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Pollination Syndromes and Pollinator Shifts in Melastomataceae

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Constantin Kopper BSc MSc

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Preamble

Pollination syndromes and disparity

Pollination of angiosperms is a fundamental ecological process essential also for global food production (Klein et al. 2007). Insects and vertebrates are among the most important pollinators of angiosperms (87.5%, Ollerton et al. 2011). To ensure successful pollination, flowers and pollinators have evolved complex interactions (Fenster et al. 2004, Waser & Ollerton 2006, Schiestl & Johnson 2013). As a result, flowers have developed specific suites of floral traits that are finely tuned to attract and interact with their most effective pollinator(s). Pollination syndromes refer to these suites of floral traits, which have evolved repeatedly across angiosperms in response to distinct functional pollinator groups (Vogel 1954, Faegri & Van der Pijl 1979). The concept of pollination syndromes implies that pollinators are clustered in functional pollinator groups, which exert similar selective pressure onto flowers due to similarities in their morphology, dietary preferences, activity patterns and/or behavior (Waser et al. 1996, Armbruster et al. 2000). Flowers pollinated by the same functional pollinator group often converge in trait space due to shared selective pressures, such as flowers evolving long corolla structures in response to moth pollinators with long proboscis (Vogel, 1954, Faegri & Van der Pijl 1979, Fenster et al. 2004, Ashworth et al. 2015). Shifts between functional pollinator groups are widely recognized as major drivers of floral trait divergence within clades. By definition, such pollinator shifts entail changes in selection regimes (Smith & Kriebel 2018, Dellinger et al., 2019) and are typically associated with the evolution of distinct floral phenotypes, differing in characteristics such as color, scent, rewards, and shape, even among closely related species (Schiestl & Johnson 2013, Gervasi & Schiestl 2017, Schiestl et al. 2018, Mackin et al. 2021). As a result, pollinator shifts are considered important drivers of floral morphological diversity, hereafter referred to as floral disparity, which needs to be clearly distinguished from species diversity (Armbruster 2014).

Although floral trait changes in association with pollinator shifts have been extensively studied (e.g., Wilson et al. 2006, Smith et al. 2008, Cardona et al. 2020), much less is known about how floral disparity evolves when lineages diversify within the same functional pollinator group. Low floral disparity and relative morphological uniformity have been reported in several large plant lineages where most species share the same functional pollinator group, Malpighiaceae (oil-collecting bees, Davis et al. 2014), *Myrcia* (Myrtaceae, Vasconcelos et al. 2019), and *Solanum* (Solanaceae; Symon 1979), the two latter taxa primarily pollinated by pollen-collecting bees. These findings suggest that lineage diversification can occur under stabilizing selection on floral traits within a 'successful' pollination syndrome (Davis et al. 2014). However, diversification in the absence of pollinator shifts does not necessarily result in low disparity. For instance, bee-pollinated clades in Melastomataceae exhibit exceptionally high floral disparity (Reginato & Michelangeli, 2016, Dellinger et al. 2019b, Kopper et al. 2024). Such patterns have been attributed to subtle divergent selection imposed by spatially heterogeneous bee pollinator communities (Macior 1971; Dellinger et al. 2019b, Wenzell et al. 2023). Additional factors, including competition for pollinators among co-flowering species and abiotic environmental variation, may also drive floral disparity in the absence of major pollination shifts (Ellis & Anderson 2012).

Floral modularity

Disparity analyses are typically based on multiple traits capturing the entire phenotype (Foote, 1994, Lupia, 1999, Brusatte et al. 2012), but recent studies have increasingly applied a modular approach, assessing disparity within functional, developmental, or evolutionary modules (Monteiro & Nogueira, 2010, Strubbs et al. 2013, Fabre et al. 2016, Chartier et al. 2017). A module is defined as set of floral traits which are highly correlated to each other but can be independent from other such sets of traits (Armbruster et al. 2014). Modules arise through different developmental pathways (Smith 2016, Irish 2017) and can evolve and respond to changing selection regimes independently (Wagner et al. 1996, Hansen 2003, Ordano et al. 2008, Claverie et al. 2013, Felice & Goswami 2018, Larouche et al. 2018). Less is known about the degree of independence and the sequence in which individual modules adapt to selective pressure while evolving a new phenotype.

Floral modules may have different functions in reproductive processes of plants, such as pollinator attraction and pollen transfer, and thus may show functional modularity (Smith & Kriebel 2018, Opedal 2019). Most flowers are comprised by three organ modules (perianth, androecium, gynoecium),, which carry different functions, underly different selection pressures and may hence evolve independently. Recent studies have expanded this classical modular

framework to include functional modules that integrate traits across two or more traditional organ systems (Diggle 2014, O'Meara 2016, Smith 2016). It has been demonstrated that selection can act on combinations of floral traits, and that analyzing such trait combinations provides a better explanation for lineage diversification rates compared to treating traits independently (Fenster et al., 2015, O'Meara et al. 2016). Using a modular approach in Ericales, Chartier et al. (2017) showed that the androecium exhibits the highest morphological disparity, while the perianth was the most conserved. This pattern likely reflects developmental and functional constraints: the perianth is generally less structurally complex than the reproductive organs (Endress & Matthews 2006). In contrast, androecium traits appear to be more evolutionarily labile (Chartier et al. 2017). In general, traits directly involved in pollination are expected to experience stronger selective pressures due to their central role in mediating plant–pollinator interactions (Stebbins 1951, Fenster et al. 2004, van der Niet & Johnson 2012).

Rates of floral trait evolution

Disparity per se does not incorporate phylogenetic information, as it is calculated solely from differences in character states among taxa, independent of their evolutionary relationships (Foote, 1999). Rates of morphological evolution, typically measured as the number of character state transitions per million years, have long been central to macroevolutionary studies (Simpson, 1944, Pagel 1994, Herting et al. 2003, Lee et al. 2013). Until now, estimates of trait evolution rates have typically focused on individual traits rather than correlated sets of traits (modules; Simpson, 1944, Pagel 1994, Herting et al. 2003, Lee et al. 2013), with few exceptions (Tarasov 2019, Porto et al. 2024). While one might intuitively expect that faster-evolving traits generate greater disparity, Herting et al. (2023) demonstrated that both high and low rates of evolution can lead to similarly high levels of disparity in Ericales, suggesting that the relationship between rate and disparity is complex and context-dependent. Herting et al. (2023) found that across angiosperms, the androecium exhibits the highest rates of evolution, whereas the gynoecium and perianth generally evolve more slowly. Interestingly, however, the two fastest-evolving individual traits (floral length and floral diameter) are associated with the perianth. As the rate of evolution is defined by the number of changes per time unit along a branch of a phylogenetic tree, a high rate of evolution suggests that divergent selection acted more strongly on these lineages (O'Meara 2016). If changes in selection regimes, i.e., environment or pollinator shifts, go along with trait changes (Schiestl & Johnson, 2013, Gervasi & Schiestl 2017, Schiestl et al. 2018, Mackin et al. 2021), we may hence expect higher rates of trait evolution in such cases (Simpson 1944, Pagel 1994, Lee et al. 2013).

Pollinator shifts and trait adaptations correlate with environmental shifts

Shifts in selection regimes may not only lead to the evolution of high disparity and potentially higher rates of evolution but also affect the evolution of mutualistic interactions themselves. In the context of pollination, for example, a major prevailing question is how evolutionary pollinator shifts happen. The composition of pollinator assemblages changes markedly along elevational gradients (Cruden 1972, Warren et al. 1988, Primack & Inouye 1993, Dellinger et al. 2021). In the Neotropics, elevational turnovers in pollinator assemblages have been shown to lead to a dominance of vertebrate (particularly hummingbird) pollination in montane cloud forests (Dellinger et al. 2023). Differences in the abiotic environmental tolerances of various pollinator groups (Ollerton 2017, Lefebvre et al. 2019) can influence their abundance and thus pollination efficiency and have been proposed as key drivers of evolutionary pollinator shifts (Cruden 1972, Arroyo et al. 1982, Krömer et al. 2006, Dellinger et al. 2021). While ectothermic insects may act as highly effective pollinators in lowland, warm, and dry habitats (Cruden 1972, Brito & Sazima 2012, McCallum et al. 2013, Classen et al. 2015, Cozien et al. 2019, Classen et al. 2020), their pollination efficiency may decline in cooler and moister environments, due to reduced activity and visitation rates. In cooler and moister environments, vertebrates, flies, and bumblebees may outperform bees as pollinators (Cruden 1972, Dellinger et al. 2021). Environmentally induced reductions in pollinator efficiency could, in theory, lead to the destabilization of ancestral pollination systems, thereby facilitating shifts toward pollinator groups that are better suited to withstanding these adverse climatic conditions (Thomson & Wilson 2008).

Although ecogeographic patterns in pollination systems suggest a reciprocal relationship between abiotic environmental conditions and evolutionary shifts in pollinators (Arroyo et al. 1982, Lefevbre et al. 2019, McCabe & Cobb 2021, Dellinger et al. 2023), this interdependence has rarely been examined at macroevolutionary scales (but see Hamilton & Wessinger 2022, Dellinger et al. 2024). The few studies that have addressed this question found support for the hypothesis that environmental shifts often precede pollinator shifts. Interestingly, both Hamilton & Wessinger (2022) and Dellinger et al. (2024) demonstrated that species retaining the ancestral pollination system were not only able to colonize new environments but also persist in them. This raises a key evolutionary question: why does colonization of novel environments sometimes lead to a shift in pollination system, while in other cases it does not?

Adaptive evolution of floral traits may play a crucial role in enabling species to retain their ancestral pollinators, even under suboptimal environmental conditions (Galen 1989, Bradshaw & Schemske 2003, Smith & Kriebel 2018, Dellinger et al. 2019). This may be particularly important when ancestral pollinators become less abundant in newly colonized habitats, for

example, the reduced presence of bees at higher elevations (Cruden 1972, Dellinger et al. 2021). In such cases, the evolution of traits that enhance pollen transfer efficiency with ancestral pollinators may be selectively favored (Thomson et al. 2000). Key traits include increased investment in overall floral display (e.g., larger flower size), which is broadly correlated with higher pollination rates across angiosperms (Dafni et al. 1997, Goodwillie et al. 2010), and modifications in pollen dispensing strategies. Despite the potential importance of these traits for understanding evolutionary transitions in pollination systems, current predictions are based largely on case studies of a limited number of species, often without accounting for environmental context. As such, it remains unclear whether floral trait modifications that optimize pollen transfer can generally serve as an alternative strategy to pollinator shifts in environments where ancestral pollinators are rare.

The main aim of my thesis is to investigate patterns of floral morphological diversity and rates of trait evolution in the context of pollination syndromes and pollinator shifts. Additionally, I aim to identify factors which trigger pollinator shifts and the associated floral trait adaptations that accompany ecological niche shifts. Using the large tropical plant family Melastomataceae as a model, in the first chapter of my thesis, I first use a machine learning approach to confirm the four proposed pollination syndrome ("buzz-bee", "nectar-foraging vertebrate", "food-body-foraging vertebrate", "generalist") for the whole family. Next, I compare patterns of floral disparity in clades characterized by shifts between different functional pollinator groups versus clades that have diversified in the absence of pollinator shifts. In the second chapter I further analyze patterns of disparity, by merging traits into functional floral modules and compare rates of trait evolution depending on pollination syndromes. Additionally, I explore the effect of different functional pollinator groups and pollinator shifts on temporal patterns of floral module trait evolution. In the third chapter I investigate potential abiotic factors triggering pollinator shifts and explore the relative timing of pollinator- and environment shifts. I also aim to investigate floral trait adaptations in bee-pollinated species growing above 1000 m that may mitigate the effects of inhabiting environments conducive to pollinator shifts, thereby enabling the maintenance of bee pollination.

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Research

High floral disparity without pollinator shifts in buzz-bee-pollinated Melastomataceae

Constantin Kopper, Jürg Schönenberger* and Agnes S. Dellinger*

Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, Vienna, 1030, Austria

Author for correspondence:
Constantin Kopper
Email: constantin.kopper@univie.ac.at

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Summary

- Shifts among functional pollinator groups are commonly regarded as sources of floral morphological diversity (disparity) through the formation of distinct pollination syndromes. While pollination syndromes may be used for predicting pollinators, their predictive accuracy remains debated, and they are rarely used to test whether floral disparity is indeed associated with pollinator shifts.
- We apply classification models trained and validated on 44 functional floral traits across 252 species with empirical pollinator observations and then use the validated models to predict pollinators for 159 species lacking observations. In addition, we employ multivariate statistics and phylogenetic comparative analyses to test whether pollinator shifts are the main source of floral disparity in Melastomataceae.
- We find strong support for four well-differentiated pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'food-body-foraging vertebrate', 'generalist'). While pollinator shifts add significantly to floral disparity, we find that the most species-rich 'buzz-bee' pollination syndrome is most disparate, indicating that high floral disparity may evolve without pollinator shifts. Also, relatively species-poor clades and geographic areas contributed substantially to total disparity.
- Finally, our results show that machine-learning approaches are a powerful tool for evaluating the predictive accuracy of the pollination syndrome concept as well as for predicting pollinators where observations are missing.

Introduction

Pollination syndromes are defined as suites of floral traits, which have evolved repeatedly in adaptation to distinct functional pollinator groups, with flowers pollinated by the same functional group converging in trait space due to shared selective pressures (i.e. due to similar sensory abilities, morphology, dietary preferences, and behavior of their pollinators; Vogel, 1954; Faegri & Van der Pijl, 1979; Fenster *et al.*, 2004; Ashworth *et al.*, 2015). Shifts among functional pollinator groups are commonly regarded as major sources of floral trait differentiation within clades. Per definition, such pollinator shifts entail shifts in selection regimes (Smith & Kriebel, 2018; Dellinger *et al.*, 2019b) and go along with the evolution of distinct floral phenotypes (i.e. differing in color, scent, reward, and floral shape) even among closely related species (Schiestl & Johnson, 2013; Gervasi & Schiestl, 2017; Schiestl *et al.*, 2018; Mackin *et al.*, 2021). Ultimately, pollinator shifts are regarded as generators of high levels of floral morphological diversity (termed 'floral disparity' from here onwards to avoid confusion with species diversity (species richness), Armbruster, 2014). While floral trait changes in relation to pollinator shifts have been studied in great detail (Wilson *et al.*, 2006; Smith *et al.*, 2008; Cardona *et al.*, 2020), we know much less about the evolution of floral disparity when lineages diversify within a functional pollinator group.

Low floral disparity and relative floral uniformity have been identified in some large plant lineages where the majority of species is adapted to pollination by the same functional group (i.e. Malpighiaceae: oil collecting bees, *Myrcia* (Myrtaceae) and *Solanum* (Solanaceae): mostly pollen-collecting bees; Symon, 1979; Davis *et al.*, 2014; Vasconcelos *et al.*, 2019). These studies are hence suggestive of lineage diversification through stabilizing selection on floral traits within a 'successful' (i.e. dominant) pollination syndrome (Davis *et al.*, 2014). Lineage diversification in the absence of pollinator shifts does not generally imply low floral disparity, however, as exemplified by the exceptionally high floral disparity reported among selected clades of bee-pollinated members of the family Melastomataceae (Reginato & Michelangeli, 2016; Dellinger *et al.*, 2019b), or divergence in floral scent profiles in moth-pollinated *Lithophragma* (Saxifragaceae, Friberg *et al.*, 2019). Subtle divergent selection in adaptation to locally variable pollinator communities has been proposed as driver of such patterns of high disparity (Macior, 1971; Dellinger *et al.*, 2019b; Wenzell *et al.*, 2023). In addition, processes such as

*These authors are joint senior authors.

competition for pollinators among co-flowering relatives, or variable impacts by the abiotic environment, may drive the evolution of floral disparity in the absence of pollinator shifts (summarized by Ellis & Anderson, 2012).

