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Low bee visitation rates explain pollinator shifts to vertebrates in tropical mountains

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Summary

Evolutionary shifts from bee to vertebrate pollination are common in tropical mountains. Reduction in bee pollination efficiency under adverse montane weather conditions was proposed to drive these shifts. Although pollinator shifts are central for the evolution and diversification of angiosperms, we lack experimental evidence of the ecological processes underlying such shifts.

Here, we combine phylogenetic and distributional data for 138 species of the Neotropical plant tribe Merianieae (Melastomataceae) with pollinator observations of eleven and field pollination experiments of six species to test whether mountain environment may indeed drive such shifts.

We demonstrate that shifts from bee to vertebrate pollination coincided with occurrence at high elevations. We show that vertebrates were highly efficient pollinators even under the harsh environmental conditions of tropical mountains, whereas bee pollination efficiency was lowered significantly through reductions in flower visitation rates. Further, we show that pollinator shifts in Merianieae coincided with the final phases of the Andean uplift and were contingent on adaptive floral trait changes to alternative rewards and mechanisms facilitating pollen dispersal.

Our results provide evidence that abiotic environmental conditions (i.e. mountain climate) may indeed reduce the efficiency of a plant clade's ancestral pollinator group and correlate with shifts to more efficient new pollinators.

Keywords: altitudinal gradient, floral evolution, pollen dosing, pollination efficiency, tropical Andes

Introduction

Pollinator shifts prevail in numerous plant radiations, occur in conjunction with prominent changes in floral morphology, and have played a paramount role in the diversification of angiosperms (Whittall & Hodges 2007, Smith et al. 2008, van der Niet et al. 2014, Lagomarsino et al. 2017, Serrano-Serrano et al. 2017, Fragoso-Martínez et al. 2018, Dellinger et al. 2019a, Kriebel et al. 2020, Phillips et al. 2020). An intriguing and hitherto poorly understood pattern in this context, is the recurrent directionality in shifts from bee to vertebrate pollinators in tropical mountains (i.e. to birds, bats, rodents; Fig. 1, Cronk & Ojeda 2008, Thomson & Wilson 2008, Abrahamzcyk et al. 2014, Dellinger et al. 2019a). The marked change in abiotic environmental conditions along altitudinal gradients (more rain, more wind, and lower temperatures at high elevations), which disproportionately impedes bee-pollinator efficiency, has been proposed as the driving force for these shifts (Cruden 1972). Recently, several authors have demonstrated that shifts to vertebrate pollination coincided with recent phases of mountain uplift and may accelerate diversification rates (Lagomarsino et al. 2016, Serrano-Serrano et al. 2017), but comparative experimental investigations of the underlying ecological processes of pollinator shifts are currently lacking. This is particularly cumbersome because only an improved understanding of such basic, and yet key ecological processes, will allow us to correctly extrapolate to the evolutionary drivers of the extraordinary diversity of (tropical) mountains worldwide (Antonelli et al. 2018).

Pollinator efficiency is measured as the product of pollinator quantity (visitation rate) and pollinator quality (rate of conspecific pollen transfer, Ne'eman et al. 2010), and reductions in pollinator efficiency were proposed as drivers of pollinator shifts (Thomson & Wilson 2008, Bezemer et al. 2019). Recently, attempts were made to formalize the process of pollinator shifts in a theoretical framework (Thomson & Wilson 2008). Generally, stabilizing selection is assumed to balance a plant species in its present pollination system (Armbruster et al. 2009). For a pollinator shift to occur, this stabilizing selection has to be overcome. Thomson & Wilson (2008) proposed a scenario where an extrinsic environmental factor first decreases the visitation frequency of the ancestral (most efficient) pollinator, leading to severe pollen limitation (i.e. receiving fewer compatible pollen grains than required for successful reproduction). Pollination by a second (previously less efficient) pollinator may then become more important, and, in the long run, selection on floral traits mediated by this new pollinator may lead to reproductive isolation and speciation (Muchhala 2019).

In tropical lowlands, bees are abundant and successful pollinators, but their diversity and abundance decrease towards high elevations (Bawa 1990, Ramos-Jilberto et al. 2010, Hoiss et al. 2012, Classen et al. 2015). As ectothermic animals, their activity (pollinator quantity) is reduced by the frequently adverse weather conditions in mountains (Cruden 1972, Brito & Sazima 2012, Classen et al. 2020). In contrast, endothermic vertebrates remain active at low temperatures and continue visiting flowers even in moderate rain (Lawson & Rands 2019). The pollinator shift scenario envisioned by Thomson & Wilson (2008) hence seems plausible, particularly when viewing pollinator shifts in the context of recent mountain uplifts. Plant lineages may have tracked the newly forming habitats by gradually adapting to changing climatic conditions during mountain uplift, thereby colonizing habitats where bee pollinator quantity is low. Colonization of montane habitats may hence have destabilized the bee-pollination systems and triggered shifts to vertebrate pollination. Although this is an intuitively appealing scenario supported by macroevolutionary studies (Lagomarsino et al. 2016, Serrano-Serrano et al. 2017, but also see Vargas et al. 2020), we lack causal empirical evidence that elevation indeed impacts bee pollination efficiency and drives pollinator shifts.

Differences in pollinator quantity may be reinforced or, alternatively, outweighed by differences in pollinator quality, intrinsic to the pollinator's behaviour, dietary preferences, morphology, degree of specialization, and its flower constancy (Castellanos et al. 2006, Muchhala & Thomson 2010). Vertebrates, for example, usually visit flowers to obtain nectar or food-body rewards while bees also actively forage for pollen (Cronk & Ojeda 2008, Dellinger et al. 2014). Naturally, active pollen foraging by bees reduces the number of pollen grains available for plant reproduction, thereby reducing the pollinator quality of bees. Plants may evolve to adjust mechanisms of pollen delivery and dispensing to the quality of their pollinators, with stricter dispensing when pollinators actively forage for pollen (Harder & Wilson 1994, Castellanos et al. 2006, Minnaar et al. 2018, Song et al. 2018). In this context, buzz-pollinated flowers with poricidal anthers (only opening by a minute pore) represent an extreme case of strict pollen dispensing (Buchmann 1983, Harder & Barclay 1994, Telles et al. 2020). In these flowers, pollen is the sole reward and can only be extracted in (usually) small doses by bees capable of applying specific vibrations (buzzes) to the anthers (Harder & Barclay 1994, DeLuca & Vallejo-Marín 2013, Dellinger et al. 2019c, Vallejo-Marín 2019, Rosi-Denadai et al. 2020). The dual function of pollen (reward and reproduction, 'pollen dilemma') is believed to have driven this strict dispensing strategy (Vallejo-Marín et al. 2010). Although buzz-pollination is widespread across angiosperms (ca. 8% of species, multiple independent origins, DeLuca & Vallejo-Marín 2013, Vallejo-Marín 2019), shifts from buzz-bee-pollination to alternative pollinators seem to be scarce and remain largely unexplored (Brito et al. 2016, Dellinger et al. 2019a). Such shifts provide the ideal set-up, however, to test whether shifts from pollen to alternative rewards may lead to a relaxation of pollen dispensing strategies as an adaptive response to changes in pollinator quality.

