagged branches (i.e. we observed the first branch for 10 min, then moved to the next one, observed that one for 10 min, and continued with this pattern until the observation time for that locality was over). All localities were observed for several hours (starting between 0600 and 0730 h and ending between 1230 and 1500 h) 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 evaluate the efficiency of each pollinator group directly, we recorded the frequency of visits, which we used to approximate their importance for pollination. This information has been proposed to be a good surrogate for the relative importance of a pollinator 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. Furthermore, all observed bees were captured and preserved in 70 % alcohol and transported to the laboratory. In the laboratory, 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, whereas North American bee specimens were identified by Sam Droege. 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 ANOVA with $\alpha = 0.05$ to compare mean differences in floral traits and pollinator groups

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

Species	Elevation (m a.s.l.)	Localities (***n***)	Flowers (***n***)	Nectar concentration (%)	Nectar volume (µL)	Corolla length (mm)	Average pollinator visits per flower				Climate	
							Bird	Bee	Fly	Butterfly	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

across *Rhododendron* species. The species mean value 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 assumptions of the test. If the ANOVAs were significant, we performed a non-parametric post-hoc Tukey's honest significance difference (HSD) test 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 visit 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 recently 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 zero (no phylogenetic signal) and one (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, and values close to zero indicate a random distribution of trait values along the phylogeny (Blomberg *et al.*, 2003). Blomberg's *K* and Pagel's λ significance was tested by comparing the observed *K* and λ values with 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 1000 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 $\log_{10}$-transformed to reach normality, and using these data, a phylogenetic generalized 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 *et al.*, 2018). PGLS was carried out using a correlation structure that accounts for phylogenetic dependencies between species based on Pagel's 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-fitting models were identified using a stepwise model selection procedure using 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 and Hijmans, 2017). The R package corrplot (Wei, 2013) was used to determine correlation coefficients between all climatic variables for the Asian or the North American species separately. Variables with 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. S2) 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 were done, the principal component (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 and PC2) as climatic predictors. For each species, the mean pollinator visits per flower averaged across localities was considered as a second predictor variable, and for each species the mean floral trait averaged across localities was used as a response variable. A best-fitting model was selected for each floral trait based on the 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 (ANOVA, $F_{20,68} = 47.25$, $P < 0.0001$), nectar volume (ANOVA, $F_{20,68} = 24.12$, $P < 0.0001$) and corolla length (ANOVA, $F_{20,68} = 91.53$, $P < 0.0001$). *Rhododendron maximum* had the highest nectar concentrations, and generally the nectar concentration in North American species (13.05–33.86 %) was higher than in Asian species (3.02–28.22 %) (Table 1). Significant pairwise differences in nectar concentration were observed between many species, but the largest difference was observed between the 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. Furthermore, the former also showed larger interspecific variations in nectar volume (1.13–81.78 μL per 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. 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 ignicauda*), 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. S3). In North America, in contrast, we observed only 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, whereas 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. S3). 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