High morphological disparity is commonly equated to 'evolutionary success' (Minelli, 2016), reflecting a lineage's potential to adapt through a broad spectrum of possible phenotypes (Foote, 1997; Chevin *et al.*, 2010; Armbruster, 2014). Alternatively, evolutionary success may be expressed through high species diversity (species richness; Minelli, 2016). Interestingly, species richness and disparity often are decoupled across both plant and animal lineages, biogeographic areas, and evolutionary time, with both high or low disparity detected in large clades or areas, irrespective of clade age (Roy & Foote, 1997; Lupia, 1999; Eble, 2000; Roy *et al.*, 2001; Neige, 2003; Oyston *et al.*, 2016; Chartier *et al.*, 2017, 2021; López-Martínez *et al.*, 2023). In the angiosperm order Ericales, for example, Chartier *et al.* (2021) found a positive correlation between floral disparity and a clade's species richness, but not with clade age or a region's species richness so that disparity peaked in tropical areas regardless of species richness. Such uneven patterns of floral disparity might be indicative of regional differences in the diversity of pollinators, developmental or evolutionary constraints, or alternative selective factors (Ellis & Anderson, 2012; Chartier *et al.*, 2021).

With the critical role pollinators play as selective agents on flowers (Caruso *et al.*, 2019), the scarcity of studies quantifying patterns of floral disparity in connection with pollinator data across large plant clades is surprising (but see, that is Dellinger *et al.*, 2019b, Kriebel *et al.*, 2020). This is effectively limiting our ability to draw conclusions on the importance of pollinator shifts vs diversification within pollination syndromes as drivers of floral disparity. The lack of such studies is, in part, due to a shortage of empirical pollinator data for large plant clades, coupled with the difficulty of collecting detailed, meaningful data on functionally relevant floral traits (Dellinger, 2020). To some extent, the concept of pollination syndromes may offer remedy to the limited availability of empirical pollinator data as a tool to predict pollinators solely based on a plant species' floral traits (Dellinger *et al.*, 2019b; Kay & Grossenbacher, 2022). Like any other classification tool, however, pollination syndromes fall short in predictive accuracy especially when only few, easy-to-score traits (e.g. color and flower size) are included (Ollerton *et al.*, 2009; Abrahamczyk *et al.*, 2017; Dellinger, 2020), or when the diversity of pollination systems occurring in a group is not translated into functional pollinator groups (i.e. bias toward specialized systems; Waser *et al.*, 1996; Hingston & Mc Quillan, 2000; Santos-Gómez *et al.*, 2022). The recent implementation of statistical classification methods such as Random Forest Analyses in syndrome-based pollinator predictions might provide a fruitful way forward here, since it allows for objectively training and validating classification models on species with available pollinator data before using such models for predictions (Johnson, 2013; Dellinger *et al.*, 2019b; Kay & Grossenbacher, 2022). These analyses quantify the prediction accuracy for species with known pollinators, rank floral traits depending on their discriminating ability, and allow for the inclusion of more species or traits, as well as for testing alternative classification models (i.e. into more/fewer syndromes) as these data become available in the future (Dellinger, 2020). Finally, such syndrome-based predictions may help identify species with unusual trait combinations (i.e. low prediction accuracy), which may be of particular interest for future empirical pollination studies (Valverde-Espinoza *et al.*, 2021).

The main aim of this study was to compare patterns of floral disparity in clades characterized by shifts between different functional pollinator groups vs clades that have diversified without any such shifts. Our study system is the species-rich, pantropical family Melastomataceae (5858 species in 173 genera and 23 tribes; Penneys *et al.*, 2022). The family is ideally suited for such a comparison since it is dominated by a single, likely ancestral pollination syndrome, 'buzz-pollination' by bees (95.5% of species; Renner, 1989; Dellinger *et al.*, 2022), from which repeated shifts to other functional pollinator groups have occurred (see below). Buzz-pollination is a functionally highly specialized pollination strategy where pollen can only be released from anthers by bees capable of producing specific vibrations applied directly to the flower (Buchmann, 1983; Vallejo-Marín, 2019). Pollen is the only reward offered by buzz-pollinated flowers and is commonly concealed in tubular anthers, which only open by small apical pores (Buchmann, 1983; Endress, 1996). Although buzz-pollination has evolved repeatedly across angiosperms (it is present in *c.* 8% of species), many lineages have converged into a single phenotype of low disparity, the so-called '*Solanum*-type' flower (Vogel, 1978; Endress, 1996; De Luca & Vallejo-Marín, 2013), characterized by actinomorphic corollas, reflexed petals, and a central staminal cone. In contrast to buzz-pollinated lineages converging on the low-disparity *Solanum*-type flower, Melastomataceae stand out as having evolved a large diversity of different buzz-flower morphologies (Dellinger *et al.*, 2019b; Melo *et al.*, 2021). Specifically, buzz-pollinated Melastomataceae may have urceolate to open corollas with the androecial arrangement varying from actinomorphic to strongly zygomorphic, prominent stamen appendages, which bees grasp during buzzing, frequent stamen dimorphisms, and structurally differentiated anther walls likely controlling pollen release dynamics (Caetano *et al.*, 2020; Bochorny *et al.*, 2021; Melo *et al.*, 2021, 2022; Dellinger *et al.*, 2022).

In seven Melastomataceae tribes (Astronieae, Pyxidantheae, Melastomeae, Merianieae, Miconieae, Sonerileae, Olisbeoideae), a small proportion of species (4.5%) have shifted from buzz-bee to alternative pollination strategies (Renner, 1989; Dellinger *et al.*, 2022), hence allowing one to assess the importance of pollinator shifts as sources of floral disparity in the family. These shifts encompass pollination by mixed assemblages of nectar-foraging vertebrates (hummingbirds, flowerpiercers, lizards, rodents, bats; Lumer, 1980; Varassin *et al.*, 2008; Dellinger *et al.*, 2019d), food-body-foraging vertebrates (passerine birds, parrots; Dellinger *et al.*, 2014), and generalist insect assemblages (flies, wasps, beetles, butterflies, bees; Kriebel & Zumbado, 2014, Brito *et al.*, 2016). As expected, these pollinator shifts go along with marked changes in floral traits, most prominently in pollinator rewards (i.e. nectar, food bodies), the evolution of non-vibratile pollen release mechanisms, and changes in corolla shape (Dellinger *et al.*, 2022). Accordingly, Dellinger *et al.* (2022) proposed

four family-specific pollination syndromes (buzz-bee, nectar-foraging vertebrate (NFV), food-body-foraging vertebrate (FFV), generalist), but these have, so far, only been thoroughly tested in the Neotropical tribe Merianieae (300 spp., Dellinger *et al.*, 2019b). It is hence unclear whether these syndromes are also applicable to Paleotropical species and the other 22 tribes. In addition, empirical pollinator data in Melastomataceae are scarce (reliable observations exist for only *c.* 4.5% of species) and skewed toward the Neotropics (only 29 of 252 observations are from the Paleotropics; Dellinger *et al.*, 2022). To mitigate these limitations, we here collected detailed information on 44 functional floral traits across 411 Melastomataceae species, sampling evenly across all genera and geographic regions. We trained and validated machine-learning-based models on the 252 Melastomataceae species with empirical pollinator observations and used these to predict pollinators for the remaining 159 species. Next, we conducted multivariate and phylogenetic comparative analyses to explore the relative importance of pollinator shifts, evolutionary history, species richness, and clade age on explaining patterns of floral disparity across pollination syndromes, biogeographic regions, and Melastomataceae tribes.

Materials and Methods

We ran all statistical analyses in R (R Core Team, 2022).

Species selection and phylogenetic hypothesis

Our analyses are based on a dataset of 411 species (7.02% of Melastomataceae), of which 252 have empirically documented floral visitors (including visitors with high potential to be legitimate pollinators to verified pollinators; Dellinger *et al.*, 2022). We selected the remaining 159 species to represent each genus by at least one species, with the aim to cover the whole morphological diversity of Melastomataceae (therefore, some particularly diverse genera are represented by several species) as well as thoroughly sampling both New- and Old-World lineages. In addition, we aimed for species represented in the most recent dated molecular phylogeny (Reginato *et al.*, 2022, 2454 tips, crown node age 68.5–75.2 million years (Myr); based on the ML topology of Penneys *et al.*, 2022). We used the MCC-tree from Reginato *et al.* (2022) and pruned the tips to the species in our trait matrix (*treedata* function, GEIGER v.2.0.11, Pennell *et al.*, 2014), yielding 334 species (81.27% of our sample). All tribes except Feliciadamieae were covered by this phylogeny. Except for Sonerileae, at least one species that has shifted pollinators in each tribe containing shifts (Dellinger *et al.*, 2022) was included in the present study. Representation of both bee-pollinated and shifted species in the different tribes is important to accurately estimate ancestral pollination systems, however. To cover the missing putative shift in Sonerileae (*Medinilla malabarica* Bedd. & C.E.C.Fisch, *M. sahyadrica* Sujanapal & Sasidh.), we selected two close relatives from the same clade (*Medinilla mannii* Hook.f, *M. micrantha* Jum. & H.Perrier) as surrogate tips. This choice was based on a currently ongoing, detailed phylogenetic treatment of the genus *Medinilla* (P. Quakenbush, unpublished). No closely related bee-pollinated taxa were included so that these surrogate tips represent an independent pollinator shift.

For subsequent analyses, we grouped species into the currently recognized 23 Melastomataceae tribes based on Penneys *et al.* (2022), and geographic regions (Africa, Asia, Central America, South America) based on Ulloa Ulloa *et al.* (2022).

Floral trait scoring

For each species, we scored 44 floral traits, explicitly focusing on functional, pollination-relevant characters (i.e. traits involved in pollinator attraction and pollen transfer; Dellinger, 2020; Supporting Information Notes S1). Of these 44 traits, three were corolla-specific, 28 described the androecium, four were gynoecium-specific, five span organ categories (e.g. *orientation of anther pore to stigma*), and four were purely related to function (e.g. *pollen expulsion mechanism*). The resulting dataset comprises traits identified as important in differentiating pollination syndromes in Neotropical Melastomataceae (i.e. *pollen expulsion mechanism*, Dellinger *et al.*, 2019b) as well as traits commonly used in pollination syndromes (i.e. *color*, Ollerton *et al.*, 2009, Notes S1). We expanded trait categories developed for Neotropical Melastomataceae (Dellinger *et al.*, 2019b) to capture the added diversity recorded here. We scored floral traits from the literature, pictures, drawings, herbarium- and ethanol specimens and stored the data and data sources in the PROTEUS database (Sauquet *et al.*, 2019, Table S1). For details regarding floral trait scoring, see Methods S1.

Predicting pollinators through machine learning

To test whether the four pollination syndromes proposed by Dellinger *et al.* (2022) can reliably predict pollinators, we used the statistical classification method of random forests (RF) implemented in the R-package RANDOMFOREST v.4.7-1.1 (Liaw & Wiener, 2002) following Dellinger *et al.* (2019b). We iteratively trained models and verified classification accuracy into the four syndromes ('buzz-bee', NFV, FFV, 'generalists') for the 252 species with documented pollinators. In a pilot attempt, we also tried classifying species into more refined groups (i.e. splitting the NFV syndrome into 'hummingbirds', 'bats', and 'other'; splitting the'buzz-bee syndrome' into 'small', 'medium', and 'large' bees), but we refrained from using these models given their higher error rates (results not shown). To quantify prediction accuracy, we calculated 100 RFs, each consisting of 500 decision trees based on four randomly chosen traits.

Next, to predict pollinators for the 159 species lacking observations, we used the function *predict* (R Core Team, 2022) on the RF model trained and validated with the species with known pollinators (model M1). As RFs cannot handle missing data, we removed two traits with > 30% missing data (*reward type*, *pollen expulsion mechanism*). As the removal of traits with high predictive power may reduce model accuracy, we first ascertained that the error rates remained low (100% prediction accuracy for 'buzz-bee' and NFV, 80% for FFV and *c.* 65% for 'generalist') by rerunning predictions of species with known pollinators on this reduced trait

dataset (M2, Fig. S1a). Note that the most important traits remained the same for M2, except for those that got removed due to missing data. Misclassifications happened only three times, where species described in the literature as 'generalist' were predicted as NFV (Table S2). In 123 species, additional characters included missing data (Table S1). For these, we ran separate predictions excluding the respective characters with missing data and collated predictions so that pollinators for each species could be predicted with the maximum number of traits available for that species. We ran all predictions 100 times to account for possible inconsistencies in group assignment. For downstream analyses, we collated the observation data for the species with empirically verified pollinators with the predictions for species with unknown pollinators. For background on RF, see Methods S1.

Evaluating floral differentiation and disparity for syndromes, regions, and tribes

We followed Chartier *et al.* (2017) to calculate a floral dissimilarity matrix on the full dataset. To visualize morphospace occupation between pollination syndromes, regions, and tribes, we performed principal coordinate analyses (PCoA) on the dissimilarity matrix. To test whether pollination syndromes, regions, and tribes occupy significantly different areas in the morphospace, we ran nonparametric analyses of variance (PERMANOVA, 9999 permutations to calculate pseudo *F*-ratios) on the dissimilarity matrix. We ran *post hoc* tests with Bonferroni corrections to account for multiple comparisons using the function *pairwiseAdonis* (PAIRWISEADONIS v.0.4.1, Martinez, 2020).

We used three different disparity metrics: mean character difference (mean*D*) as a measure of average disparity between taxa, the maximum pairwise dissimilarity (*R*) as measure of maximal disparity between taxa, and partial disparity (PD) to quantify the relative contribution of taxa to total disparity (following Foote, 1993). We tested for differences in mean *D* using the function *betadisper* in VEGAN v.2.6-2 (Oksanen *et al.*, 2018) and compared disparity among syndromes, clades, and geographic areas. To explore the relation between species richness, clade age, and disparity, we correlated mean *D*, *R*, and PD with tribe size (from Penneys *et al.*, 2022) and tribe age (Reginato *et al.*, 2022) using Spearman rank correlations.

To evaluate whether differences in species numbers in each syndrome, region, and tribe affect our results, we reran the pairwise- and partial disparity analyses, randomly subsampling to 13 spp./syndrome, 42 spp./region, and 12 spp./tribe 100 times. We then calculated the mean *D*, *R*, and PD across the 100 runs. For more details, see Methods S1.

Pollination syndrome evolution and ancestral character estimation

For the 334 species covered in the phylogenetic hypothesis of Reginato *et al.* (2022), we tested for phylogenetic signal in pollination syndromes (Pagel, 1999) and compared five tree transformation models: no transformation, lambda, early burst (EB), kappa, delta using the *fitDiscrete* function GEIGER v.2.0.7 (Pennell *et al.*, 2014). We compared model fit using weighted AIC scores (Table S3).