In this study, we combined macroevolutionary analyses with empirical observations and field experimental data in an ancestrally buzz-pollinated Neotropical plant clade (Merianieae, Melastomataceae) to investigate the effect of the abiotic environment on pollination efficiency and, potentially, in driving pollinator shifts. First, using occurrence data of 138 species, we show that shifts from bee to vertebrate pollination correlate with occurrence in tropical mountains (Fig. 1). These pollinator shifts go along with shifts in reward type and pollen-release mechanisms (Dellinger et al. 2019a). Buzz-pollinated Merianieae species are pollen rewarding, and buzzing bees apply vibrations to extract pollen by seizing the prominent stamen appendages (Fig. 1, Dellinger et al. 2019a). As we have shown earlier (Dellinger et al. 2019a), Merianieae are characterized by repeated independent shifts from ancestral bee-buzz-pollination to either mixed assemblages of vertebrate pollinators (i.e. hummingbirds and bats, hummingbirds and rodents) and nectar rewards (Dellinger et al. 2019c) or to passerine pollinators and food-body rewards (Dellinger et al. 2014). Here, we demonstrate that the shifts in reward type correlate with a relaxation in pollen-dispensing strategies through modifications in stamen structure, potentially as an adaptation to increased pollinator quality of non-pollen foraging pollinators. Then, building on these macroevolutionary patterns, we provide empirical ecological evidence for eleven selected species that bee-pollinator quantity (visitation rate) is reduced significantly along a gradient from lowland rainforests to montane cloud forests. Monitoring pollen transfer in six of those eleven species, we then show that vertebrates are highly efficient pollinators in tropical mountains, while bees are not. We thus provide empirical evidence for the hypothesis that pollination efficiency of a plant clade's ancestral pollinator group (bees in the case of Merianieae) decreases under certain environmental conditions (i.e. mountain climate) and that ancestral pollinators are frequently replaced by alternative and more efficient pollinators (vertebrates in the case of Merianieae). Whether such pollinator shifts were a pre-requisite or rather a corollary of the colonization of montane habitats remains to be investigated, however. At a general level, our results support that alterations in pollinator quantity (through abiotic environmental conditions) as driver of pollinator shifts is not merely an abstract concept, but a biologically realistic scenario, which is crucial both for understanding the evolutionary history of angiosperms as well as for predicting future responses to climate change and anthropogenic influence.

Material and methods

Estimating pollinators using pollination syndromes

For 138 Merianieae species, we recorded 15 to 17 pollination-relevant floral traits identified as informative in characterizing pollination syndromes in in the tribe (Dellinger et al. 2019a; Supporting Information Methods, data deposited at http://phaidra.univie.ac.at/o:1176744). We evaluated trait states from ethanol preserved material, herbarium vouchers, schematic drawings, photos, and taxonomic literature (Supporting Table S1). Next, we used machine-learning algorithms (Random Forest Analyses, RF) to characterize pollination syndromes based on 26 species with empirically documented pollinators (Supporting Table S2) and 15 floral traits, and used the trained models to predict pollinators for the remaining 112 species. The approach follows Dellinger et al. 2019a, and we provide a detailed protocol in the Supporting Information Methods 1. For further analyses, we collated the empirical data with the prediction data.

Relating pollination strategy to altitude

To test whether pollination syndrome correlates with occurrence in mountains, we downloaded GBIF occurrence data for 134 Merianieae species on 10.09.2020, yielding 23’094 records (GBIF Secretariat 2019, Chamberlain et al. 2020; for details on data source see Supplementary Literature). We pruned these data by first removing all values lacking information on longitude or latitude, leaving 16’989 occurrence points. We then used custom filtering techniques implemented in the R-package *CoordinateCleaner* (Zizka et al. 2019), flagging records with equal longitude/latitude, zero coordinates, coordinate – country mismatches, records located in country centroids, in the sea or around GBIF headquarters as well as duplicates, leaving 7’055 pruned coordinates (Supporting Table S3). Finally, we removed all records lacking elevation data, leaving 5’809 records for further analyses. To reduce potential bias due to misidentification, we then plotted occurrence data of each species separately to check whether it corresponds to the known distribution range.

Four additional taxa included in our trait dataset are local varieties (*M*. aff. *drakei*, *M*. aff. *sanguinea*) or recently described species (*M. ardyae*, *M. zunacensis*) and were not represented in GBIF. We added occurrence information for those species from our own field records (Dellinger et al. 2019c, Fernández et al. 2020). In addition, we added occurrence data for two Cuban species (*M. angustifolia*, *M. albiflora*) from Michelangeli et al. 2015, which had only been represented by one or two records after filtering the GBIF data (Supporting Table S3). The final number of occurrence records was 5’853.

We then calculated median latitude and median elevation per species. We used the R-package ggpubr (Kassambra 2019) to visualize the association between pollination syndrome (as estimated through RF) and elevation across latitude. We used Kruskal-Wallis ANOVA with Dunn’s test as post-hoc test (correcting for multiple comparisons) to test whether shifts to vertebrate pollination are significantly associated with growth at high elevations. To account for phylogenetic relatedness (see below), we repeated this test using a phylogenetic ANOVA (*RRPP*, Collyer & Adams 2018). We repeated this test excluding specimens occurring in the Greater Antilles, since the well-documented island effect decreases elevation zones in oceanic islands, potentially blurring our results (Irl et al. 2016).

Pollinator shifts in a phylogenetic context

To analyse pollinator shifts and trait evolution in a phylogenetic context, we used the seven-marker Bayesian phylogeny recently developed for Merianieae (Dellinger et al. 2019b). For our analyses, we used both the maximum clade credibility (MCC) tree and a subsample of 100 trees from the posterior distribution to account for phylogenetic uncertainty. We pruned the phylogeny to the 138 tips included in our trait matrix using the *treedata*-function (*geiger*, Harmon et al. 2008). This sample includes ca. 46% of Merianieae (ca. 51% of the focal clade where pollinator shifts occurred), and covers the morphological and taxonomic diversity as well as geographic distribution of the tribe. Previous analyses have identified the ‘equal-rates’ model as optimal for reconstructing ancestral pollination syndromes in Merianieae (Dellinger et al. 2019b). We hence used the ‘equal-rates’ model to estimate ancestral pollination syndromes along the MCC-tree and across the sample of 100 trees, using 1000 iterations of Bayesian stochastic character mapping per tree in the *make.simmap* function (*phytools*, Revell 2012). We counted the number of pollinator shifts (transitions between states) and the time spent in each state for each tree, and calculated the mean and median number, as well as the range of transitions across all 100 trees. Finally, we mapped ancestral pollination syndromes and present-day median elevation to the MCC tree.