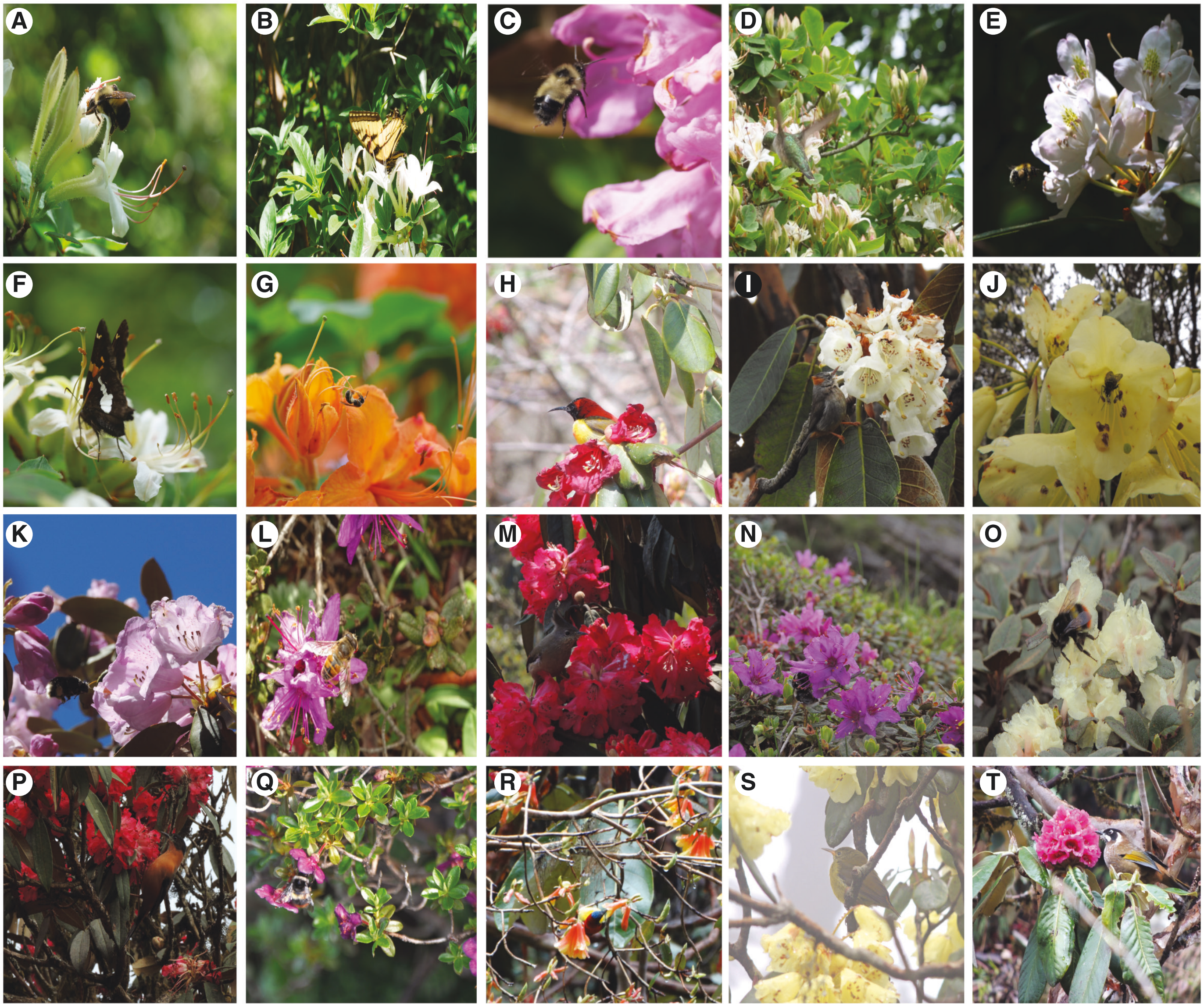

FIG. 2. Species observed as pollinators of *Rhododendron* in North America (A–G) and Asia (H–T). (A) *Bombus* sp. visiting *R. arborescens*. (B) Eastern tiger swallowtail on *R. arborescens*. (C) *Bombus* sp. and *R. catawbiense*. (D) Ruby-throated hummingbird and *R. arborescens*. (E) *Bombus* sp. and *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*.

tiger swallowtails (*Papilio glaucus*), silver-spotted skippers (*Epargyreus clarus*) and great spangled fritillary (*Speyeria cybele*) (Fig. 2).

At the interspecific 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 preferred strongly by bees and thus showed the largest difference in bee visitation when compared with the Asian *R. hodgsonii*, *R. campanulatum* and *R. barbatum*, which were less preferred by bees. High-elevation Asian species such as *R. anthopogon* were significantly preferred by fly visitors when compared with 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 of 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, whereas corolla length showed a significant signal only for Pagel's λ.

No apparent phylogenetic signal existed in nectar volume across species (Table 2). Likewise, 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.

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

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

The PGLS results indicated that nectar concentration and volume were significantly correlated 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 relationships 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 Data Table 3).