To estimate ancestral pollination systems in Melastomataceae, we constructed three different multi-state speciation-extinction models (MuSSE), allowing for symmetric transitions among states (SYM), different transition rates for each state (ARD), and constrained rates, following Smith & Goldberg (2015). Then, we compared the weighted AIC of the models using *aic.w* and tested for significant differences between the models using ANOVA (Fig. S2a). We selected the model with lowest wAIC and highest log likelihood (SYM) to use in a an Markov chain Monte Carlo (MCMC) framework. To quantify the impact of differences in tree calibration, we reran the MCMC (5000 steps) over 100 randomly subsampled trees from Reginato *et al.* (2022). We calculated the mean for each parameter across the 100 trees and used *profiles.plot* to visualize the model parameters. We compared parameter estimates between the consensus and the average across the 100 random trees. Since we found no difference between the consensus and the 100 randomly sampled trees (Fig. S2a), we used the consensus tree and the symmetric MuSSE model to map pollination syndromes onto the phylogeny. Finally, we calculated and visualized the log likelihood for the 5000 steps. For details regarding SSE models and MCMC parameters, see Methods S1 and Table S4.

Do pollinator shifts change selection regimes?

To test whether pollinator shifts in Melastomataceae correspond to the expected macroevolutionary changes in selection regimes, we extracted morphospace scores along PC axes 1–3 and modeled trait evolution under an Ornstein–Uhlenbeck (OU) process (Hansen, 1997) using L1OU v.1.43 (Khabbazian *et al.*, 2016). We used a Least Absolute Shrinkage and Selection Operator (LASSO) procedure, following Tibshirani (1996) to estimate shifts in phenotypic optima once for the three PC-axes together and once for each axis separately without an *a priori* definition of where regime shifts may have occurred (OU-M1) using *estimate_shift_configuration*. Note that in this context, 'optimum' is defined as optimum of floral trait evolution (θ) and does not include empirical fitness estimates (Hansen, 1997). We then evaluated convergence of regime shifts using *estimate_convergent_regimes* and evaluated model fit through the phylogenetic Bayesian information criterion (pBIC). Finally, we reran the analyses on morphospaces calculated separately for each tribe with shifted pollinators to explore whether the detection of regime shifts is blurred by the generally wide scattering of buzz-pollinated flowers at the family level.

Results

Predictive accuracy of pollination syndromes in Melastomataceae

Training and validating classification models with the 252 species with empirical pollinator observations showed high predictive accuracy (median OOB error rate 0.8% over 100 RFs). All

'buzz-bee' (209 ssp.), FFV (6 ssp.), and NFV (28 spp.) species iteratively used for training were correctly classified. Prediction accuracy was lower for 'generalists' (9 ssp., median error rate 25%, Fig. 1a), with two generalist species predicted as pollinated by NFVs with 86% (*Miconia corymbiformis* Cogn.) and 58% probability (*Miconia brevitheca* Gleason, Table S2 for details), and one species (*Miconia pusilliflora* DC.) predicted as pollinated by bees with 11% probability.

Given the overall high accuracy of our training model, we next attempted to classify the 159 species lacking observations. Group assignment over 100 RFs was 100% consistent in 149 species, ranged between 96% and 98% in six species, and between 61% and 84% in four species (Table S2).

In total, 350 species were classified into the 'buzz-bee' syndrome, covering all Melastomataceae clades. A total of 61 species across seven clades were placed in syndromes other than 'buzz-bee': 38 as NFV across six clades (Astronieae, Pyxidantheae, Melastomateae, Merianieae, Miconieae, Sonerileae), six as FFV in one clade (Merianieae, Tables S1, S2), and 17 as 'generalist' across four clades (Astronieae, Miconieae, Olisbeoideae, Sonerileae).

Floral characters differentiating pollination syndromes

The traits recovered as particularly informative in differentiating pollination syndromes related to pollinator rewarding (*reward type*) and efficient pollen transfer/flower-pollinator-fit (*pollen expulsion mechanism*, *corolla shape*, *structure of adaxial thecal wall*, *orientation of flowers*, *relative position of stigma to corolla opening*; Fig. 1b). The predictive power of traits varied among syndromes so that a trait of high importance (measured by the Gini index) in one syndrome may be of little importance in another syndrome. Corolla shape, for example, was highly important for characterizing all but the 'generalist' syndrome (Fig. 1b,c).

Geographic distribution of pollination syndromes in Melastomataceae

We found pollinator shifts in each geographic region, but buzz-pollination dominating everywhere (Africa: 41 'buzz-bee', one 'generalist'; Asia: 62 'buzz-bee', four 'generalist', six NFV Central America: 37 'buzz-bee', two 'generalist', 11 NFV, one FFV; South America: 209 'buzz-bee', 10 'generalist', 21 NFV, five FFV; Table S2).

Floral differentiation and disparity across syndromes, regions, and tribes

The floral morphospace (PCoA) showed no clear grouping according to pollination syndromes, but a wide and continuous cloud of 'buzz-bee' species, with shifted species occupying various areas mostly toward the space's margins (Fig. 2a). Despite this broad scattering, all syndromes occupied significantly different areas in the morphospace ($P < 0.05$, Table S5d). The first three PCoA axes explained 31.96% of variation.

The 'buzz-bee' syndrome occupied the largest area of morphospace, contributing most to total disparity (PD 79.21%). Species of the NFV syndrome, which was the second most disparate (PD 13.7%), formed two clusters. One of these clusters (Fig. 2a, group 1: Astronieae, Pyxidantheae, Melastomateae, Miconieae) is clearly separated from the 'buzz-bee' syndrome, while the other group (group 2: Merianieae, Sonerileae) spread along the upper and lower margins of the 'buzz-bee' syndrome (Figs 2a, S3, S4). Most species with a 'generalist' syndrome grouped between the 'buzz-bee' and the NFV syndrome along the negative side of PC1 (Fig. S1b). The 'generalist' syndrome ranked third in terms of disparity (PD 5.37%). Finally, the FFV syndrome, confined to tribe Merianieae, was the least disparate (PD 1.73%) and clustered at the margin of the 'buzz-bee' syndrome along positive PC1/PC2 (Fig. 2a). When randomly subsampling syndromes to $n = 17$ per syndrome (to account for differences in species richness), the NFV syndrome contributed most to total disparity (PD 30.56%), followed by the 'buzz-bee' (PD 29.57%), the 'generalist' syndrome (PD 25.9%) and the FFV syndrome (PD 13.98%).

Comparing mean pairwise disparity as a measure of average floral differentiation per syndrome, the NFV syndrome ranked first (mean $D = 0.381$; Fig. 3a), followed by the 'buzz-bee' (mean $D = 0.365$), 'generalist' (mean $D = 0.295$), and FFV syndrome (mean $D = 0.186$; Fig. 3a). All but the 'buzz-bee' and the NFV syndromes were significantly different in mean disparity ($P < 0.01$; Fig. 3a; Table S5b). These results were largely consistent when subsampling to 17 species per syndrome (Table S5b, c). The 'buzz-bee' syndrome contained the most disparate species pair ($R = 1$), followed by the NFV syndrome ($R = 0.81$).

Comparing morphospace occupation among geographic regions, we found significant differences between South America and all other regions (Table S6d), with South American species scattered widely across the morphospace (Fig. 2b). South America was the region that contributed most to total disparity (PD 51.68%), followed by Asia (PD 22.84%), Africa (PD 13.69%), and Central America (PD 11.65%). Asia had the most disparate species on average (mean $D = 0.412$), followed by Africa ($D = 0.392$), Central America ($D = 0.378$), and South America ($D = 0.366$; Fig. 3a). When randomly subsampling regions to the same number of species ($n = 42$/region), less speciose regions contributed more to total disparity (Asia PD 29.3%, Africa PD 27.26%, Central America PD 22.14%, South America PD 21.31%). Asia and South America as well as Central and South America differed significantly from each other (Table S6c). Results for mean dissimilarity remained the same. For more detailed results and maximum disparity (R), see Notes S2.

Assessing morphospace occupation of the 23 Melastomataceae tribes, 40 of the 253 possible pairwise comparisons were significant (Table S7). Several tribes scattered widely (e.g. Miconieae, Melastomateae), while others occupied distinct areas in the space (e.g. Pyxidantheae, Merianieae; Fig. S5b). The four tribes that contributed most to total disparity all included species that shifted from bee to at least two other pollination syndromes (PD: Melastomateae 21.17%, Sonerileae 19.66%, Miconieae 18.33%, Merianieae 8.74%, Fig. 3b). Partial disparity was strongly

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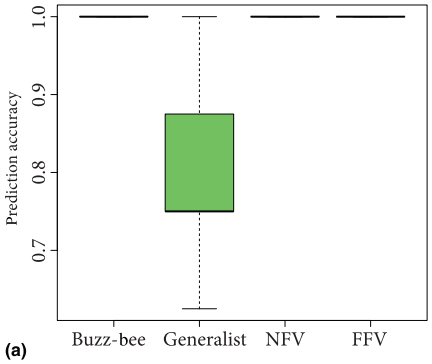
positively correlated with tribe species richness ($\rho = 0.90$, $P < 0.0001$), but uncorrelated with tribe age ($\rho = -0.031$, $P = 0.89$, Fig. S5a). Mean pairwise dissimilarity was slightly correlated with tribe size ($\rho = 0.44$, $P = 0.035$, Fig. S6a) but not with tribe age ($\rho = 0.043$, $P = 0.85$, Fig. S5a), with highest mean D in Trioleneae (0.522), Sonerileae (0.385), and Dissochaeteae (0.377, Fig. 3). Of the 253 possible pairwise comparisons of mean D, 68 were significantly different, with 28 comparisons of clades with pollinator shifts showing higher dissimilarity (Tables S8, S9). When randomly subsampling to an



(a)

Across all syndromes

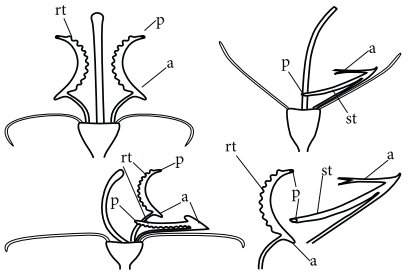
Character	MeanGini
Pollen expulsion mechanism	13.466
Reward	7.858
Structure of adaxial thecal wall	5.735
Corolla shape	5.575
Relative position of stigma to corolla opening	4.560
Orientation of flowers in inflorescence	3.943

(b)

(c)

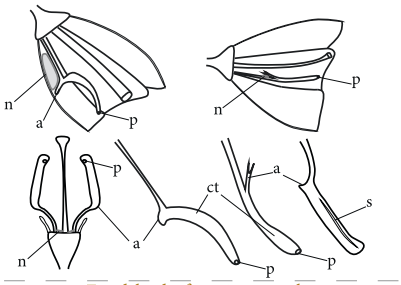
Buzz-Bee

Character	MeanGini
Pollen expulsion mechanism	18.230
Structure of adaxial thecal wall	13.080
Reward	11.512
Corolla shape	9.258
Orientation of flowers in inflorescence	9.014



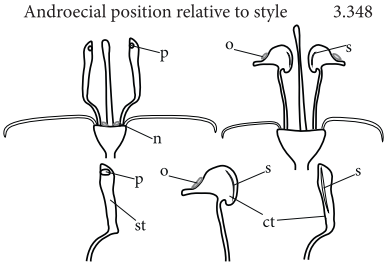
Nectar-foraging vertebrate

Character	MeanGini
Pollen expulsion mechanism	15.906
Reward	12.933
Relative position of stigma to corolla opening	12.722
Corolla shape	12.305
Orientation of flowers in inflorescence	9.338



Generalist

Character	MeanGini
Pollen expulsion mechanism	14.473
Structure of adaxial thecal wall	10.914
Petal length	8.548
Anther length of antesepalous stamens	5.760
Anther length of antepetalous stamens	5.401
Androecial position relative to style	3.348



Food-body-foraging vertebrate

Character	MeanGini
Pollen expulsion mechanism	13.076
Reward	12.552
Dorsal appendage bulbous or not	12.319
Dorsal appendage length of antesep. Stamens	6.193
Corolla shape	5.338
Dorsal appendage length of antepet. Stamens	4.816

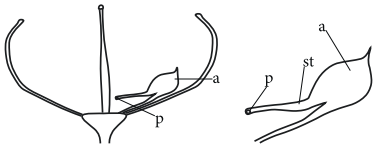


Fig. 1 Prediction accuracy and trait importance of random forest (RF) analyses. (a) Prediction accuracy of best RF model using a total of 100 RF; each forest consisting of 500 trees; the analysis is based on 252 species with known pollinators, 44 functional floral trait, and four pollination syndrome categories ('buzz-bee', 'nectar-foraging vertebrate' (= NFV), 'generalist' and 'food-body-foraging vertebrate' (= FFV), whiskers show range of prediction accuracy). (b) Trait importance (mean Gini Index) for all syndromes together (i.e. traits that are most discriminating between pollination syndromes). (c) Trait importance for the different syndromes separately and schematic drawings of most important traits and most common floral displays; the 'buzz-bee' syndrome is characterized by pollen as reward, buzzing as pollen release mechanism, ruminate or smooth thecal wall, a flat to reflexed corolla and upright to horizontal flowers; the 'nectar-foraging vertebrate' syndrome is characterized by nectar as reward, saltshaker as pollen release mechanism (pollen released when anthers compressed, Dellinger *et al*., 2019b), crumpled thecal wall and a usually pseudo-campanulate, pendent corolla; the 'generalist' syndrome is characterized by nectar/oil as reward, messy pollen expulsion (on touch via large pores), smooth thecal wall when anthers open via pores and crumpled thecal wall when anthers open via longitudinal slit, stamen arrangement is actinomorphic and a flat and small corolla; the 'food-body-foraging vertebrate' syndrome is characterized by food body as reward, bellows mechanism for pollen expulsion (Dellinger *et al*., 2014), large appendages, smooth thecal wall and a bowl-like corolla. a, appendage; ct, crumpled thecal wall; n, nectar; o, oil gland; p, pore; rt, ruminate thecal wall; s, slit; st, smooth thecal wall.

equal number of species per tribe, smaller tribes contributed more to total disparity (Astronieae PD 9.8%, Dissochaeteae PD 8.94%, Sonerileae PD 8.54%; Fig. S5c). We found similar results for mean dissimilarity (Fig. S6b). For more detailed results and maximum disparity (*R*), see Notes S2 and Tables S10, S11.

Ancestral pollination syndrome and floral evolution

Exploring patterns of syndrome evolution, we recovered the delta ARD model as best fit (delta = 2.99, aic.w = 0.78, Table S3), indicating that pollinator shifts happened late (toward the tips) in the evolutionary history of Melastomataceae. Further, we detected strong phylogenetic signal (best lambda model ARD, lambda = 1, aic.w = 0.99, Table S3) for pollination syndromes, indicating that closely related taxa share similar pollination syndromes.