The reward type (pollen, nectar or food-body rewards) and mechanism of pollen release (buzzing, salt-shaker or bellows-mechanism) constitute the traits most important in discriminating pollination syndromes in Merianieae (Dellinger et al. 2019a). We could only reliably score these traits for 68 species since they require studying species in the field, or detailed structural features (i.e. nectar secreting tissue) on well-preserved material (Dellinger et al. 2019a, c). We had hence excluded these traits from estimating pollination syndromes (Supporting Information Methods 1), and tested for correlated evolution between pollination syndrome and reward type (thereby avoiding circularity) in 68 species, using Pagel's method for discrete characters (Pagel 1994). We binarized the traits (pollination syndrome: bee or vertebrate, reward type: pollen or other) for analyses and performed likelihood-ratio tests to select the best-fit model. Further, stamen appendage size/shape and structure (i.e. structure of the thecal wall) are tightly associated with the mechanism of pollen release in Merianieae, and hence with pollination strategy. Our trait "primary appendage shape" consists of six different states. To test for correlated evolution between reward type and appendage size, we simplified this trait to small (states "acuminate", "knob") and large (states "bulbous", "crown-like", "fusiform", "pyramidal") appendages. We also tested for correlated evolution between reward type and stamen structure (binarized to "corrugated" or "other"), again using Pagel's method. Finally, we mapped trait states for reward type, appendage shape and thecal wall structure onto the MCC tree (Fig. 2).

Field experiments on pollination efficiency

We conducted field work in eleven selected species with different pollinators in Colombia, Costa Rica and Ecuador from 2015 to 2020 (Supporting Table S4). We selected seven buzz-bee-pollinated species (*Adeloborys adscendens*, *Graffenrieda penneysii*, *Meriania hernandii, M. maguirei, M. maxima, M. nobilis, M. speciosa*), two mixed-vertebrate pollinated species (*M. sanguinea* (hummingbirds, rodents), *M. tomentosa* (hummingbirds, bat)) and two passerine-pollinated species (*Axinaea confusa, A. costaricensis). Ad. adscendens* is a lowland species while all other species occur in montane cloud forests (Supporting Table S4). In *M. hernandii*, we compared pollination efficiency at two sites, one at *M. hernandii*'s natural habitat in the montane forest and the second in an orchid garden to which *M. hernandii* had been transplanted from the natural site by locals. Although these sites are only approximately 4 km apart, they differ markedly in their environmental conditions since the natural site is exposed to heavy Amazonian rainfalls (mean annual precipitation in the nearby town Jondachi 3,915 mm) and continuous clouds and fogs, while the transplant site is sheltered from rain, receiving ca. 1,300 mm less rain (mean annual precipitation in Cosanga 2,624 mm, www.climate-data.org) and counts with sunnier and warmer conditions. The transplant site hence is more similar to lowland conditions favourable to bee activity.

Pollinator visitation rates were readily available for *Axinaea confusa* (Dellinger et al. 2014), *Meriania sanguinea* and *M. tomentosa* (Dellinger et al. 2019c). Additional information on visitation rates is provided here for the other seven species (Supporting Table S5). To assess visitation rates, we monitored flowers of one to four individuals in each species using video cameras (Sony HDR-CX190 camcorders). We placed cameras on tripods approximately 2 m away from the flowers and filmed individual inflorescences during daytime (06:00-18:00). We used the Sony software Play-Memories-Home to replay videos. Since all inflorescences presented more than one open flower, we could monitor multiple open flowers simultaneously and calculated visitation rates per flower per hour, yielding a total of 916.71 flower observation hours (201.02 total observation hours, Table 1; Muchhala et al. 2009 for similar approach). We scored visitors as pollinators if they triggered pollen release from stamens and contacted the stigma. We classified pollinators into the functional groups “bees”, “passerine birds” and “mixed-vertebrate”.

Next, we monitored pollinator quality in six of the ten study species (Table 1). We could not include five of the montane bee-pollinated species in our assessment of pollinator quality (*Graffenrieda penneysii*, *Meriania maguirei*, *M. maxima*, *M. nobilis*, *M. speciosa*), since they only presented a limited number of accessible flowers (many Merianieae are trees), limiting our capacity of arriving with sample sizes required for studying pollen transfer. To evaluate pollinator quality, we first monitored the duration of anthesis by checking the same flowers at 6 am and 6 pm on consecutive days and partitioned anthesis in each species into three stages: early, intermediate and late (shortly before withering; Supporting Table S4). We then marked an average of 69 pre-anthetic buds per species on the petals with a waterproof pen and a-priori assigned each flower to be collected at a specific stage (Supporting Table S6). Once the pre-assigned anthetic stages were reached, we collected the flower and carefully removed all stamens with tweezers to avoid pollen spilling and put them into a 1.5 ml Eppendorf tube with 70% ethanol. Since passerines remove entire stamens in *Axinaea*, we recorded the number of stamens left at the different anthetic stages. We also removed the style of the same flower and stored it in a separate 1.5 ml Eppendorf tube with 70% ethanol. This approach allowed us to assess whether pollen removal and deposition saturated over time and whether rates of pollen transfer differed between species with different pollinators. In order to compare pollen-removal across species, we also collected virgin (i.e.

anthetic, but unvisited) flowers. We bagged 13 pre-anthetic buds per species with fine mesh bags (Dellinger et al. 2019c) and collected all stamens once flowers became anthetic.

We then assessed pollen removal by analysing how much pollen was left in stamens after the different anthetic stages. We randomly selected three stamens per flower and placed each stamen into a separate 1.5 ml Eppendorf tube filled with 1 ml of purified water. We squeezed each stamen with a micro pestle to rip thecal walls to release pollen. To assure complete pollen removal and to break up potential pollen clumps, we additionally sonicated the tubes for 15 minutes in an ultrasonic bath and vortexed them for two seconds. We then left samples for 10 minutes to let potential air bubbles settle out. Before measuring, we gently inverted samples three times to disperse pollen evenly throughout the liquid. We subsequently injected 100 µl of the solution into a TOPAS particle counter (FAS-362). To assure accuracy of measurements, particle concentration has to be below 5000 particles/cm³. We ran test samples for each species and when particle concentrations were equal to or higher than 5000 particles/cm³, we diluted all subsequent samples of these species 1:9 a second time (100 µl sample: 900 µl filtered water). The FAS-362 TOPAS particle counter partitions counts into 64 size classes from 2-200 µm. For our analyses, we only included size classes that covered the pollen grain sizes to exclude smaller or larger particles, which may represent plant debris formed during sample preparation (Dellinger et al. 2019d). For choosing the accurate size classes, we plotted the count data for ten random samples of each species and selected the size classes which showed the highest detected particle numbers and which overlapped with the pollen size ranges measured in Dellinger et al. 2019a (Supporting Table S7). We also inspected selected samples under the light microscope to rule out possible bias due to sample preparation (e.g. incomplete pollen release or pollen clumping) or differences in pollen grain sizes, which would affect the size-range selection from the particle counter. We could confirm complete pollen release by our preparation technique, the presence of single pollen grains instead of clumps and no variation in pollen grain sizes.

We assessed deposited pollen by counting pollen grains on stigmas under a fluorescence microscope following the methodology of Dellinger et al. (2019d). We cut stigmas from styles, put them into a droplet of lactic acid on a microscope slide, squashed them and counted pollen grains in three selected areas from the edge to the centre at 20x magnification. We estimated the total number of pollen grains by multiplying the mean number of pollen grains per µm² with the total squashed stigmatic area (Dellinger et al. 2019d). To understand whether stigmas received enough pollen grains to fertilize all ovules or suffered from pollen limitation, we additionally estimated ovule numbers. We removed the hypanthium and dissected ovaries (on average, 8 per species, Supporting Table S7) to expose the ovules. We prepared ovaries for HRX-CT scanning following Staedler et al. (2018) and scanned all samples using a Zeiss MicroXCT-200 system. We reconstructed 3D-image-stacks from the raw scan data (XMReconstructor Zeiss) and used the 3D-imaging software AMIRA 5.5.0 to create 3D-models of ovaries (Fig. S1). We calculated isosurface models, adjusting thresholds so that only the most strongly contrasted tissue (i.e. ovules) were visible (Staedler et al. 2018). We counted all ovules of one carpel by hand on tomographic reconstruction. As ovule numbers between carpels were relatively constant, we multiplied the number of ovules of one carpel by the total number of carpels to estimate total ovule number per flower.