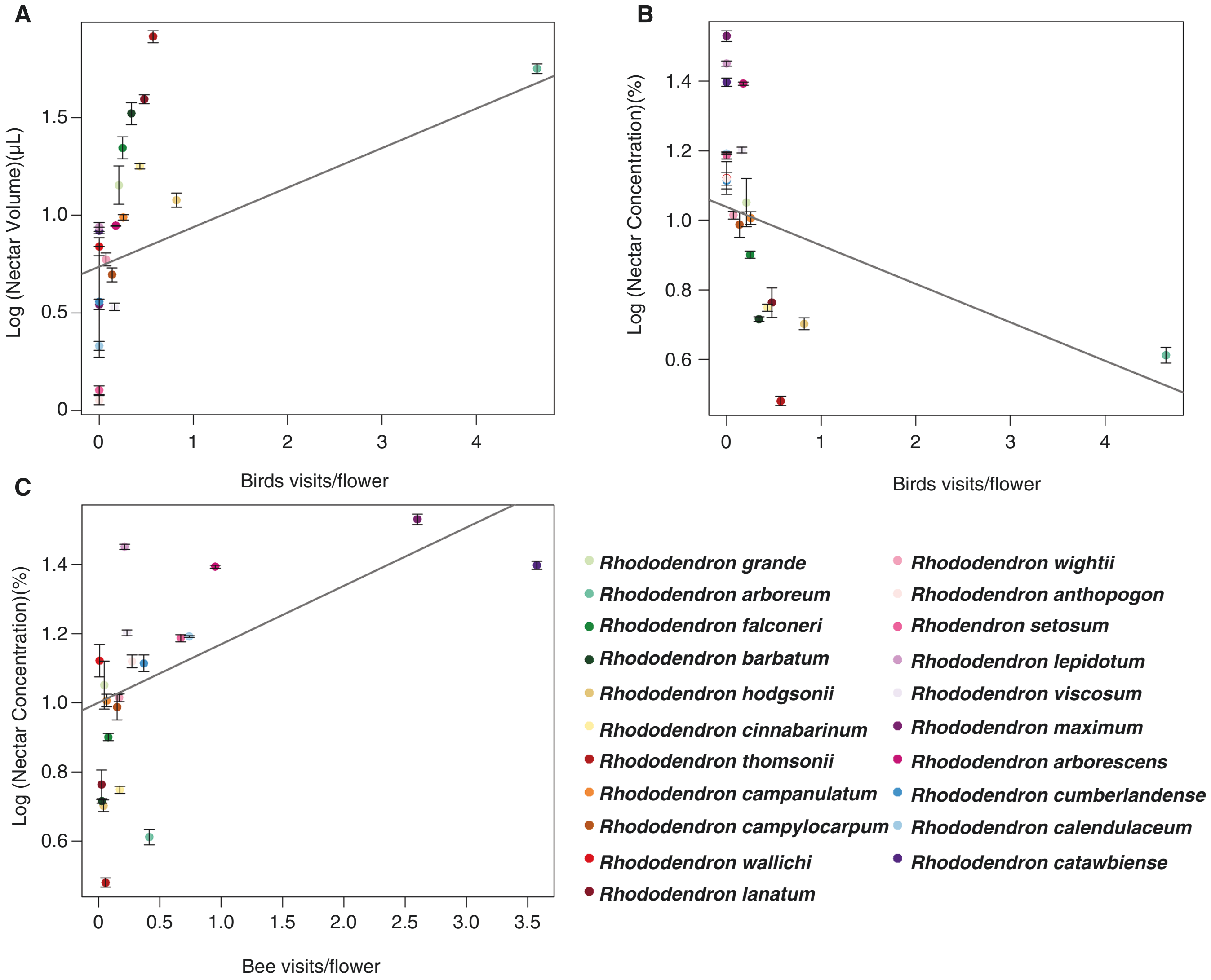

Fig. 3. Relationships between floral traits and pollinator groups. Points represent species means ± s.e. Continuous lines show the trend in floral traits as measured using phylogenetic generalized least squares.

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 the direction of the effect (positive or negative) of significant variables.*

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										

*$P < 0.05$ and **$P < 0.01$.