We found strong support for the last common ancestor of Melastomataceae belonging to the 'buzz-bee' syndrome (SYM MuSSE model best fit, Figs 4, S7). The generally few shifts between the 'buzz-bee' and other syndromes likely occurred within the past 15 Myr (low transition rates: 0.02441–0.1251, Fig. S2b). Transition rates among NFV and 'generalist' syndromes were up to 26 times higher than all other transitions (3.399), but with a lower probability (Fig. S2b). We found the lowest transition rates from NFV and 'generalist' to FFV (0.0000024–0.0000034, Fig. S2b). Parameter estimates remained the same across the random 100 trees with slightly variable dating (Fig. S2b).

In our dataset, we recovered two independent shifts from 'buzz-bee' to FFV pollination (both in Merianieae), 13 independent shifts to NFV pollination (four in Melastomateae, two each in Merianieae, Miconieae, Pyxidantheae and one each in Olisbeoideae, Astronieae, Sonerileae), and nine independent shifts to 'generalist' pollination (two in Astronieae, two in Olisbeoideae, and five in Miconieae, Fig. 4). Except for two shifts among the NFV and 'generalist' syndrome, other syndromes always emerged from the 'buzz-bee' syndrome. Our dataset included one reversal from NFV to 'buzz-bee' (*Miconia reducens* Triana, Fig. 4).

Shifts in phenotypic optima did not coincide with pollinator shifts (Fig. 4), even when we reran the analyses on morphospaces calculated for each tribe with shifted species separately (Figs S8, S9). Instead, we found one major shift in phenotypic optima in the evolutionary history of Melastomataceae (Fig. 4), along the branch separating the Olisbeoideae and Kibessioideae (gray, Fig. 4) from Melastomatoideae (light cyan). For more details, see Notes S2.

Discussion

With the central role flowers play in the reproductive process of angiosperms, understanding the sources of their morphological diversity has formed a hallmark in botanical research (Sauquet *et al*., 2019; Sauquet & Magallón, 2018; López-Martínez *et al*., 2023). We identify a combination of diversification within the likely ancestral, functionally specialized 'buzz-bee' pollination syndrome, together with repeated independent shifts to vertebrate or different insect pollinators, as sources of floral disparity. Further, our study emphasizes the usefulness of the pollination syndrome concept as a tool for studying patterns of flower macroevolution and disparity in a pollination biological context (Ashworth *et al*., 2015; Dellinger, 2020).

Pollination syndromes as a predictive tool for macroevolution

Pollination syndromes have been criticized for their low predictive accuracy, especially when few, easy-to-score traits are used as basis for model development (Ollerton *et al*., 2009; Abrahamczyk *et al*., 2017). Particularly when employing pollination syndromes at large scales (i.e. across angiosperms), finding meaningful traits which can be scored across all species can be challenging, limiting researchers to relatively coarse, generalized traits (Dellinger, 2020). In the present study, we used floral traits specific to Melastomataceae and with known functional relevance in the pollination process of the group (based on previous field studies, Dellinger *et al*., 2014, 2019b), in combination with traits commonly used in pollination syndromes. Of the six traits required to accurately delineate Melastomataceae-wide syndromes (Fig. 1), three were specific to Melastomataceae and related to the pollen transfer process (*pollen expulsion mechanism*, *thecal wall structure*, *stigma position*), while three were traditional syndrome traits related to pollinator rewarding and flower-pollinator fit (*reward*, *corolla shape*, *floral orientation*).

Overall, the predictive accuracy of our models, tested on species with empirically verified pollinators, was high (0.8% error rate), with lowest accuracy in the 'generalist' syndrome (25%

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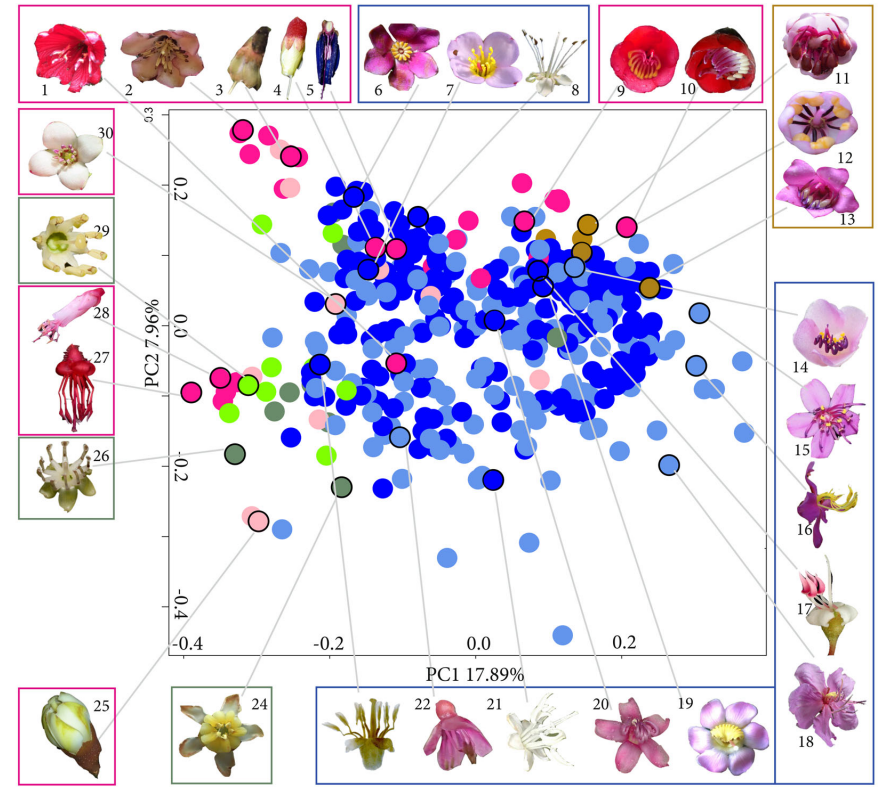
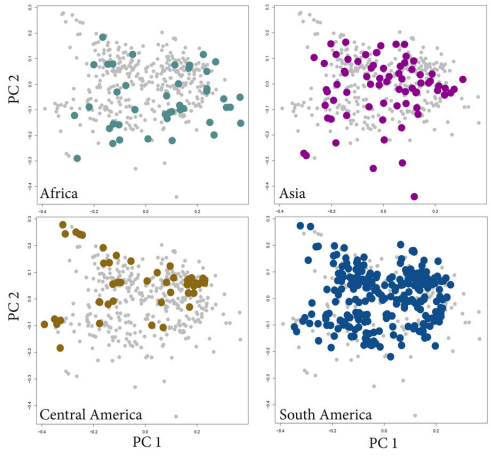
error rate, Table S2). This reduced prediction accuracy of generalist flowers can be explained by their relative 'intermediacy' to accommodate a wide variety of pollinators with different mouthpart morphology and behavior (reflected in scattering across the morphospace, Fig. 2a; Momose *et al.*, 1998, Brito *et al.*, 2016, Karuppusamy, 2019). This prediction inaccuracy is further interesting since it emphasizes the power of statistical prediction models in identifying species of particular interest for empirical



(a) Syndrome

Buzz-bee
Nectar-foraging vertebrate
Generalist
Food-body-foraging vertebrate
Buzz-bee pred.
Nectar-foraging vertebrate pred.
Generalist pred.



(b) Continent

Fig. 2 Morphospace of pollination syndromes, geographic region, and tribes using 411 species and 45 functional floral traits. (a) Syndromes: Morphospace of the four pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'generalist', and 'food-body-foraging vertebrate'); saturated colors indicate known pollinators and dull colors predicted pollination syndromes. The 'buzz-bee' syndrome is clustering in the center of the space with some species exploring the lower right edge of the space. The 'nectar-foraging vertebrate' syndrome is scattered in at least two groups (group 1 (flowers 1–5, 25, 27–28, 30), group2 (flowers 9, 10)). The 'generalist' syndrome falls between the 'buzz-bee' and the 'nectar-foraging vertebrate' syndrome space. The 'food-body foraging vertebrate' syndrome clusters in one group at the upper right edge. The flowers of selected species are shown to illustrate the morphological diversity of the family: (1) Chaetogastra grossa (L.f.) P.J.F.Guim. & Michelang., (2) Chalybea macrocarpa (L.Uribe) M.E.Morales & Penneys, (3) Blakea chlorantha Almeda, (4) Brachyotum ledifolium (Desr.) Triana, (5) Brachyotum naudinii Triana, (6) Blakea tuberculate Donn.Sm., (7) Nerophila clandestina (Jacq.-Fél.) Ver.-Lib. & R.D.Stone, (8) Mouriri pusa Gardner, (9) Meriania radula Triana, (10) Meriania costata Wurdack, (11) Axinaea sclerophylla Triana, (12) Axinaea macrophylla Triana, (13) Meriania macrophylla (Benth.) Triana, (14) Medinilla multiflora Merr., (15) Nothodissotis barteri (Hook.f.) Ver.-Lib. & G.Kadereit, (16) Tigridiopalma exalata S.Jin Zeng, Y.C.Xu & D.F.Cui, (17) Blastus cochinchinensis Lour., (18) Eleotis welwitschii (Cogn.) Ver.-Lib. & R.D.Stone, (19) Blakea maurofernandeziana (Cogn.) Penneys & Almeda, (20) Miconia reducens Triana, (21) Miconia ibaguensis (Bonpl.) Triana, (22) Lijndenia roborea (Naudin) Jacq.-Fél., (23) Miconia lacera (Bonpl.) Naudin, (24) Astronia ferruginea Elmer, (25) Beccarianthus pulcherrimus (Merr.) J.F.Maxwell, (26) Miconia sintenisii Cogn., (27) Miconia melanotricha (Triana) Gleason, (28) Miconia leblondii Judd & Skean, (29) Miconia alpina Cogn., (30) Pleroma cleistoflora (Ule) P.J.F.Guim., M.F.Oliveira da Silva & Michelang. (b) Geographic regions: Morphospace of different regions; colors highlight the species of a specific region; Africa and Asia explore a large area of the space although they harbor fewer species than Central- and South America. The morphospace of tribes can be found in Supporting Information Fig. S5.

studies and hence in generating testable hypotheses. For example, RF models predicted *Meriania macrophylla* (Benth.) Triana, which has traits characteristic of both the buzz-bee and the FFV syndrome, as passerine-pollinated (Dellinger *et al.*, 2019d). Through follow-up fieldwork, we could confirm passerine pollination, thereby also confirming an independent shift into the FFV syndrome (Valverde-Espinoza *et al.*, 2021).

Is there a 'generalist' syndrome?

First, we want to clarify that we use 'generalist' for systems encompassing a wide diversity of insect pollinator taxa from *multiple different* functional groups (Ollerton *et al.*, 2007; flies, wasps, bees, beetles, and butterflies in Melastomataceae; Brito *et al.*, 2017; Dellinger *et al.*, 2022). We consider 'specialized' systems as being pollinated by a *single* functional group of one to many taxa ('buzz-bee', FFV) or representing 'bimodal' systems where a small set of taxa from a maximum of two functional groups acts as pollinators (NFV, Dellinger *et al.*, 2019a,d). We thus do not restrict specialization to obligate one-on-one interactions (compare Waser *et al.*, 1996, Ollerton *et al.*, 2015).

The generalist species distinguished with high accuracy in our study all show distinct traits with adaptations to a broad spectrum of unspecialized pollinators. These traits include upright open flowers, easily accessible nectar, and isomorphic stamens with large pores or slits for facilitated pollen release, arranged in a circle. The generalist species with lower predictive accuracy, however, show interesting crossovers to either the 'buzz-bee' syndrome (i.e. enlarged stamen appendages for buzzing) or the NFV syndrome (i.e. larger, pendant flowers, Fig. 2a), and might indicate a generalization continuum from the specialized Melastomataceae flowers. The proportionally higher evolutionary lability between the generalist and the bimodal NFV syndrome (Fig. 4) indicates that the evolution of nectar secretion is a critical step in adapting to multiple functional pollinator groups. Refined analyses of nectar and scent, paired with empirical field observations, will be essential to resolve the boundaries between these syndromes in Melastomataceae in the future.

Floral disparity across syndromes, tribes, and regions

The high disparity of the 'buzz-bee' syndrome (Figs 2a, 3; Table S5a) is remarkable as it is one of few examples of high floral disparity in the absence of shifts of functional pollinator groups (also e.g. the *Gorteria diffusa* complex, Asteriaceae, Ellis & Johnson, 2009). By contrast, several examples for low disparity in clades without pollinator shifts are known (e.g. *Solanum*, Solanaceae, Endress, 1996; *Myrcia*, Myrtaceae, Vasconcelos *et al.*, 2019). With the notion that high disparity reflects a lineage's ability to adapt through a diversity of phenotypes (Minelli, 2016), we want to emphasize that > 50% of all known bee species can buzz flowers (> 10 000 species; Cardinal *et al.*, 2018). Melastomataceae are hence exposed to a large diversity of bees, offering tremendous potential for floral divergence in adaptation to different bees, without shifting to a different functional pollinator group (Vogel, 1975; Renner, 1989). While there is empirical evidence of effective specialization on distinct buzzing bees among co-flowering Melastomataceae (Mesquita-Neto *et al.*, 2018), we do not know at this point whether patterns of floral disparity differ among 'buzz-bee' syndrome species specialized on few vs many bee species. It will thus be essential to re-evaluate floral disparity in Melastomataceae once enough detailed, empirical pollinator data become available to not only categorize species into four distinct syndromes, but to delineate more refined subsyndromes, that is through trait-matching models (Pichler *et al.*, 2020). Such approaches will also help to surmount shortcomings of the pollination syndrome concept such as the artificial binning of species into 'functional groups', thereby glossing over more subtle processes of floral divergence.