To test for significant differences in pollen removal across the different functional pollinator groups, we constructed generalized linear mixed-effects models (GLMMs) using *lmerTest* (Kuznetsova et al. 2017). First, we tested the hypothesis that buzz-pollination becomes less efficient under montane weather conditions by comparing pollen removal across anthesis between the lowland bee pollinated *Adelobotrys adscendens*, the montane population of *Meriania hernandii* and the transplant site of *M. hernandii.* Second, we tested the hypothesis that vertebrate pollinators are more efficient than bees at high elevations by comparing pollen removal from the natural montane population of *M. hernandii* with *M. sanguinea* and *M. tomentosa* (the two *Axinaea* species were not included in this model since entire stamens were removed from flowers by passerine birds). To assure comparability between the different species, we standardized the pollen count data by the average of all anthetic stages within each species. We treated plant species (or site in the case of *M. hernandii*) and the different anthetic stages (virgin, early, intermediate, late) as fixed effects and flower ID as random effect and specified a Gamma-distribution with an identity link given the positive right skew of the data. Since the initial models did not converge, we first ruled out problems due to singularity and gradient conversion. We achieved convergence updating the models using the 'bobyqa'-optimization from the *glmerControl*-function (*lme4*, Bates et al. 2015). We validated model fit by plotting residuals against fitted values and predictors (Zuur et al. 2009). We estimated marginal means (*emmeans,* Russell 2020) between the different anthetic stages and species, p-values were adjusted for multiple comparisons using the Tukey method. We further contrasted anthetic stages per species (*phia*, Rosario-Martínez 2015). We also calculated the average percentage of pollen remaining at each anthetic stage from the raw data.

To compare pollen deposition on stigmas within and among species, we divided stigmatic pollen counts by the average number of ovules per species. A value smaller than 1 indicates pollen limitation, a value larger than 1 indicates pollen saturation. Since the continuous, positively right-skewed data included zeros (not allowed in a traditional Gamma GLMM), we specified Gamma hurdle models with a log link (*glmmTMB*, Brooks et al. 2017). We constructed GLMMs treating plant species and time as fixed effects and plant ID as random effect. Again, we ran two separate GLMMs, one comparing the three bee pollinated species and the second one comparing the natural montane population of *M. hernandii* against *A. confusa*, *A. costaricensis*, *M. sanguinea* and *M. tomentosa*. We validated model fit by plotting residuals against fitted values and predictors and detected strong heteroscedasticity among species. We hence updated the initial models by including the per-species variance structure, which significantly improved model fit (bee-model: AIC without weights -113, with weights -247; bee-vertebrate model: AIC without weights -240, with weights -15162). We estimated marginal means between the different anthetic stages and species and adjusted p-values for multiple comparisons using the Tukey method.

Finally, we estimated per-visit pollination effectiveness. We calculated effectiveness in pollen removal for each time interval for each species as follows: $(\bar{x}_{\text{virgin}} - \bar{x}_{\text{stage[i]}}) / h_{[i]}$ / visitation rate; $\bar{x}_{\text{virgin}}$ being the average number of pollen grains in virgin stamens, $\bar{x}_{\text{stage[i]}}$ being the average number of pollen grains at a specific anthetic stage, $h_{[i]}$ being the hours a flower could potentially have received pollinator visits at the specific anthetic stage (e.g. a flower with an intermediate anthetic stage of 36 hours, but only visited diurnally, would be assigned 24 hours in this calculation since it would not be visited for the 12 hours at night time, also see Ne'eman et al. 2010), and the visitation rate being the average visitation rate per flower per hour (Table 1). As no pollinators visited flowers in the montane population of *M. hernandii* during monitoring, we used the average visitation rate (0.024) across all montane bee-pollinated species in order to obtain an estimate (Table 1). For the two passerine pollinated species, we multiplied the average number of pollen grains per stamen by the number of stamens removed at each anthetic stage to arrive at estimates of pollen removal. Finally, we averaged across the three anthetic stages and calculated proportional per-visit pollen removal to compare among species. We further calculated per-visit pollen deposition as: $\bar{x}_{\text{stigmatic pollen load [i]}}$ / $h_{[i]}$ / visitation rate and again took averages for each species across the three anthetic stages.

Results

Vertebrate-pollinated Merianieae occur at high elevations

Using machine-learning algorithms trained on 26 Merianieae species, we predicted the most probable pollinators for the remaining 112 species with high accuracy (96.4% of species were consistently classified into one pollination syndrome; all species were classified into one syndrome with more than 50% accuracy, Supporting Table S1, S2). This yielded a total of 94 bee-buzz syndrome species, 22 mixed-vertebrate syndrome species and 22 passerine-syndrome species (Fig. 2).

Pollination syndrome significantly associated with elevation (Kruskal-Wallis ANOVA: X^2 *38.8, df = 2, p < 0.001*; phylogenetic ANOVA: *F 3.69, df = 2, p = 0.031;* Fig. 1, Supporting Table S8). Bee-pollinated species occurred across all elevations, but predominated below 1.900 masl (Fig. 1). Vertebrate-pollinated species were restricted to elevations above 1.900 masl, with exceptions found on oceanic islands (Greater Antilles) and in the Atlantic Forest of Brazil, where vertebrate-pollinated species occurred below 1000 masl.

Pollinator shifts are correlated with shifts in reward type and pollen dispensing strategy

Consistent with previous findings (Dellinger et al. 2019a), buzz-pollination by bees was supported as ancestral by Bayesian stochastic character mapping (Fig. 2, 84.9% of evolutionary time across all branches of the phylogeny). Across a random sample of 500 phylogenetic trees and 138 species, 12.7 pollinator shifts occurred, with approximately 6.7 shifts from bee to vertebrate-pollinated lineages (bee to mixed-vertebrate: 3.15, bee to passerine: 3.66; each pollination syndrome making up ca 7.55% of evolutionary time across all branches, Supporting Table S9). We also detected a high number of potential reversals from mixed-vertebrate to bee-pollination (4.13) and from passerine to bee-pollination (1.60, Supporting Table S9). However, these reversals are restricted to terminal branches and should be taken with care given phylogenetic uncertainty at shallow levels of the phylogeny (see Discussion).

The shifts from bee to vertebrate pollination are restricted to a single major subclade of Merianieae (marked with asterisk in Fig. 2, called "core"-clade from here onwards), which has colonized the montane tropical Andes, the Atlantic Forest of Brazil, and the Greater Antilles. The pollinator shifts temporally coincide with the final phases of the Andean uplift (ca. 5 Million years ago, Fig. 2, Hazzi et al. 2018). Shifts from pollen to nectar or food body rewards are restricted to this clade (correlated evolution between reward type and pollination strategy, assessed across 68 species: *likelihood-ratio 13.6, p = 0.001*). Neither pollinator shifts nor shifts in reward type have occurred outside of the core-clade, despite several species of other Merianieae clades occurring at high elevations (Fig. 2).