Climatic preferences

The pPCA results of the Himalayan species demonstrated that 99 % of the total variance was explained by the first principal component (pPC1, 99.83 % and pPC2, 00.15 %) (Supplementary Data Fig. S4A, B). The largest contribution to pPC1 was that of temperature seasonality (bio4), whereas mean annual air temperature (bio1) and precipitation seasonality (bio15) were the most important in pPC2 (Supplementary Data Fig. S4B). The results indicate that *R. arboreum*, *R. grande*, *R. barbatum* and *R. falconeri* occupy a different climatic niche from the rest of the Himalayan species, with higher pPC1 scores (Table 1), which indicates that they occur in regions with higher temperature seasonality, whereas the opposite is the case for *R. lepidotum*, *R. wightii* and *R. anthopogon* (Supplementary Data Fig. S4B). Likewise, 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. S4C, D). The strongest contributor to pPC1 was mean annual air temperature (bio01), whereas those of pPC2 were mean precipitation of the driest month (bio14) and mean diurnal air temperature range (bio02) (Supplementary Data Fig. S4D). *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. In contrast, *R. calendulaceum* and *R. cumberlandence* shared similar climatic spaces, positively associated with higher precipitation during the driest month (Supplementary Data Fig. S4D).

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 either pollinator identity or climate (Table 3; Supplementary Data Table 4).

In North American species, nectar volume showed a significant positive relationship with bee pollination (slope = 0.314, AIC = 11.66, $P < 0.042$, $\lambda = 1.82$). Likewise, 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 Data Table S4).

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 between bee pollination and nectar concentration. Although climatic patterns and their contributions were different across the two regions, significant relationships between floral traits, pollinators and climate were observed only among Himalayan species.

DISCUSSION

The primary aim of this study was 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 secondary aim 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, whereas North American species are most commonly pollinated by butterflies and bees, indicating biogeographical 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. These last 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 ability of the plants to sustain fitness in 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) in order to evaluate whether floral traits relate to different pollinator groups. Before our study, our knowledge on *Rhododendron* pollinators was restricted and had a strong spatial bias. For example, birds had been reported as common pollinators in several Eastern Himalaya and Asian high-elevation species, with specialist nectarivores (e.g. sunbirds, *Aethopyga gouldiae* and *A. ignicauda*) and non-specialist nectarivores (e.g. warbling white-eye, *Zosterops japonicus*; leaf-warbler, *Phylloscopus affinis*; and black-faced laughing thrush, *Trochalopteron affine*) described as common pollinators of several *Rhododendron* species in Sikkim-India, China and Nepal (Georgian *et al.*, 2015; Huang *et al.*, 2017; Basnett *et al.*, 2019*b*; Ollerton *et al.*, 2020). 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.*, 2019*b*). Although 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 30 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 *Papilio glaucus*) 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 regions. Here, we identified some similarities and differences across regions. Although 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 ones, and butterfly pollination is restricted to North American species.

In the Himalayas, we found bumblebee pollination to be common in high-elevation species and bird pollination to be common among low- and mid-elevation taxa. Our results recovered pollination patterns that agree with 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. Furthermore, we recovered a relationship between general biodiversity patterns and floral visitation in the Himalayas. The Eastern Himalayan Mountain system supports a hyperdiverse bird community, with maximal diversity recorded mainly at mid-elevations (Price *et al.*, 2014), where the highest *Rhododendron* diversity is also found (Basnett and Ganesan, 2022). Coincidentally, this is where we identified *Rhododendron* most commonly to be 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.

In contrast to the Himalayas, (bumble)bees and butterflies were the most common pollinators of North American species, whereas 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 is 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. Furthermore, their wing-flapping behaviour allowed contact with both the male and female reproductive organs, contributing to pollen transfer (pers. obs. S. Basnett). 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. Although the east coast of North America is comparatively poorer in bird richness than the Himalayas (Quintero and Jetz, 2018), the Himalayas have a high diversity of butterflies. However, our results might also be reflective of the evolutionary and biogeographical history of this pollinator group. Butterflies have been shown to have originated in North and Central America, colonizing Asia only later on in their evolution (Kawahara *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 might 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 biogeographical pattern we observe. S. Basnett.

Along with butterflies, (bumble)bees were the most common pollinators of North American *Rhododendron*, visiting flowers for both nectar and pollen, and probably contributing to pollen deposition through buzz pollination and their constant movement from the male reproductive parts to the nectar pouches (pers. obs. S. Basnett). The case was slightly different for smaller *Andrena* bees, which were abundant at <1000 m a.s.l. and which probably contribute less than bumblebees to pollination, given that they rarely touch the female reproductive organs (pers. obs. S. Basnett). 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 on which they were observed, although this will have to be tested explicitly in future studies.