While adaptation to distinct buzzing bees might explain the high floral disparity of Melastomataceae, it does not explain why other large buzz-pollinated lineages such as the genus *Solanum* do not have equally disparate flowers. One feature that clearly sets Melastomataceae apart from the low-disparity *Solanum*-type flower (Symon, 1979; Endress, 1996) is the loosely arranged androecium (Melo *et al.*, 2022). Since the tightly arranged anther cones of *Solanum*-type flowers release higher doses of pollen and place it more accurately than loosely arranged stamens

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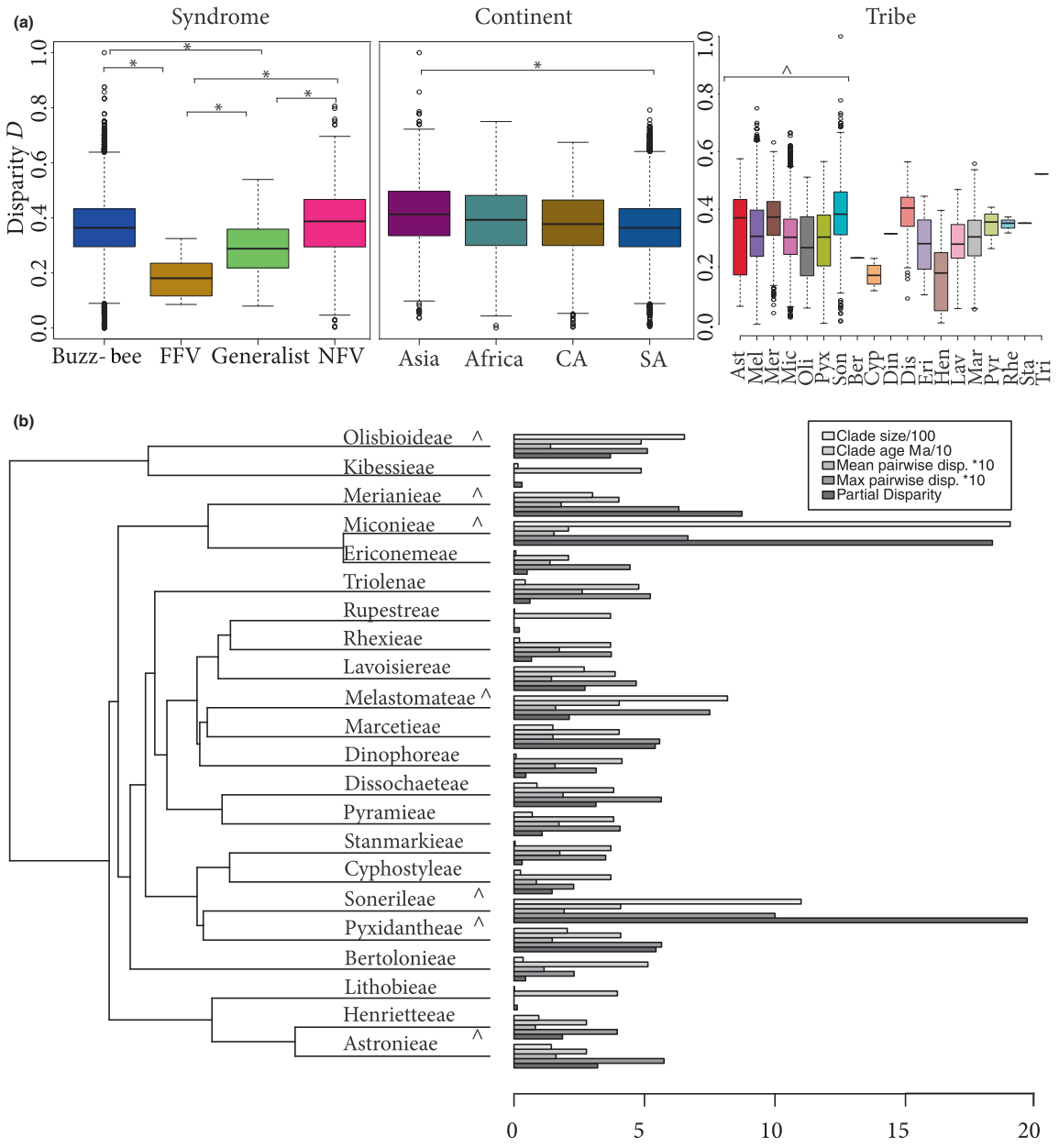

Fig. 3 Floral disparity (= morphological diversity) with respect to syndromes, geographic regions, and tribes. (a) Left graph: mean pairwise disparity (*D*) of syndromes; 'nectar-foraging vertebrate' (= NFV) with highest pairwise disparity; all syndromes are significantly different from each other, except for the 'buzz-bee' and the 'nectar-foraging vertebrate' (NFV) syndrome. Middle graph: pairwise disparity (*D*) of regions; Asia with highest pairwise disparity; Asia significantly different from South America. Right graph: pairwise disparity (*D*) of tribes, for significance (Table S8), we used the function betadisper to test for signific differences among groups; horizontal lines represent the mean; whiskers represent the range; circles represent outliers. (b) Left: Phylogenetic tree from Reginato *et al*. (2020) reduced to one species per tribe. Right: maximum and mean pairwise disparity; partial disparity; clade age (according to Reginato *et al*., 2020) and number of species (according to Penneys *et al*., 2022) per tribe; we performed transformations in order to balance the scale bar (partial disparity values range from 1 to 100 while pairwise disparity value range from 0 to 1, also species number of Tribes vary between 1 and 92). * Indicates significance; ^ indicates tribes that contain pollination syndromes other than the ancestral buzz-bee syndrome.

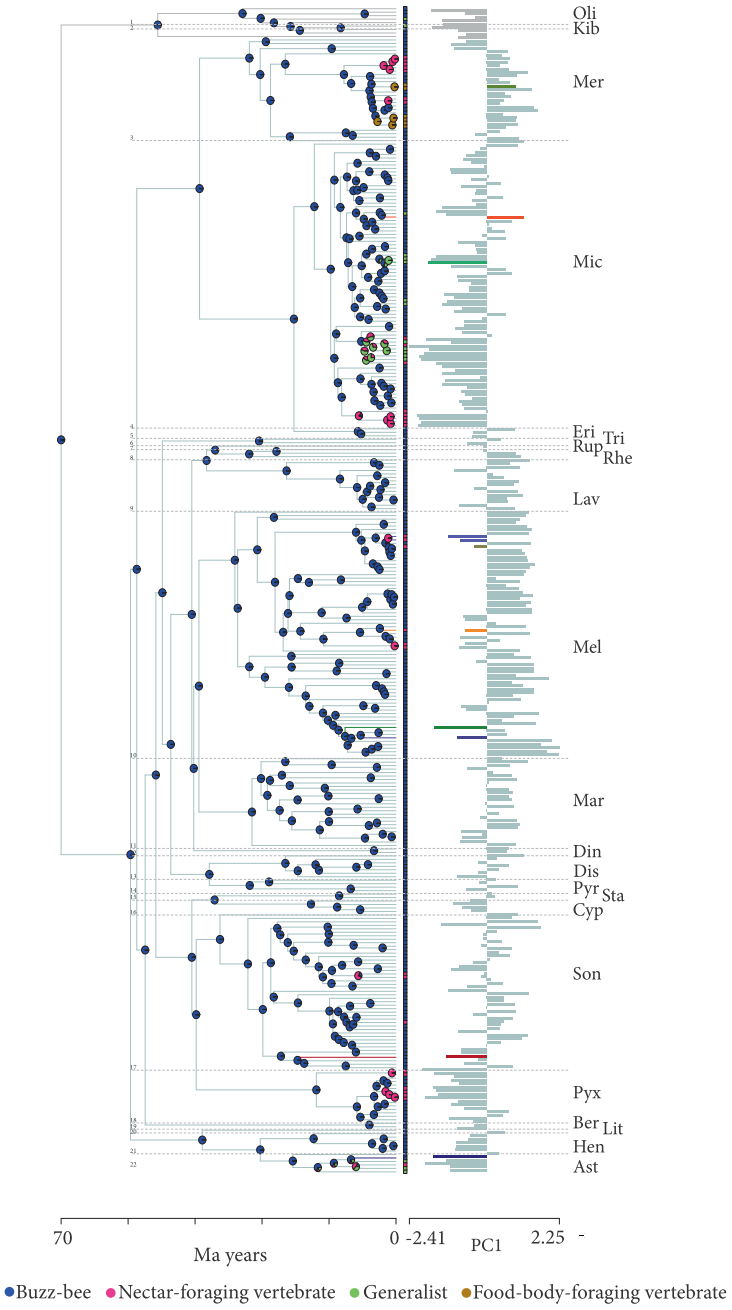

Fig. 4 Estimation of ancestral pollination strategies (left) and results of OU-modeling on first PC axis (right) testing for shifts in floral phenotypic optima across Melastomataceae. Left graph: dated phylogeny with 334 tips, based on the tree of Reginato *et al*. (2020); tip labels indicate pollination strategy; node labels represent probabilities of different pollination syndromes at that node; branch color corresponds to values of the first three PC axis (right), different colors indicate different phenotypic optima (= separate regimes). Right graph: scores from PC1 using 44 functional floral traits, colored bars (and the correspondent branches in the phylogenetic trees) represent separate regimes (optima) from the best shift configuration under the pBIC model selection criterion. These regime shifts correspond to broad patterns of floral trait differentiation, such as slit/slit-like anther dehiscence (instead of pores), pollination syndromes do not correspond to shifts in phenotypic optima, numbers at the bottom of the graph show range of PC-values, actinomorphic androecia and oblong, straight anthers in group 1 (gray), highly variable species in group 2 (light cyan) the rest of the family. Dashed lines separate tribes: 1, Olisbeoideae (Oli); 2, Kibessieae (Kib); 3, Merianieae (Mer); 4, Miconieae (Mic); 5, Eriocnemeae (Eri); 6, Trioleneae (Tri); 7, Rupestreae (Rup); 8, Rhexieae (Rhe); 9, Lavoisiereae (Lav); 10, Melastomateae (Mel); 11, Marcetieae (Mar); 12, Dinophoreae (Din); 13 Dissochaeteae (Dis); 14, Pyramieae (Pyr); 15, Stanmarkieae (Sta); 16, Cyphostyleae (Cyp); 17, Sonerileae (Son); 18, Pyxidantheae (Pyx), 19; Bertolonieae (Ber); 20, Lithobieae (Lit); 21, Henrietteeae (Hen); 22, Astronieae (Ast), for more details see Supporting Information Fig. S7.

(Vallejo-Marín & Vallejo, 2021), it is possible that this highly effective pollination system contributed to constraining *Solanum*-type flowers within a relatively narrow phenotypic range, while the loosely arranged stamens of Melastomataceae allowed for placing pollen on different parts of bees' bodies, increasing potential for phenotypic differentiation.

We found floral disparity to be correlated with species richness across syndromes, tribes, and geographic regions (Figs S5a, S6a),

underlining the importance of floral trait divergence in the evolutionary history of plants (Mander, 2016; Chartier *et al.*, 2017; López-Martínez *et al.*, 2023). Tribes differed in how they contributed to disparity (Fig. 3), however, with large tribes exploring unique areas of trait space through pollinator shifts (high PD), and both speciose and species-poor tribes containing highly distinct species (high maximum pairwise disparity). Similarly, while the most species-rich area, South America, contributed most to total disparity (51.68%, 2743 spp.; Ulloa Ulloa *et al.*, 2022), randomly subsampling to equal species numbers per region showed that even comparatively few species (Asia: 1472, Africa 675) contributed substantially to family-wide disparity through unique phenotypes (Fig. 3). To what extent these patterns of disparity relate to general differences in the bee fauna between the Neo- and Paleotropics (i.e. no bumblebees in sub-Saharan Africa and parts of Asia, Williams, 2007, Orr *et al.*, 2021), or general differences in vertebrate pollinator assemblages (i.e. restriction of hummingbirds and hovering flower bats to Neotropics, Fleming & Muchhala, 2008) remains to be tested.

Buzz-bee pollination is likely ancestral and pollinator shifts were rare in Melastomataceae

We recovered the 'buzz-bee' syndrome as most likely ancestral in Melastomataceae, with a high probability for infrequent but independent, recent pollinator shifts (Fig. 4). This is particularly remarkable considering the rarity of shifts away from buzz-pollination in angiosperms with poricidal anthers and only pollen as reward (e.g. in the former *Cyphomandra* (35 spp.) within mega-genus *Solanum* (*c.* 1500 spp.); Sazima *et al.*, 1993). These low rates of transitions away from buzz-pollination might be directly related to the poricidal anther morphology and pollen rewarding, requiring the evolution of complex alternative pollen expulsion mechanisms and reward types upon shifts to non-vibrating pollinators (Dellinger *et al.*, 2019a). Although not formally tested, this hypothesis is indirectly supported by the high frequency of shifts in lineages with nectar-producing buzz-pollinated flowers where anthers dehisce either via longitudinal slits or pores (e.g. *Erica*, Moquet *et al.*, 2017). Despite poricidal anthers potentially representing a developmental/evolutionary constraint, the recent shifts in Melastomataceae confirm that functionally specialized buzz-pollination is not always an evolutionary dead-end (i.e. *Ruellia*, Tripp & Manos, 2008). To fully evaluate the role of poricidal anthers in dynamics of floral evolution beyond Melastomataceae, however, an angiosperm-wide analysis of lineages with and without buzz-pollination is required.

No shifts in selection regimes with pollinator shifts?

Per definition, shifts among functional pollinator groups represent shifts in selection regimes, detectable as shifts in floral phenotypic optima (Smith & Kriebel, 2018; Dellinger *et al.*, 2019d). Although our RF analyses yielded clear support for this tenet, we did not recover syndrome-specific phenotypic optima in our analyses (Fig. 4). We caution, however, against dismissing the existence of syndrome-specific optima at this point. Our OU-models are based on the first three axes of floral morphospace (31.9% of variation explained), with considerable overlap among syndromes given the wide scattering of the bee-pollinated species. Thus, some bee-pollinated and shifted species have similar PC scores, and hence appeared similar also in OU-models. Since previous analyses focusing on Merianieae (Dellinger *et al.*, 2019a) detected distinct optima with pollinator shifts, we believe that the lack of syndrome-specific optima in this analysis may be caused by our broad-scale approach of recording floral traits informative at the family level, rather than traits capturing the subtleties of each tribe (see Dellinger, 2020). More refined, tribe-specific analyses, ideally including additional traits such as scent and reward quality, as well as more detailed pollinator information, will help clarify this question.

Conclusions and future perspectives

In this study, using Melastomataceae as a model, we show that high floral disparity can evolve in the absence of shifts of functional pollinator groups through floral differentiation within a single pollination syndrome. This result is important since previous studies in other clades (i.e. Myrtaceae, Malpighiaceae) have documented relative floral uniformity when lineages diversify within a pollination syndrome. Whether the high disparity (i.e. diversity of corolla shapes, stamen architectures and stamen appendages) characteristic to the 'buzz-bee' syndrome in Melastomataceae is adaptive in specialization to different buzzing bees, and why patterns of floral disparity are uneven across lineages diversifying within pollination syndromes, awaits experimental testing. Besides diversification within a syndrome, we confirmed the general idea that pollinator shifts contribute to disparity through the evolution of unique floral trait combinations. Importantly, by using refined, system-specific floral traits in combination with objective classification algorithms, we could show that pollination syndromes initially proposed for a single Neotropical Melastomataceae tribe could be accurately applied to the whole family and across the tropics. By training and evaluating classification models on species with empirical pollinator data, we could then predict pollinators for species lacking observations. Clearly, documenting pollinators for these species, as well as refining trait collection for species with lower predictive accuracy, are critical next steps for further validating and refining our prediction models. Finally, we are convinced that an open, integrative research approach that links careful examination of interactions and plant as well as pollinator morphology with large-scale macroevolutionary assessments of trait evolution, embedded in a well-supported phylogenetic framework, has the potential of substantially advancing our understanding of the processes generating floral disparity.

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Competing interests

None declared.

Author contributions

CK, JS and ASD conceived the study. CK scored floral traits and compiled the data matrix. CK and ASD carried out statistical analyses. CK wrote the manuscript, and all authors contributed to finalizing the manuscript. JS and ASD are joint senior authors.

ORCID

Agnes S. Dellinger https://orcid.org/0000-0003-1394-3414
Constantin Kopper https://orcid.org/0000-0002-5567-1720
Jürg Schönenberger https://orcid.org/0000-0001-6791-2731

Data availability

All datasets, r-codes, and the floral trait matrix have been deposited in the open access repository phaidra (https://phaidra.univie.ac.at/o:2052567).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Prediction accuracy of reduced trait set (to test prediction accuracy for species having missing data) and principal coordinate analysis using PC-axis 3.

Fig. S2 ANOVA of different multi-state speciation-extinction models and Markov chain Monte Carlo parameters.