Following our expectation, the shifts in reward type (release from 'pollen dilemma') coincide with mechanisms facilitating pollen-release by vertebrates: significant modifications have occurred in the size and shape of stamen appendages, and in the structure of the stamen thecal wall (Fig. 2). Conspicuously enlarged appendages, i.e. functional in the bellows' mechanism of pollen release in passerine-pollinated flowers, have only evolved in the clade where pollinator shifts occurred (correlated evolution with reward-type tested across 68 species: *likelihood-ratio 10.3, p = 0.006*, Fig. 2, Supporting Fig. 2). Finally, thecal wall structure, a trait crucial for pollen dispensing, also evolved in a correlated manner with reward type (*likelihood-ratio 13.4, p = 0.001,* Supporting Fig. 3). Bee-buzz-pollinated species have strongly corrugated to smooth thecal walls, often with persistent septa between the two pollen sacs of a theca (Fig. 1, 2). Smooth thecal walls and thecae without persistent septa have evolved mostly in connection with passerine-pollination. Soft, easily deformable thecal walls, again without persistent septa in the thecae, associate with mixed-vertebrate pollination (Fig. 2).

Experimental evidence: bees are inefficient pollinators at high elevations

Monitoring pollinator quantity (visitation rate) of eleven Merianieae species (six focal study species plus five additional montane bee-pollinated species), we found that bee-pollinator quantity decreased dramatically from low to high elevations (Table 1). In three out of six montane bee-pollinated species, we did not record any bees during observation times (Table 1). In the other three montane bee-pollinated species, we recorded less than 0.1 visits per flower per hour. When monitoring visitation rates of montane bee-pollinated *Meriania hernandii* which had been transplanted out of its natural occurrence to a garden with warm and dry conditions, bee visitation rates were comparable to lowland species. The visitation rates of vertebrate pollinators were two to three magnitudes higher than those of bees in tropical mountains (Table 1).

Next, we compared pollinator quality (efficiency in removing and depositing pollen) of two bee-, two mixed-vertebrate-, and two passerine-pollinated species. Bees were significantly more efficient in removing pollen in the lowland bee-pollinated species *Adelobotrys adscendens* and in the transplanted individual of the originally montane *Meriania hernandii*, than in the natural montane population of *M. hernandii* (Fig. 3a, GLMM for interaction between species and time: *F-value 12.86, sum of squares 17.6,* $p < 0.01$*, logLik -475.5,* R^2 *0.46,* Supporting Tables S10, S11, S12*)*. Pollen was removed fastest in the transplanted site of *M. hernandii*, with approximately 71% of pollen removed early in anthesis and only 18% of pollen remaining in old stamens (Supporting Tables S10, S13). In its natural montane habitat, pollen removal of *M. hernandii* was not significant across anthesis and approximately 95% of pollen remained in the anthers (Supporting Tables S10, S13). In *Ad. adscendens*, substantial amounts of pollen were removed across anthesis, with ca. 60% of pollen grains remaining in old stamens (Supporting Tables S10, S13). The unexpected increase in countable pollen grains from virgin to early stage stamens (Fig. 3a) is likely due to staggered pollen maturation in early anthesis (Dellinger et al. 2019d).

Pollen removal in the montane cloud-forests was significantly faster in the two vertebrate-pollinated species than in the montane bee-pollinated species (Fig. 3b, GLMM for interaction between species and time: *F-value 6.2, sum of squares 8.1,* $p < 0.01$*, logLik -371.7,* R^2 *0.43*, Supporting Tables S14, S15). Pollen was removed most quickly and completely by hummingbirds and bats (*Meriania tomentosa*; only 26% of pollen grains left in old flowers, Supporting Tables S10, S16), followed by hummingbirds and rodents (*M. sanguinea;* 45% of pollen remaining in late stage stamens, Supporting Tables S10, S16). In the two passerine pollinated species, approximately half of the stamens (and hence pollen) were removed until the end of anthesis (Supporting Table S1).

Patterns for pollen deposition on stigmas were similar to pollen removal. Bees were significantly more efficient in depositing pollen on stigmas in the lowland bee-pollinated *Adelobotrys adscendens* and the transplanted individual of *Meriania hernandii* than at the natural montane occurrence of *M. hernandii* (Fig. 3c, *logLik 135.7*, X^2 *187.86,* $df = 4$*,* $p < 0.01$, R^2 *0.831*, Supporting Tables S17, S18, S19, S20). Pollen deposition was fastest in the transplanted site of *M. hernandii,* with pollen saturation reached already at early anthesis (pollen load:ovule ratio 1.82, *F-*

ratio 0.27, p *0.76*, Fig. 1c, Supporting Table S17, S20). At the natural montane occurrence, *M. hernandii* was strongly pollen limited even at the end of anthesis (pollen load:ovule ratio 0.114, Supporting Table S17). With a pollen load:ovule ratio of 0.4, lowland bee-pollinated *Ad. adscendens* was significantly less pollen limited than montane *M. hernandii* (Supporting Table S19).

Amounts and rate of pollen deposition in cloud forests were significantly higher in the vertebrate-pollinated species than in bee-pollinated *M. hernandii* (Fig. 3d, *logLik 7599.1*, X^2 *524.8*, $df = 8$, $p < 0.001$, R^2 *0.7*, Supporting Tables S17, S21). Pollen deposition was fastest and highest in hummingbird- and bat-pollinated *Meriania tomentosa* (pollen load:ovule ratio 1.43 at intermediate stages, *F-ratio 17.6*, $p < 0.0001$, Supporting Tables S15, S21, S22, S23), followed by passerine pollinated *Axinaea confusa* (pollen load:ovule ratio 0.9 at intermediate stages, *F-ratio 9.3*, $p < 0.0001$, Supporting Tables S15, S21, S22). Pollen deposition increased more gradually over anthesis in hummingbird- and rodent-pollinated *M. sanguinea* (pollen load:ovule ratio 0.52 late in anthesis, Supporting Table S22, *F-ratio 300.6*, $p < 0.0001$). In passerine-pollinated *A. costaricensis*, pollen deposition was very low (pollen load:ovule ratio 0.09 in late anthesis; Supporting Table S17) and not significantly higher than in bee-pollinated *M. hernandii* at any anthetic stage (Fig. 3d, Supporting Table S22).

When estimating single-visit pollination effectiveness from the quantity and quality data, we found highest effectiveness in both pollen removal and pollen deposition by passerine birds in the two *Axinaea* species, followed by bees in montane *M. hernandii* (Table 1). Single-visit effectiveness of bees was low in the lowland species *Adelobotrys adscendens* (Table 1). Hummingbirds and bats had very low single-visit effectiveness in *M. tomentosa* (1.1%) while hummingbirds and rodents removed ca. 8.5% of pollen per visit in *M. sanguinea* (Table 1).