Pollinators and floral traits

Considerable evidence indicates that pollinators can drive the evolution of plants and flowers (Van der Niet *et al.*, 2014). Floral morphology has been shown to evolve in response to the selection induced by suites of pollinators. As a result, over time, it might lead to morphological convergence in floral morphology in association with a pollinator group (Fenster *et al.*, 2004). The convergence in floral traits has been shown to occur commonly at the family and genus levels 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 (Neves *et al.*, 2020; Weber *et al.*, 2020). Our transcontinental field-based study suggests that the large floral and species diversity of *Rhododendron* is likely to be driven, at least in part, by their pollinators, suggesting that pollinators might be playing and/or might 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 *et al.*, 2008; Muchhala *et al.*, 2014; García *et al.*, 2020). Thus, our results agree with predictions made by the pollinator syndromes hypothesis.

Specifically, our PGLS identified nectar volume to be correlated significantly and positively 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 (Georgian *et al.*, 2015; Huang *et al.*, 2017; Basnett *et al.*, 2019*a*), colours usually related to bird pollination syndromes (Faegri and van der Pijl, 1979; Fenster *et al.*, 2004). Furthermore, among bird-visited species, taxa such 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 and warblers), which could also indicate that it is not only 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, probably owing 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. arboretum* and *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* and *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 upward-reflexed 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. Furthermore, we also found that Himalayan species with high nectar volumes and long corollas (*R. arboreum*, *R. grande*, *R. falconeri* and *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 survival of the species.

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.*, 2018;

Kuppler and Kotowska, 2021). Although the literature is not conclusive, the relative content of sugars in nectar can vary in response to temperature; some studies describe no correlation between nectar concentration and this environmental factor (Clearwater *et al.*, 2018), whereas others suggests that increases in temperature can decrease nectar secretion (Takkis *et al.*, 2018), and still others find that moderately elevated temperatures can increase it (Nocentini *et al.*, 2013). Furthermore, 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). Also, 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 (González *et al.*, 2009; Ortega-Jimenez and Dudley, 2012). Our results recovered trends that agree with many of these studies. In the Himalayas, species experiencing the highest temperature seasonality, mean annual air temperature and precipitation seasonality were those that displayed the longest corollas and largest nectar volumes and that 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.*, 2019*a*). Our results support the idea that in this region both climatic conditions and pollinator identity can interact, leading to the trends we observed of 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 be attributable 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 MD and NC starts in late April and peaks between June and July, and during this time the region also experiences high temperature. Warm climatic conditions and clear days are known to have a positive influence on butterfly visitation, because butterflies are ectothermic, deriving their internal heat almost exclusively through basking (Heinrich, 1993). Bumblebees, in contrast, 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 showed that bird-visited plants display large nectar volumes, whereas bee-visited flowers have flowers with high nectar concentration. We also found substantial evidence of a role played by climatic variables in explaining floral traits, especially in the Himalayas. We observed interactions between bird visitation and temperature seasonality in most Himalayan species, whereas 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 the 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. Furthermore, we focused here on main pollinator groups, and future studies could expand 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, in addition to long-term evolutionary trends in these floral traits using phylogenetic tools. Such work would allow understanding their roles in the diversification of the genus and would provide a predictive framework to evaluate impact and develop conservation strategies for these species in the face of climate change.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Table S1: non-parametric post-hoc Tukey's honest significance difference test result for floral traits. Table S2: non-parametric post-hoc Tukey's honest significance difference 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: the length of corolla, indicated by the black arrow, includes corolla tube length and length of its lobes. Fig. S2: correlation plot of bioclimatic variables for Asia (A) and North America (B). Fig. S3: relationship between floral traits and pollinator groups. Different symbols represent within-group pollinator compositions, as follows: 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. S4: 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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AUTHOR CONTRIBUTIONS

S.B. conceived the idea, conducted the fieldwork and laboratory work, analysed the data and wrote the manuscript. J.K. assisted with fieldwork and laboratory work. A.E. conceived the idea, provided input and revised the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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