Fig. S3 Morphospace of the four pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'generalist', and 'food-body-foraging vertebrate') highlighting each tribe separately (Astronieae, Bertolonieae, Pyxidantheae Pyramieae, Cyphostyleae, Dinophoreae, Dissochaeteae, Eriocnemeae, Feliciadamieae, Henrietteeae, Kibessioideae, Lithobieae).

Fig. S4 Morphospace of the four pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'generalist', and 'food-body-foraging vertebrate') highlighting each tribe separately (Marcetieae, Melastomateae, Merianieae, Miconieae, Lavoisiereae, Olisbeoideae, Rhexieae, Rupestreeae, Sonerileae, Stanmarkieae, Trioleneae).

Fig. S5 Correlation plots of partial disparity of tribes and clade age/ clade size and principal coordinate analysis of tribes as well as corrected partial disparity.

Fig. S6 Correlation plots of disparity and clade age/ clade tribe of tribes and corrected mean/maximum disparity values of tribes.

Fig. S7 Ancestral state estimation and Ornstein–Uhlenbeck modeling

Fig. S8 Morphospace and Ornstein–Uhlenbeck models of the four pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'generalist', and 'food-body-foraging vertebrate') plotted separately for tribes Miconieae, Olisbeoideae, and Sonerileae where not all species belong to the 'buzz-bee' syndrome. Colored bars in the Ornstein–Uhlenbeck models indicate different phenotypic optima.

Fig. S9 Morphospace and Ornstein–Uhlenbeck models of the four pollination syndromes ('buzz-bee', 'nectar-foraging vertebrate', 'generalist', and 'food-body-foraging vertebrate') plotted separately for tribes Astronieae, Pyxidantheae, Melastomateae and Merianieae where not all species belong to the 'buzz-bee' syndrome. Colored bars in the Ornstein–Uhlenbeck models indicate different phenotypic optima.

Methods S1 Details on floral trait scoring, random forest, and disparity analyses as well as speciation-extinction model specification.

Notes S1 Character code with all traits and trait − states.

Notes S2 Detailed results of disparity analyses and OUwie models.

Table S1 Traitmatrix.

Table S2 Summary of random forest predictions.

Table S3 Pagel's tree transformations.

Table S4 Markov chain Monte Carlo parameter constraints.

Table S5 Results from disparity analysis of syndromes.

Table S6 Results from disparity analysis of regions.

Table S7 Results from pairwise adonis analysis for tribes.

Table S8 Results from betadisper analysis for tribes.

Table S9 Results from max and mean pairwise disparity analysis for tribes.

Table S10 Results from pairwise adonis analysis for tribes when correcting for different number of species.

Table S11 Results from betadisper analysis for tribes when correcting for different number of species.

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Pollination syndromes shape evolutionary rates, floral disparity and modularity

Constantin Kopper, Jürg Schönenberger*, Agnes S. Dellinger*

Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, Vienna 1030, Austria

* Joint senior authors

Author for correspondence: Constantin Kopper

Email: constantin.kopper@univie.ac.at

Social Media:

Agnes Dellinger: @the_kunsze

Abstract

Flowers are composed of distinct developmental modules (perianth, androecium, gynoecium), each serving specialized functions. While these modules must act in concert to ensure reproductive success, studies have shown that they can evolve relatively independently from each other. Such modular independence may lead to differences in disparity and rates of trait evolution among floral modules, potentially facilitating adaptation to different functional pollinator groups.

In this study, we assembled a comprehensive dataset comprising 44 floral traits scored across 326 species within the Melastomataceae family. We use this dataset to investigate three key questions: (1) do floral modules differ in their morphological disparity; (2) can differences in disparity and rates of trait evolution be linked to distinct functional groups of pollinators; and (3) do these pollinator groups differentially influence the tempo and mode of floral evolution.

We find (1) that floral modules differ in their disparity and rates of trait evolution and that (2) these differences depend on pollination syndromes. Also, we find (3) that flowers may evolve in a modular manner in response to selection by different functional pollinator groups, and that these different modules may not only differ in how much, but also in when in the evolutionary history of lineages, they diversify.

Our findings highlight how shifts in selective regimes, such as those driven by pollinator transitions, can promote the evolution of divergent floral phenotypes. It is plausible that major

bursts of floral diversification in angiosperms were similarly driven by shifts in selection regimes.

Introduction

Floral diversity is one of the most striking features of angiosperms and plays a central role in the evolution of plant-pollinator interactions (Stebbins 1970, Fenster et al. 2004, van der Niet & Johnson 2012). Disparity, a measure of morphological diversity, can be used to compare floral diversity among different plant families (Chartier et al. 2017) or pollination syndromes (Kopper et al. 2024). One major driver of high floral disparity is thought to be shifts among different functional pollinator groups (Armbruster 2014). However, the absence of pollinator shifts does not necessarily equate to low disparity, as demonstrated by the remarkably high floral disparity observed within bee-pollinated Melastomataceae (Reginato & Michelangeli 2016, Dellinger et al. 2019b, Kopper et al. 2024). Subtle divergent selection in response to locally variable pollinator communities has been proposed as a key mechanism behind such patterns (Macior 1971, Dellinger et al. 2019b, Wenzell et al. 2023, Kopper et al. 2024). Additionally, factors such as competition for pollinators among co-flowering relatives and heterogeneous abiotic environments may also drive floral disparity in the absence of major pollinator shifts (Ellis & Anderson 2012).

Comparative analyses of disparity, combining data from a large number of traits across a large set of species with phylogenetic information, although rarely conducted, are powerful tools for exploring the evolutionary dynamics of complex, multi-trait phenotypes (Foote 1994, Lupia 1999, Brusatte et al. 2012). Flowers are generally constructed through different developmental modules (sterile perianth, male androecium, female gynoecium) which carry different functions (Endress 1996; perianth – protection, pollinator attraction; androecium – pollen production; gynoecium – seed production). Besides the different functions inherent to each floral module, the modules have to function together as one integrated phenotype in order to achieve successful reproduction (Monteiro & Nogueira 2010, Strubbs et al. 2013, Diggle 2014, Fabre et al. 2016, O'Meara 2016, Smith 2016, Dellinger et al. 2019b). Despite this functional integration and synorganization (sensu Endress 2016), several studies have demonstrated considerable independence among floral traits (and modules), indicating that floral traits may respond differently to selection and evolve at different rates (or "speeds", trait changes per unit time), and show elevated rates of evolution at different times in the evolutionary history of a lineage (O'Meara et al. 2016, Dellinger et al. 2019). To what extent pollinators, the primary agents of selection on floral phenotypes (Barrett & Harder 1996, Caruso et al. 2018), drive such different patterns and speeds of floral trait evolution has rarely been investigated, however.

Given that shifts among functional pollinator groups per definition signify changes in selection regimes on flowers (Smith & Kriebel 2018), lineages featuring repeated pollinator shifts are well suited for studying how pollinators affect the tempo and mode of floral diversification. Accordingly, we may expect higher rates of evolution in adaptive floral traits in lineages undergoing pollinator shifts (Schiestl & Johnson 2013, Gervasi & Schiestl 2017, Schiestl et al. 2018, Mackin et al. 2021). While not evaluated in a pollination-biological context, a recent analysis of rates of floral evolution across angiosperms identified the androecium as fastest evolving component, while gynoecium and perianth traits showed lower rates of evolution (Herting et al. 2023). Importantly, the latter study also found that the two fastest-evolving single traits are "floral length" and "floral diameter", two traits defined by the size of the perianth organs, which are critically important in pollinator attraction and mediating pollinator fit. Similarly, Dellinger et al. (2019b) found that in a plant group with frequent pollinator shifts (Merianieae, Melastomataceae), corolla shape evolved significantly faster than the remaining floral architecture (i.e., spatial arrangement of androecium and gynoecium), in line with studies demonstrating the critical role of corolla shape in mediating pollinator fit and restricting reward access (Mucchala 2007, Muchhala & Thomson 2009, Campos et al. 2015, Mackin et al. 2021). While we may expect that such faster-evolving traits lead to higher disparity, support for this expectation is equivocal, and both high and low rates of evolution can result in high floral disparity (Herting et al. 2023). Furthermore, it remains unclear *how* floral phenotypes evolve, i.e., whether traits belonging to one developmental module evolve jointly or independently, and whether such patterns hold across different selection regimes (i.e., different pollinators). Further, rates of evolution have rarely been reconstructed so far, and when they have, it was typically for individual traits rather than for correlated sets of traits (modules).

We here use the species-rich, pantropical plant family Melastomataceae (5858 species in 173 genera and 23 tribes; Penneys et al 2022) as a model to test for differences in disparity and evolutionary rates across floral organ modules and pollination syndromes. The family is well suited for this assessment since it provides the required evolutionary replicates of pollinator shifts. Specifically, our data encompass at least 21 independent pollinator shifts across 53 species from all seven tribes where pollinator shifts have been reported (Dellinger et al. 2022, Kopper et al. 2024). These shifts away from the ancestral "buzz-bee" pollination syndrome (most common, ca. 96% of species) include shifts to vertebrates (i.e., birds, bats, rodents; Lumer 1980, Dellinger et al. 2014, 2019a) or generalist insect pollinators (i.e., flies, wasps, beetles, butterflies, Kriebel & Zumbado 2014, Brito et al. 2016). Regardless of pollination system, Melastomataceae are characterized by poricidal anthers, whereby most anthers only dehisce through a small apical pore (Endress 1996). The ancestral "buzz-bee" syndrome is characterized

by pollen as reward which is only released via the apical pore when vibrations are applied. Species with the "generalist" syndrome produce nectar as a reward and anther pores are enlarged to facilitate pollen removal by a wide range of non-buzzing pollinators (Brito et al. 2016, Kopper et al. 2024). Species of the "nectar-foraging vertebrate" syndrome also produce nectar as a reward, are mostly pendant and have pseudo-campanulate corollas, probably ensuring pollinator fit (Dellinger et al. 2019, Kopper et al. 2024). Similarly, corolla shape probably has adapted to improve flower-pollinator fit in the "food-body-foraging vertebrate" syndrome, where birds feed off sugary stamen appendages which simultaneously function as a "bellows" mechanism for pollen dispersal (Dellinger et al. 2014). Additionally, species of the family can be found along a wide elevational gradient ranging from sea level to over 3500 m, and pollinator shifts have almost exclusively happened in clades which colonized montane environments (Kopper et al 2025). Hence, comparing tribes that contain species which shifted pollinators ("shifted tribes"), their bee-pollinated ancestors and tribes which remained bee-pollinated ("non-shifted tribes"), offers the opportunity for exploring patterns of floral evolution both in light of pollinator shifts and adaptation to different bee pollinator communities along elevational gradients.

For the present study, we have compiled a data set comprising 44 floral traits (3 traits pertaining to the corolla, 30 to the androecium, 4 to the gynoecium, plus 7 functional traits spanning more than one organ category) that we scored for 326 species (273 "buzz-bee", 31 "nectar-foraging vertebrate", 6 "food-body-foraging vertebrate", 16 "generalist") from across the Melastomataceae family. We use this data set to assess whether floral organ modules differ in their disparity, whether differences in disparity may be attributed to different functional groups of pollinators and whether these pollinators differentially affect the tempo and mode of floral evolution. Since pollinator shifts are an important source of floral diversification, we expect that tribes which contain species that shifted away from bee pollination show higher rates of floral evolution than tribes lacking such pollinator shifts. Similarly, bee-pollinated species from shifted tribes are expected to show higher evolutionary rates than bee-pollinated species from exclusively bee-pollinated tribes, given that they colonized wider abiotic niches, hence also exposing them to more varied pollination niches. Finally, given that the different pollination systems in Melastomataceae require highly differentiated trait functioning, we expect to find differences in the sequence and rate of trait evolution among the four pollination syndromes, with highest rates of evolution in traits critical in mediating pollinator fit (corolla shape in vertebrate-pollinated species, androecial arrangement in bee-pollinated species), pollen dispersal mechanism and pollinator rewarding.

Material and Methods

We ran all statistical analyses in R (R Core Team 2022).

Species selection, pollination mode and phylogenetic hypothesis

Our analyses were based on a dataset of Kopper et al. (2024), removing species with more than 50% missing traits. This resulted in 326 species (5.57% of Melastomataceae), for 205 of which floral visitors were empirically documented (including visitors that were verified as pollinators or were shown to have at least a high potential to be legitimate pollinators; Dellinger et al. 2022). For the remaining 121, we have predicted pollinators in a previous study using machine learning-based classification methods (Random Forest analyses), where we trained initial models on 44 floral traits and 252 species with documented pollinators. Then we used these trained and validated (high prediction accuracy) models to predict pollinators for the remaining species (Kopper et al. 2024). Of the 326 species used in this study, 53 species across seven tribes with at least 21 independent origins have shifted pollinators. These shifted pollination systems can be summarized into three syndromes: "generalist" (flies, wasps, beetles, butterflies, bees), "nectar-foraging vertebrate" (hummingbirds, flower piercers, lizards, rodents, bats) and "food-body-foraging vertebrate" (passerine birds, parrots, Dellinger et al. 2022, Kopper et al. 2024).

We used the most recent dated molecular phylogeny for Melastomataceae to investigate patterns of trait diversification (Reginato et al. 2022, 2454 tips, crown node age 68.5 – 75.2 my; based on the ML topology of Penneys et al. 2022). We used the MCC-tree from Reginato et al. (2022) and pruned the tips to the species in our data matrix (treedata function, geiger v2.0.11, Pennell et al. 2014). All tribes except Feliciadamieae were covered by this phylogeny. Except for Sonerileae, at least one shifted species from each tribe containing shifts (Kopper et al. 2024) was included in the phylogeny, a critical pre-condition for accurately estimating the evolution of pollination systems. The shifts observed in Sonerileae (*Medinilla malabarica* Bedd. & C.E.C.Fisch, M. sahyadrica Sujanapal & Sasidh.) which are not included in the phylogeny originate from the same subclade. In order to also represent this sub-clade (and hence the shifts) in our analysis, we selected two close relatives as surrogate tips from the same sub-clade (Medinilla mannii Hook.f, M. micrantha Jum. & H.Perrier). This choice was based on a currently ongoing, detailed phylogenetic treatment of the genus Medinilla (Quakenbush, pers. com.). No bee-pollinated *Medinilla* taxa from the same sub-clade were included, so that these surrogate tips represent an independent pollinator shift and do not confound our analyses.

For subsequent analyses, we grouped species into the currently recognized 23 Melastomataceae tribes based on Penneys et al. (2022). Taxonomy strictly follows phylogenetic principles, i.e.,

all tribes are monophyletic. Note that we use the term tribe throughout the paper to refer to these 23 taxonomic tribes, while we use the term clade for groups of closely related species within a tribe (i.e., shifted and non-shifted clades within tribes).

Defining floral organ modules

We classified our traits into the three ontogenetic floral organ modules corolla (three traits), androecium (30 traits), gynoecium (four traits) and additionally functional traits (seven). We define functional traits as traits which are directly linked to pollination (i.e., *pollen expulsion mechanism*, *reward type*) and traits which fall into two floral organ modules (e.g., *orientation of anther pore to stigma*).

Partial disparity of organ modules

To quantify the relative contribution of taxa to total disparity for the four floral organ modules (corolla, androecium, gynoecium, functional) separately for each pollination syndrome we calculated partial disparity (PD) (Foote 1993), following Chartier et al. (2017).