Discussion

Our results corroborate the hypothesis that changes in abiotic environmental factors (i.e. across altitudinal gradients) differentially impact the efficiency of distinct pollinator groups and may, hence, constitute important drivers of pollinator shifts. The dramatic decrease in pollination efficiency of bees from lowland rainforests to montane cloud forests occurred through a reduction in visitation frequency (Table 1), while vertebrates were highly efficient pollinators at high altitudes. While there undoubtedly is a strong association between montane environment and shifts from bee to vertebrate pollination in many angiosperm lineages (Cronk & Ojeda 2008, Smith et al. 2008, Thomson & Wilson 2008, Serrano-Serrano et al. 2017, Fragoso-Martínez et al. 2018, Dellinger et al. 2019a), we caution that the macroevolutionary drivers underlying pollinator shifts remain largely unexplored. The occurrence of several bee-pollinated Merianieae species at high elevations, for example, indicates that mountain environment alone does not consistently cause shifts. Instead, shifts to vertebrate pollination may be partly constrained as they are contingent on extensive modifications in floral traits (reward type, pollen dispensing).

Differences in pollination efficiency were primarily driven through the quantity component (extremely low visitation rates of bees in tropical mountains) rather than qualitative differences between different pollinators (high single-visit effectiveness of bees, Table 1). Generally, buzz-pollination by bees is regarded as an evolutionarily highly successful pollination strategy (Berger et al. 2015, Reginato & Michelangeli 2016, Dellinger et al. 2019a). Our data support this idea and, although evolutionary shifts towards other pollination systems appear triggered by harsh environmental conditions, buzz-pollinated species across the phylogeny persist in tropical mountains (Fig. 1a, 2). We propose that the large floral diversity observed among bee-pollinated species (Fig. 2, Dellinger et al. 2019b) reflects adaptions to locally variable bee-pollination niches (i.e. larger bees at high elevations) both in lowlands and in mountains. In connection with this, we observed marked differences in buzzing behaviours of bees, with bees buzzing the entire androecium at once in flowers with small appendages, but sequentially buzzing single stamens in flowers with large appendages (Fig. 1b, also compare Mesquita-Neto et al. 2017, Konzmann et al. 2020). We argue that allocating pollen to ten separate buzzing units (two whorls of five stamens each in pentamerous flowers), as in large-appendage flowers, is an adaptive strategy to optimize pollen dispensing and delivery. Bees have to take different positions in the flower in order to buzz each stamen individually, which increases chances of contacting the stigma with different body parts, and total handling-time per flower (Solís-Montero & Vallejo-Marín 2017). The complex stamen morphology, with (mostly) corrugated thecal walls, together with persisting septa between the two pollen sacs of a theca (Fig. 2), and the narrow shape of the thecae, hinders pollen passage and allows for the release of only small proportions of pollen per buzz (Dellinger et al. 2019d). Future experimental investigations are planned to explore the links between stamen morphology and pollen release dynamics in depth, and, ultimately, to establish why some bee-pollinated lineages underwent pollinator shifts in mountains while others did not.

Pollinator shifts are contingent on adaptive floral trait evolution (Thomson & Wilson 2008, Opedal 2019). Our results underline that selective pressures imposed by the environment most likely triggered pollinator shifts, which in turn need to be accompanied by changes in various traits (i.e. reward type, pollen dispensing). The evolution of large stamen appendages possibly was an important pre-adaptation to permit shifts to passerine-pollination with stamen appendages serving as food-body rewards and as triggers for the bellows-mechanism of pollen release (Dellinger et al. 2014). Like in bee-pollinated species with single-stamen buzzing, in passerine-pollinated flowers pollen is allocated to ten stamens, which are removed individually by the birds. In contrast to the bee-pollinated species, however, pollen may only be released once per stamen since each stamen is ripped out of the flowers. Thus, all pollen should ideally be released at once. The smooth thecal walls in passerine-pollinated species likely are an adaptation to permit unhindered pollen expulsion and maximum pollen release. Similarly, the soft and easily deformable thecal walls in mixed-vertebrate-syndrome species represent an adaptation to allow pollen removal without buzzing (Dellinger et al. 2019c). This relaxed pollen dispensing may be regarded as an adaptation to the high pollination efficiency of the mixed-vertebrate pollinator assemblages (Castellanos et al. 2006).

Worldwide, biodiversity peaks in mountains and increased ecological opportunity through the formation of highly heterogeneous environments across small spatial scales is regarded as major driver of this diversity (Wellborn & Langerhans 2015, Rangel et al 2018). Our results support the idea that the formation of new habitats through mountain uplift may indeed have spurred pollinator shifts, since shifts from bee to vertebrate pollination in Merianieae temporally coincide with late phases of the Andean uplift (Fig. 2). However, the link between pollinator shifts and paleoenvironmental variation, and their potential impact on diversification in Merianieae, remain to be explored. In addition, according to our current understanding of the phylogeny of Merianieae (Fig. 2), there were at least five reversals from vertebrate to bee-pollination in mountains. All of these reversals are located at tip branches of the phylogeny, however. While such dynamic transitions are possible and merit further investigation, we emphasize that they should be treated with care at this point, since tip relationships in Merianieae remain weakly supported given their recent diversification.

Finally, we emphasize that our results provide an exciting baseline to test hypotheses on the drivers of pollinator shifts in other tropical mountain ecosystems and other plant lineages. While abiotic environmental conditions affect pollination efficiency (in mountains) worldwide, the responses may be different. Besides prominent pollinator shifts, for example, increased generalization (i.e. using many different pollinators) with elevation has been reported (Classen et al. 2020, Gavrutenko et al. 2020, Mertens et al. 2020). Hence, the experimental comparative investigation of pollination efficiency in mountains worldwide will ultimately help to obtain a more comprehensive understanding of the ecological processes underlying (tropical) mountain plant diversification (Rahbek et al. 2019, Raven et al. 2020), and potential responses to climate change in the future.

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Author Contribution

ASD and JS conceived the study, ASD and DMF-F carried out the fieldwork. ASD carried out the lab work, ASD and RP-B analysed the field results. DSP and FAM provided sequence data and the molecular phylogeny. ASD wrote the manuscript, all authors contributed on finalizing the manuscript.

Data availability

All datasets from fieldwork and the floral trait matrix have been deposited in the open access repository phaidra (http://phaidra.univie.ac.at/o:1176744).

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Table 1. Pollinator quantity (visitation rates) and quality (per-visit pollen removal and deposition) of the different pollinator groups in the six main study species and visitation rates for five additional bee-pollinated species (*Meriania maguirei*, *M. maxima, M. nobilis, M. speciosa, Graffenrieda penneysii)*.

	Species	Pollinator	Elevation of study site [m asl]	Visitation rate per flower per hour	Average no. of pollen grains removed per visit	Average proportional share of pollen removed per visit [%]	Average no. of pollen grains deposited per visit	Average proportional share of stigmatic pollen saturation per visit [%]
1	***Ad. adscendens*** **(Sw.) Triana**	bees	70 – 200	**1.39** (110.3)	**13‘386** (439)	**2.9**	**77** (63)	**3.9**
2	***M. hernandii*** **L. Uribe -** ***garden***	bees	1‘950	**0.78** (105.8)	**146‘161** (142)	**5.0**	**613** (42)	**13.9**
2	***M. hernandii*** **L. Uribe -** ***mountain***	bees	2‘000 – 2‘100	**0.00** (60.5)	**262‘704** (206)	**9.0**	**525** (78)	**11.9**
7	***M. maguirei*** **Wurdack**	bees	2‘800	**0.01** (77.7)				
8	***M. maxima*** **Markgr.**	bees	2‘200	**0.00** (152.8)				
9	***M. nobilis*** **Triana**	bees	2‘630	**0.07** (28.0)				
10	***M. speciosa*** **(Bonpl.) Naudin**	bees	1‘885	**0.00** (32.5)				
11	***G. penneysii*** **Michelang. & C. Ulloa**	bees	2‘539	**0.07** (121)				
3	***M. tomentosa*** **(Cogn.) Wurdack**	hummingbirds bats	2‘160 – 2‘250	**3.19** (23.5) **0.73** (20.5)	**50‘399** (264)	**1.1**	**152** (83)	**1.7**
4	***M. sanguinea*** **Wurdack**	hummingbirds rodents	2‘740 – 3‘100	**0.25** (52) **0.03** (63.7)	**62‘930** (274)	**8.6**	**157** (87)	**5.4**
5	***Ax. confusa*** **E. Cotton & Borchs.**	passerines	1‘800 – 2‘000	**0.16** (850.2)	**97‘354** (77)	**20.8**	**239** (63)	**16.9**

6	***Ax. costaricensis* Cogn.**	passerines	2‘500 – 2‘600	**0.13** (228.3)	**99‘352** (76)	**15.3**	**38** (64)	**3.1**

For visitation rate, flower observation hours are given in brackets, for pollinator quality, sample sizes (number of stamens (number of flowers in the case of *Axinaea*) and number of stigmas) are given in brackets. asl, above sea level.

Figure 1. Relation between pollination syndrome and elevation, and the six focal study species. A. Median elevation and latitude of occurrence of 138 Merianieae species. Bee-pollinated species (blue) occur from lowland rainforests throughout to montane cloud forests, while vertebrate-pollinated species (mixed-vertebrate (i.e. hummingbirds, bats, rodents; salmon) and passerine-pollinated (yellow)) are restricted to high elevations mostly above 1900 masl. Mixed-vertebrate-pollinated species in the North occur in oceanic islands, where elevation zones are usually lower than on the mainland (63); passerine-pollinated species in the South occur in the Brazilian Atlantic Rainforest. B. The six study species used to assess differences of pollination efficiency across altitude: 1) lowland *Adelobotrys adscendens*, where bees buzz (vibrate) all stamens together to obtain pollen rewards; 2) montane *Meriania hernandii*, where bees buzz single stamens by biting into the enlarged (yellow) stamen appendages to obtain pollen rewards; 3) montane hummingbird-bat-pollinated *Meriania tomentosa*, providing nectar rewards between stamens (47); 4) montane hummingbird-rodent-pollinated *Meriania sanguinea*, providing nectar rewards; 5) montane passerine-pollinated *Axinaea confusa*, providing food-body rewards (the bulbous inflated yellowish-white stamen appendages, 29); 6) montane passerine-pollinated *Axinaea costaricensis*, providing food-body rewards.

Figure 2. Bayesian stochastic character mapping of pollination syndrome evolution in Merianieae, in association with median elevation of occurrence, and floral traits important in pollinator attraction (reward type) and pollen delivery (stamen structure and stamen appendage shape and size). Bee-buzz-pollination is ancestral in Merianieae, and 3.2 shifts to mixed-vertebrate pollination and 3.7 shifts to passerine pollination have occurred. Pollinator shifts happened relatively recently (late Pliocene), and only in correlation with changes from pollen to nectar or food-body rewards, and adaptations to facilitate pollen delivery (i.e. changes in thecal wall structure) with vertebrate pollinators. Vertebrate-pollinated species have smooth or crumpled thecal walls without septa, while bee-pollinated species usually have corrugated thecal walls and septa, restricting pollen release. Bee-pollinated species do also occur at high elevations, and pollinator shifts are restricted to the core clade (marked with *), which has evolved large stamen appendages and single-stamen buzzing. Numbers 1-6 indicate the six species where we studied pollinator quantity and quality (Figure 1b) and 7-11 indicate the additional five species where we studied pollinator quantity only. asl, above sea level; myr, million years ago.

Figure 3. Differences in pollination efficiency between the different pollinators estimated by GLMMs. A) Bees barely removed pollen in montane *Meriania hernandii* while they removed significantly larger amounts of pollen in lowland *Adeloborys adscendens* and transplanted *M. hernandii*, n = 787. B) Mixed-assemblages of vertebrate pollinators were significantly more efficient pollinators than bees in mountains, n = 747. C) Montane *M. hernandii* was strongly pollen limited while transplanted *M. hernandii* received a surplus of pollen and lowland *Ad. adscendens* was only moderately pollen limited, n = 183. D) Mixed assemblages of vertebrates were most efficient in depositing pollen while passerine birds varied in their pollen-deposition efficiency and *A. costaricensis* was equally pollen limited as montane bee-pollinated *M. hernandii,* n = 375. In A and B, y-axes represent the amount of pollen remaining in stamens, standardized by the mean; in C and D, y-axes represent the stigmatic pollen load relative to the average number of ovules per flower; given the strong variance among species, values are on the log-scale; x-axes represent flower age, error bars represent the 95% confidence interval.

Supporting Information

Fig. S1 Gynoecia mounted for HRX-CT scanning and isosurface models of single carpels.

Fig. S2 Pagel's test for correlated evolution between reward type and appendage size.

Fig. S3 Pagel's test for correlated evolution between reward type and thecal wall structure.

Table S1 138 species of Merianieae included in this study

Table S2 26 Merianieae species with empirically documented pollinators

Table S3 Number of GBIF occurrence records for the 138 species

Table S4 Details on field localities, experimental period and basic information on data collection.

Table S5 Sample sizes for the assessment of pollinator quantity (visitation rates).

Table S6 Sample sizes for the pollination efficiency (pollinator quality) experiment.

Table S7 Pollen grain sizes (mean, min, max) and selected size classes from TOPAS-particle counter.

Table S8 Pairwise comparison of median elevation of occurrence of the three pollination syndromes.

Table S9 Number of transitions between pollination strategies inferred through Bayesian ancestral character estimation.

Table S10 Number and percentage of pollen grains remaining in single stamens at the four anthetic stages.

Table S11 GLMM on pollen removal in the buzz-bee pollinated *Meriania hernandoi* (mountain) *M. hernandoi* (transplant) and *Adelobotrys adscendens*

Table S12 Pairwise comparison of GLMM on pollen removal of three buzz-bee-pollinated species.

Table S13 Chi²-test for significant differences in pollen removal.

Table S14 GLMM on pollen removal from the buzz-bee pollinated montane *M. hernandoi* and the vertebrate pollinated cloud forest species *M. sanguinea* and *M. tomentosa*.

Table S15 GLMM on pollen removal.

Table S16 Chi²-test for significant differences in pollen removal.

Table S17 Number of pollen grains deposited on stigmas.

Table S18 GLMM on stigmatic pollen:ovule ratios.

Table S19 Estimated Marginal means from GLMM.