To evaluate whether differences in species numbers in each syndrome and trait numbers in each floral organ module affect our results, we reran the partial disparity analyses, randomly subsampling to 6 spp./syndrome, and 3 traits/module. We then calculated PD across the 100 runs.

Comparing pairwise disparity of floral organ modules

To compare the disparity of the four floral organ modules of the different syndromes we calculated pairwise disparity (D) as a measure of disparity between tax of a specific group (Foote 1993). As our organ-specific disparity analyses differ in the number of traits, their respective disparities cannot be compared directly. Following Chartier et al. (2017), we first investigated whether the pairwise disparity for each of the floral organ modules increased with increasing disparity of the whole flower. To do so, we performed a Mantel test using the function *mantel* in VEGAN v.2.6-2 (Oksanen et al. 2018), to test for a correlation between the pairwise disparity matrices (D for each taxa pair) calculated for each of the modules' character sets, respectively, and the pairwise disparity matrix calculated for the whole character set. Taxa pairs for which D could not be computed for one of the functional modules, due to missing data, were pruned from the matrices before performing the test.

To assess whether the corolla, androecium, gynoecium, and functional organ modules are more or less disparate than the rest of the flower, we compared the pairwise disparity (D) of the whole dataset associated with the corolla, the androecium, the gynoecium and the functional organ

modules to the distributions of D calculated for random character sets of the same sizes, following Chartier et al. (2017). Using the total taxon set, each of these distributions was obtained by calculating D for 1000 matrices of respectively 3 (to be compared with the corolla), 30 (to be compared with the androecium), four (to be compared with the gynoecium) and seven (to be compared with the functional module) characters randomly sampled without replacement in the character set. Corolla, androecium, gynoecium, and functional modules were considered as significantly more or less variable than the rest of the flower if D of corolla, androecium, gynoecium, and functional modules were higher than 97.5% or lower than 2.5% of the 1000 randomly sampled D values of the whole flower.

We used the above-described pipeline for all species together but also ran analyses for each syndrome separately.

Trait variation

Following Chartier et al. (2017), we compared the variation among the 44 functional floral traits, by averaging the differences between taxa, for each trait. The resulting values, here noted Dchar, increase with the variation of a character in the dataset. Again, we calculated Dchar for all species together, as well as separately for each syndrome.

Rates of floral organ modules evolution

To evaluate the rate of evolution of the four different floral organ modules and the entire phenotype, we used the PARAMO pipeline following Tarasov et la (2019) and Porto et al. (2024). PARAMO takes a dated phylogeny, a phylogenetic character matrix, and character annotations to anatomy ontology terms (Tarasov et al. 2019, Porto et al. 2024). As PARAMO is only working with categorical traits, we converted continuous traits into categorical traits (i.e. corolla length). Additionally, we merged character states with more than seven categories as reconstructing traits with many states results in weakly supported node probabilities (ZITAT), resulting in a final trait matrix including 44 traits (corolla: 3, androecium: 30, gynoecium: 4, functional: 7) and 326 species across all 23 tribes.

In a first step, we linked traits to one of the four floral organ modules. Next, we fitted multiple Markov models (ARD, SYM, ER) of discrete character evolution using the functions *corHMM* (corHMM, Beaulieu et al. 2013) and chose the best model using model selection. We used the function *makeSimmap* (corHMM, 1000 simulated maps for each trait) to simulate stochastic maps for each character using the best fit model. Then, we used the function *paramo* (ontophylo, Porto et al. 2024) to stack the stochastic maps for a set of anatomy ontology terms (i.e., corolla, androecium, gynoecium, functional) and *paramo.list* (ontophylo) to stack

stochastic maps for the entire phenotype. To plot the resulting simulated stochastic maps, we used the function *plotSimmap* (phytools, Revell 2024).

To estimate the evolutionary rates of amalgamated traits we used the pNHPP (piecewise Non-Homogeneous Poisson Process) method in ontophylo. First, we calculated Hamming distances between state vectors using the function *path_hamming_over_trees_KDE* (ontophylo) and estimated Kernel Density Estimator (KDE) using *estimate_edge_KDE* (ontophylo). Next, we calculated smoothing and normalize KDE data using the functions *loess_smoothing_KDE* and *normalize_KDE* (ontophylo). Then, we calculated the lambda statistics for Non-Homogenous Poisson distribution and retrieved the posterior distribution using the functions *posterior_lambda_KDE* and *make_postPois_KDE* (ontophylo). To prepare our data for plotting the estimated evolutionary rates of the four floral organ modules and the entire phenotype we used the functions *make_contMap_KDE* and *edge_profiles4plotting* (ontophylo). To plot the data, we used the function *edgeplot* (ontophylo). To better visualize the rate differences among the four different floral organ modules we adapted the *make_contMap_KDE* function to scale the plotting colors to a global range (*make_contMap_KDE_scale*). To plot the output of *make_contMap_KDE_scale* we also adapted the *edgeplot* function (edgeplot_custom_scale).

We extracted the number of changes with their respective rate values along each edge of the phylogeny (equals the rate of evolution along each branch) for downstream analyses and visualizing the rates of evolution. To make the plots better comparable between syndromes we divided the frequency of rates by the number of species present in a syndrome (Fig. 6).

We performed Kruskal-Wallis chi-squared tests in combination with pairwise Wilcoxon tests to check for significant differences in the rate of evolution between the different organ modules for the whole data set, among shifted tribes and tribes which remained bee-pollinated, among syndromes, and among bee-pollinated species from shifted tribes and bee-pollinated species from tribes which remained bee-pollinated.

Timing of rate shifts

To analyze potential temporal patterns of evolutionary rate shifts of the different floral organ modules across pollination syndromes, we retrieved ages and evolutionary rates of specific nodes of the phylogeny calculated during PARAMO analyses. Specifically, for shifted species (NFV, FFV, generalist) we traced back edges until reaching a node with 100% probability of being bee-pollinated, while for bee-pollinated species, shifted tribes and non-shifted tribes we traced back the nodes until reaching the last common ancestor of the tribe. To visualize temporal patterns of rate shifts we used the function *ggplot* (ggplot2, Wickham 2016), showing age on the x-axis and rate of evolution on the y-axis.

Rates of initial single traits

To also evaluate evolutionary patterns at the level of single traits, we used the output files of the *makeSimmap* function (see above) for each trait. We wrote a custom function (process_simmap_rates) which automatically loads the required files and retrieves the total time spent in each state along each edge for all 1000 simulated trees (mapped.edges), Q (rate matrix) and the length of edges. To get the expected number of changes along an edge, we used mapped.edges and the exit rate (rate of leaving a state) retrieved from Q. Next, we divided the number of expected changes by the branch length to get the rate of evolution for each trait along each edge and calculated the mean across the 1000 simulated trees. Next, we wrote a function, called *plot_simmap_rates* where we implemented *ggplot* (ggplot2) to plot the rate of evolution for each induvial trait colored by the four different floral organ modules. We performed Kruskal-Wallis chi-squared tests in combination with pairwise Wilcoxon tests to check for significant differences in the rate of evolution among the individual traits and among the different organ modules.

Results

The disparity of floral organ modules differs among pollination syndromes.

We found that the most species-rich "buzz-bee" syndrome showed the highest partial disparity (PD) in all floral organ modules (Fig. 1, Tab. 1). The "nectar-foraging vertebrate" syndrome showed the second highest disparity, especially in corolla and gynoecium traits (ca. 50% of "buzz-bee" disparity). Disparities of the "generalist" and "food-body-foraging vertebrate" syndrome were less than half that of the "buzz-bee" syndrome. These results were matched when randomly subsampling to the same number of traits per organ module (n = 3) when comparing syndromes (Fig. 1a, S1, Tab. S1).

Table 1: Partial disparity (PD) across pollination syndromes and floral modules. NFV = "nectar-foraging vertebrate"; FFV = "food-body-foraging vertebrate".

Syndrome	Total	Corolla	Androecium	Gynoecium	Functional
Bee	75.55	66.34	80.49	67.84	64.40
Generalist	6.73	4.38	5.92	6.04	14.41
NFV	15.24	24.89	11.13	25.11	20.00
FFV	2.44	4.37	2.47	1.01	1.19

These results were matched when randomly subsampling to the same number of traits (n = 3, Fig. 1b). When randomly subsampling to the same number of species per syndrome (n = 6), we found the lowest total PD in the "buzz-bee" syndrome, confirming that the high floral disparity of bee-pollinated species is a result of their high species richness (Fig.1c, S1, Tab. S2). The

"nectar-foraging-vertebrate" syndrome showed the highest PD in corolla and gynoecium traits, while the "food-body-foraging vertebrate" syndrome peaked in PD for androecium traits, and the "generalist" syndrome showed the highest disparity in diverse traits linked to flower functioning such as reward types and pollen release mechanisms.

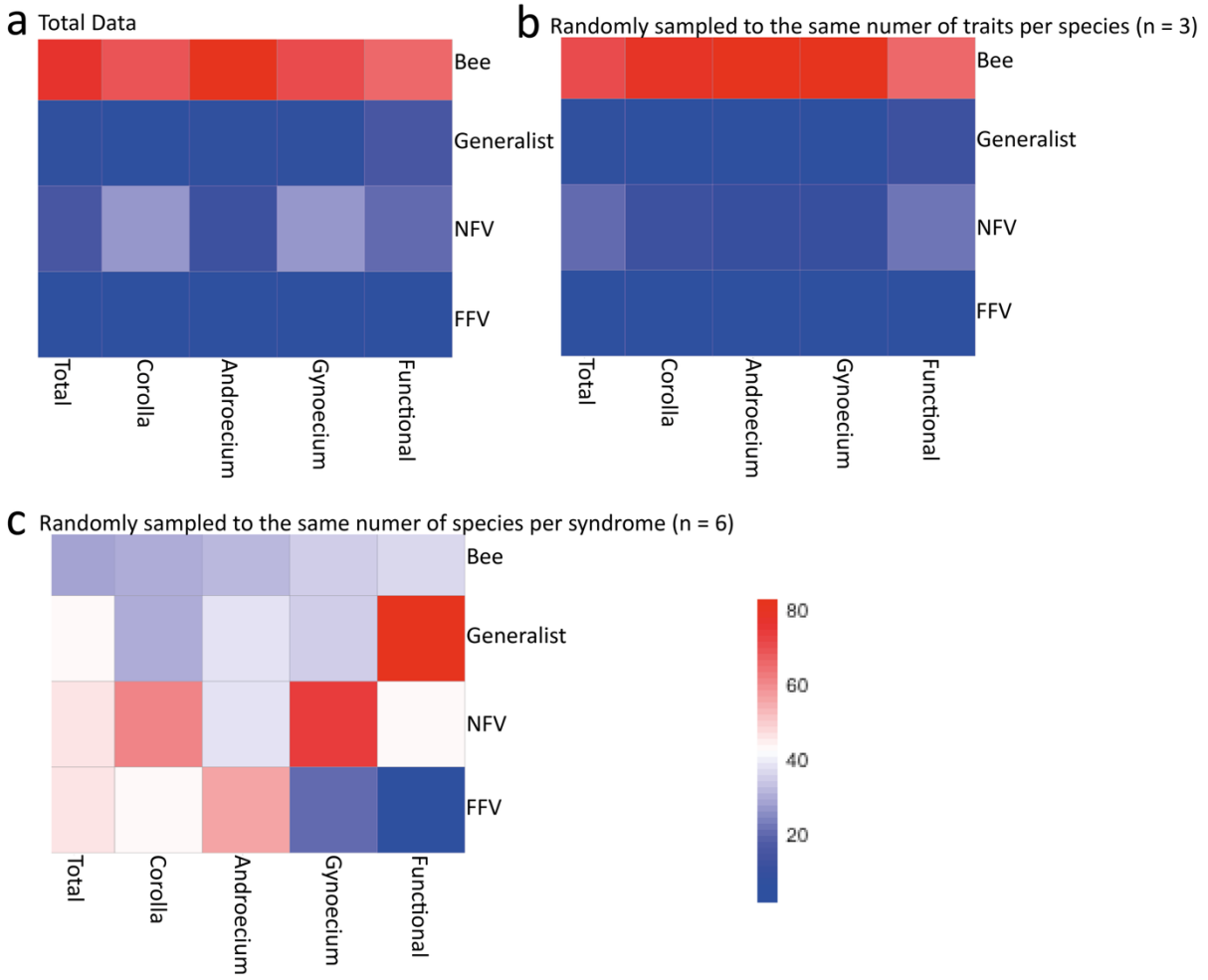

Figure 1: Heatmap of partial disparity (PD). (a) total data; (b) randomly subsampled (100 runs) to the same number of traits per module (n = 3); (c) randomly subsampled (100 runs) to the same number of species per syndrome (n = 6).

Corolla and androecium are most disparate

The Mantel test revealed that the disparity of the androecium is strongly positively correlated to the total floral disparity (R^2 0.88, p = 0.001). This strong positive correlation between androecial and total floral disparity was found in each pollination syndrome. None of the other organ modules showed similarly strong correlations with total floral disparity (Tab. S3).

Comparing the pairwise disparity (D) of the corolla, the androecium, the gynoecium and the functional trait modules to the distributions of D calculated for random character sets of the same sizes, we found that none of the organ modules were significantly more disparate than expected by chance. The only cases where modules were significantly different from a random expectation were the corolla (more disparate) in the "nectar-foraging vertebrate" syndrome and

functional and androecium traits in the "food body-foraging vertebrate" syndrome (Fig. 2). These results indicate that diverse corollas (i.e., shape, color) exist in flowers visited by nectar-foraging vertebrates, while the androecium and its functioning (bulbous stamens with bellows mechanism serving as food-body reward) is highly conserved in the "food-body-foraging" vertebrate syndrome.