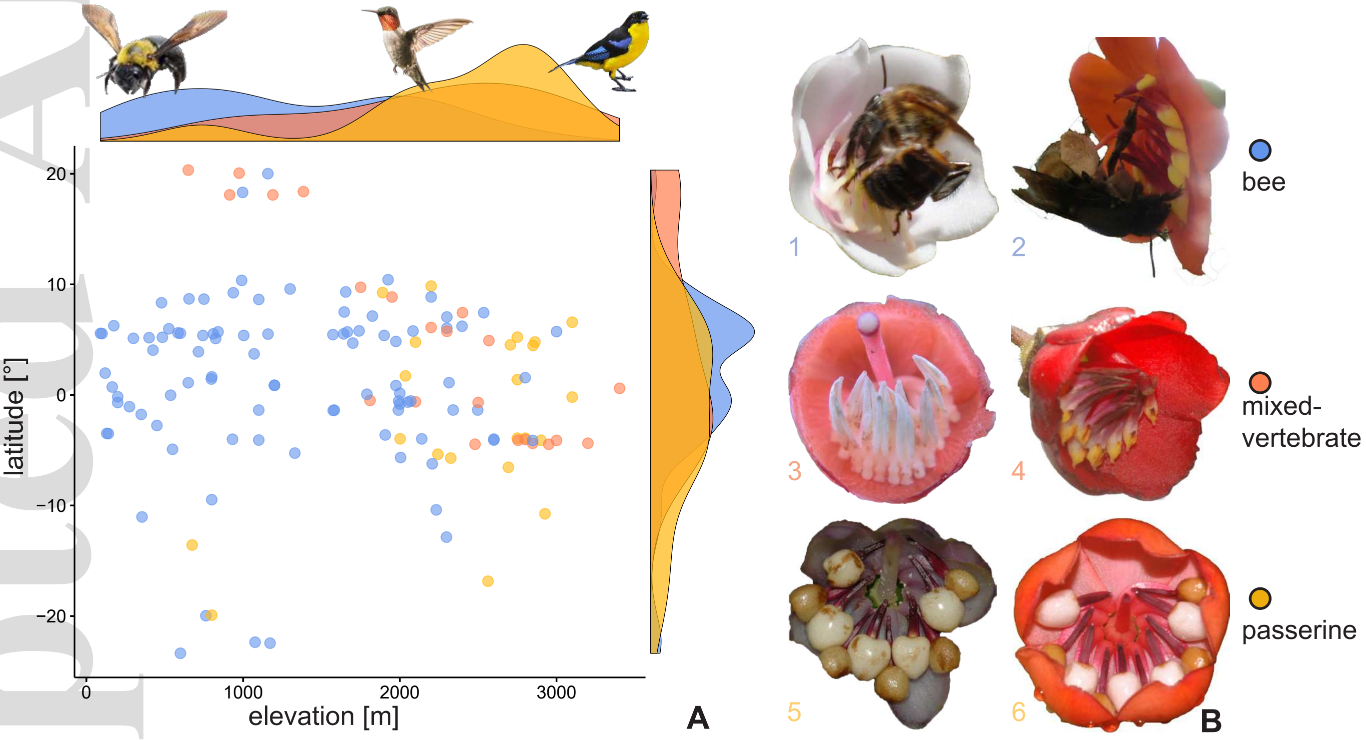
Table S20. Differences in stigmatic pollen loads across anthesis.

Table S21 GLMM on stigmatic pollen load:ovule ratios.

Table S22 GLMM on stigmatic pollen load:ovule ratios.

Table S23. Differences in stigmatic pollen loads across anthesis.

Methods S1 17 floral characters recorded for Merianieae and used for pollination predictions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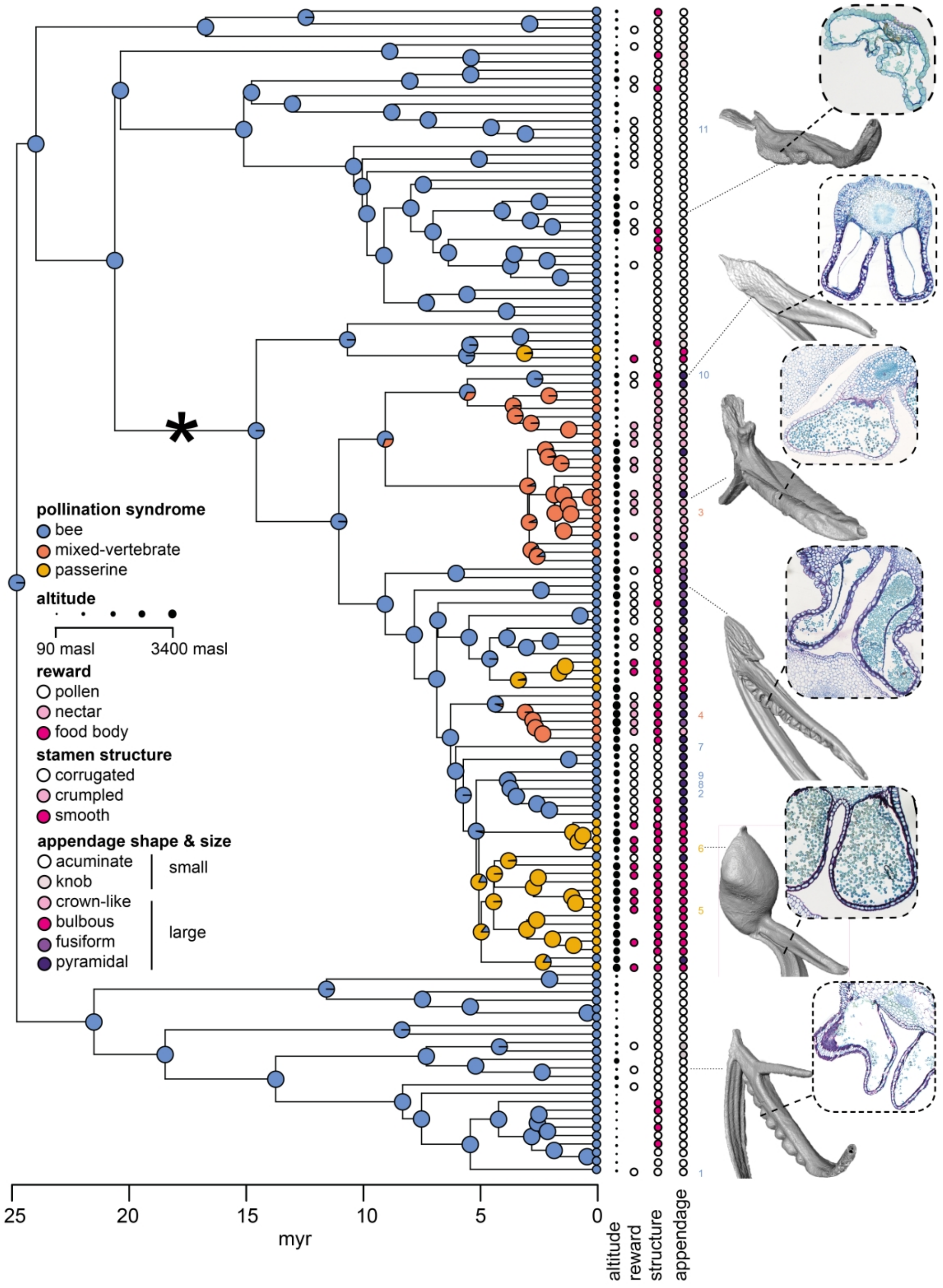

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