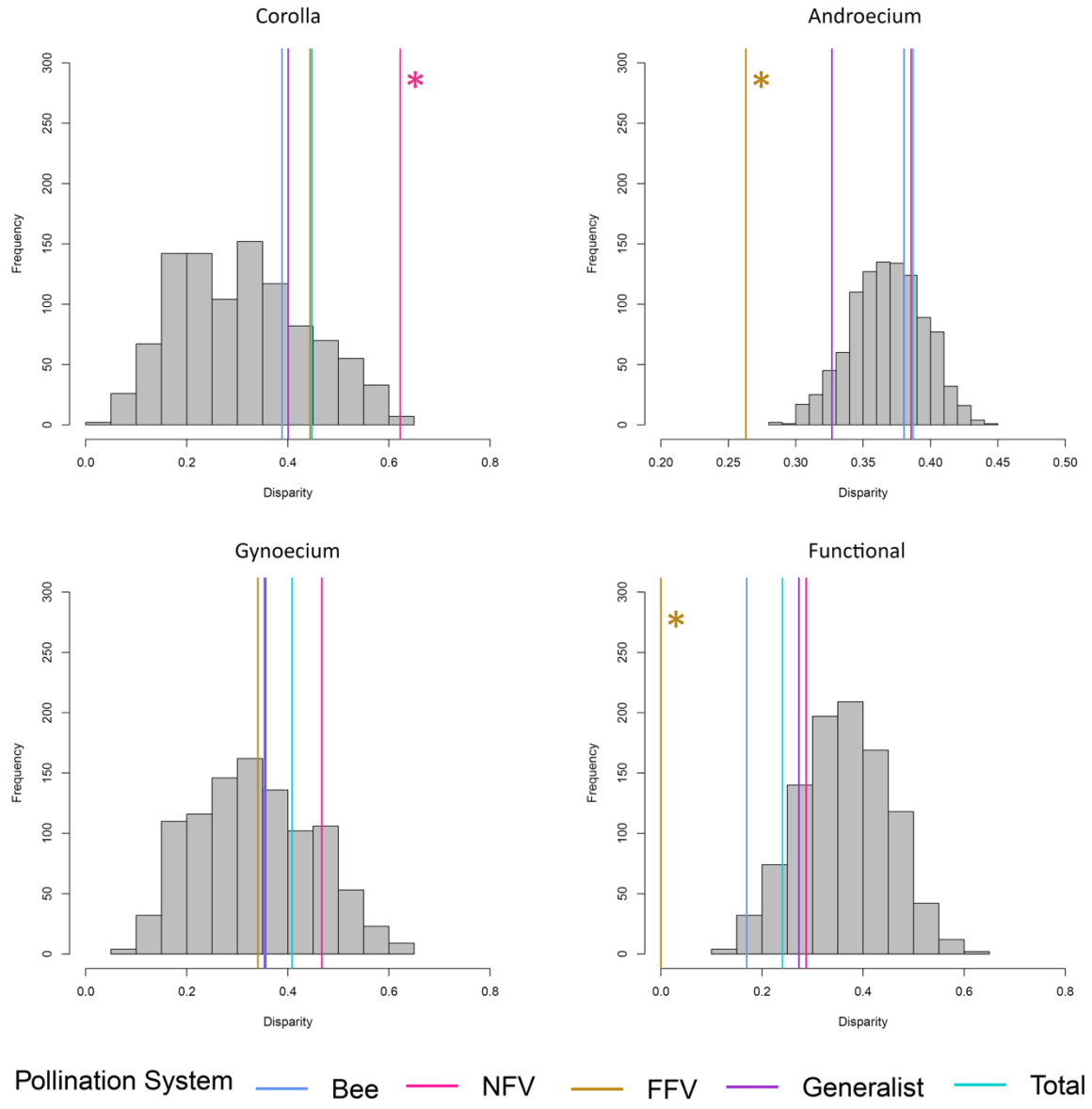

Figure 2: Comparing pairwise disparity (D) of the whole dataset associated with the corolla, the androecium, the gynoecium and the functional organ modules to the distributions of D calculated for random character sets of the same sizes, following Chartier et al. (2017). Significant values (≤ 25th or ≥ 75th percentile of the null distribution) are indicated with an asterisk (*).

When comparing trait variation (Dchar) of the floral organ modules, we could show that the corolla- and androecium traits showed the highest variation, with corolla color being most variable, followed by five androecium traits (*shape of dorsal stamen appendage of antepetalous stamen, location of stamen pore of antepetalous stamen in relation to floral center, location of stamen pore of antesepalous stamen in relation to floral center, shape of dorsal stamen*

appendage of antesepalous stamen, level of anther pore). Functional floral traits showed intermediate variability (Fig. 3). These patterns mostly held also when analyzing trait variation within syndromes, with androecium and corolla traits being most variable in the "buzz-bee", "generalist" and "nectar-foraging-vertebrate" syndrome, and gynoecium and corolla traits being most variable in the "food-body-foraging" vertebrate syndrome (Fig. S2, S3).

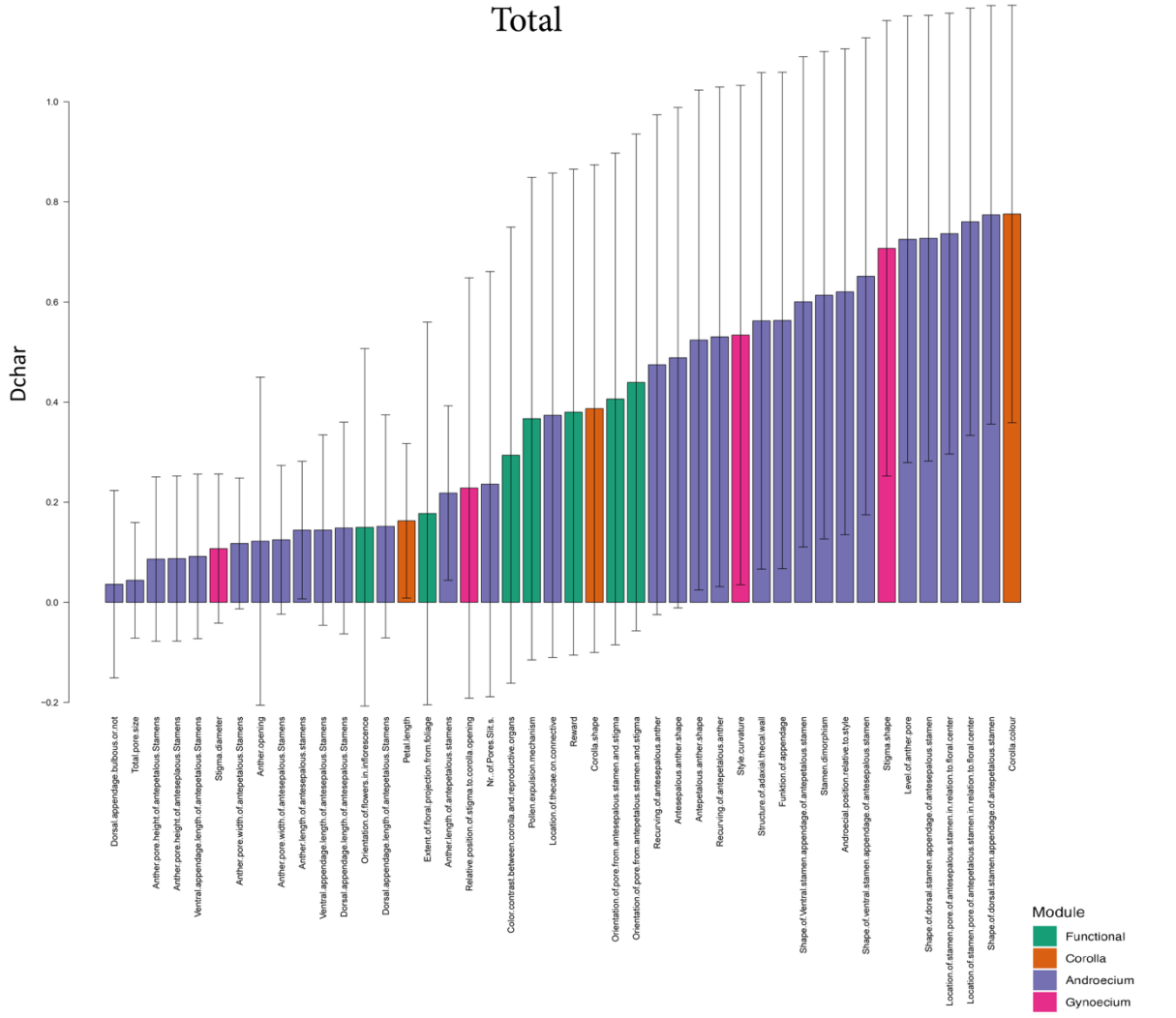

Figure 3: Trait variation of the 44 floral traits used in this study following Chartier et al. (2017). Dchar, increase with the variation of a character in the dataset.

Single trait reconstructions

When looking at the rate of evolution of the initial single traits which define the different floral organ modules, we found that androecium and gynoecium traits showed the highest rates of evolution followed by functional and corolla traits (Fig. S4). Specifically, orientation and size of the anther pore and the stigma (*location of pore in relation to floral center, anther pore width, stigma shape, level of anther pore in relation to stigma, anther curvature*, and *appendage shape*), important in mediating pollen transfer, showed the highest rates of evolution. Among corolla traits, corolla color and petal length (likely involved in pollinator attraction) showed higher rates of evolution than corolla shape.

Timing of rate shifts across syndromes

Across the Melastomataceae phylogeny, we observed temporally variable shifts in rates of trait evolution. Notably, lineages rarely exhibited elevated rates in more than one floral module at the same time. Instead, modules tended to evolve in a specific sequence, shaped by whether lineages retained the ancestral "buzz-bee" syndrome or shifted to novel pollinators (Fig. 4, S5).

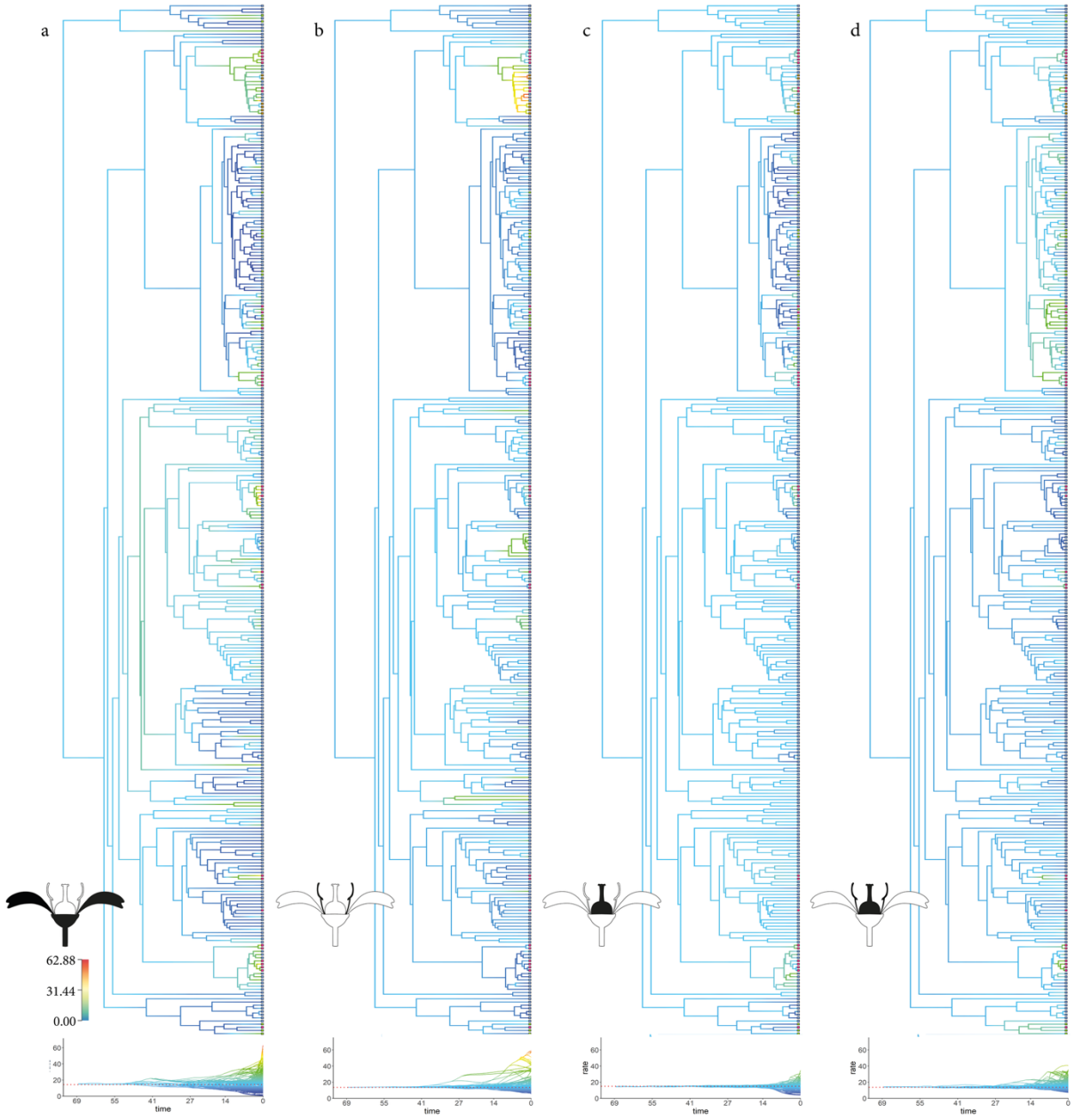

Figure 4: Reconstructed rates of trait evolution of four floral models (corolla, androecium, gynoecium, functional) and corresponding rates through time plots using PARAMO (Tarasov et al. 2019, Porto et al. 2024) based on the most recent phylogeny of Reginato et al. (2020); tip labels indicate pollination syndromes(blue = bee-pollinated, pink = nectar-foraging vertebrate, green = generalist, gold = food-body-foraging vertebrate.(a) corolla module; (b) androecium module; (c) gynoecium module; (d) functional module. Drawings of flowers correspond to modules. Colored branches represent the speed of module evolution.

When comparing rates of floral evolution among shifted and non-shifted tribes, we found an earlier and stronger decoupling in evolutionary rates in non-shifted than in shifted tribes (Fig.

5a, b). In non-shifted tribes (that remained bee-pollinated), corolla traits exhibited elevated rates of evolution early on (around 60 Mya), remaining high until about 30 Mya, after which rates declined sharply until 20 Mya. Rates of corolla evolution then increased again, reaching levels above the median by 5 Mya. Notably, between 5 Mya and the present, only corolla traits maintain elevated evolutionary rates. Androecium traits began to increase around 40 Mya, peaked around 35 Mya (higher rates than any other traits), and declined again by 30 Mya. Unlike corolla traits, androecium rates did not increase again closer to the present. Functional and gynoecium modules showed relatively stable, low-to-moderate rates throughout the family's history (Fig. 5a). Among shifted tribes (analyzing all pollination syndromes together), all floral modules showed moderate continuous rates of evolution until about 17 Mya, when the functional trait module started to increase slowly, while the corolla and androecium module, and later (12 Mya) the gynoecium module decreased slightly in rate, before accelerated rates of evolution at around 10 Mya (7 Mya for the gynoecium, Fig. 5b).

When splitting the dataset into the distinct pollination syndromes, rates of trait evolution of bee-pollinated species mostly mirror the patterns described for shifted tribes (continuous moderate rates before 20 Mya, followed by a dip and marked increase in evolutionary rates of all modules), and also hold when only analyzing bee-pollinated species of shifted tribes (Fig. 5c, S6). In NVF lineages, corolla traits showed a continuous increase, with a marked acceleration around 10 Mya (Fig. 5e). Androecium and gynoecium traits also began to increase rapidly at this time, while functional traits started rising earlier, around 13 Mya. Notably, all modules showed rates above the median. In FFV lineages (Fig. 5d), trait modules exhibited contrasting trends. Androecium traits started at very high levels (above 50) but declined steeply until 1.5 Mya. In contrast, gynoecium and corolla traits showed sharp increases during the same period, peaking around 1.5 Mya, after which their rates declined rapidly. Androecium traits, however, started rising again after this point. Functional traits began to increase steadily around 3.5 Mya. Again, all modules showed rates above the median (Fig. 5d). In the "generalist" syndrome (Fig. 5f), corolla traits started with elevated rates (above 20) but showed a consistent decline over time. Functional traits, in contrast, steadily increased until around 5 Mya, followed by a slight decline. Androecium and gynoecium modules remained relatively stable, with androecium traits increasing slightly and gynoecium traits showing a slight decrease (Fig. 5f).

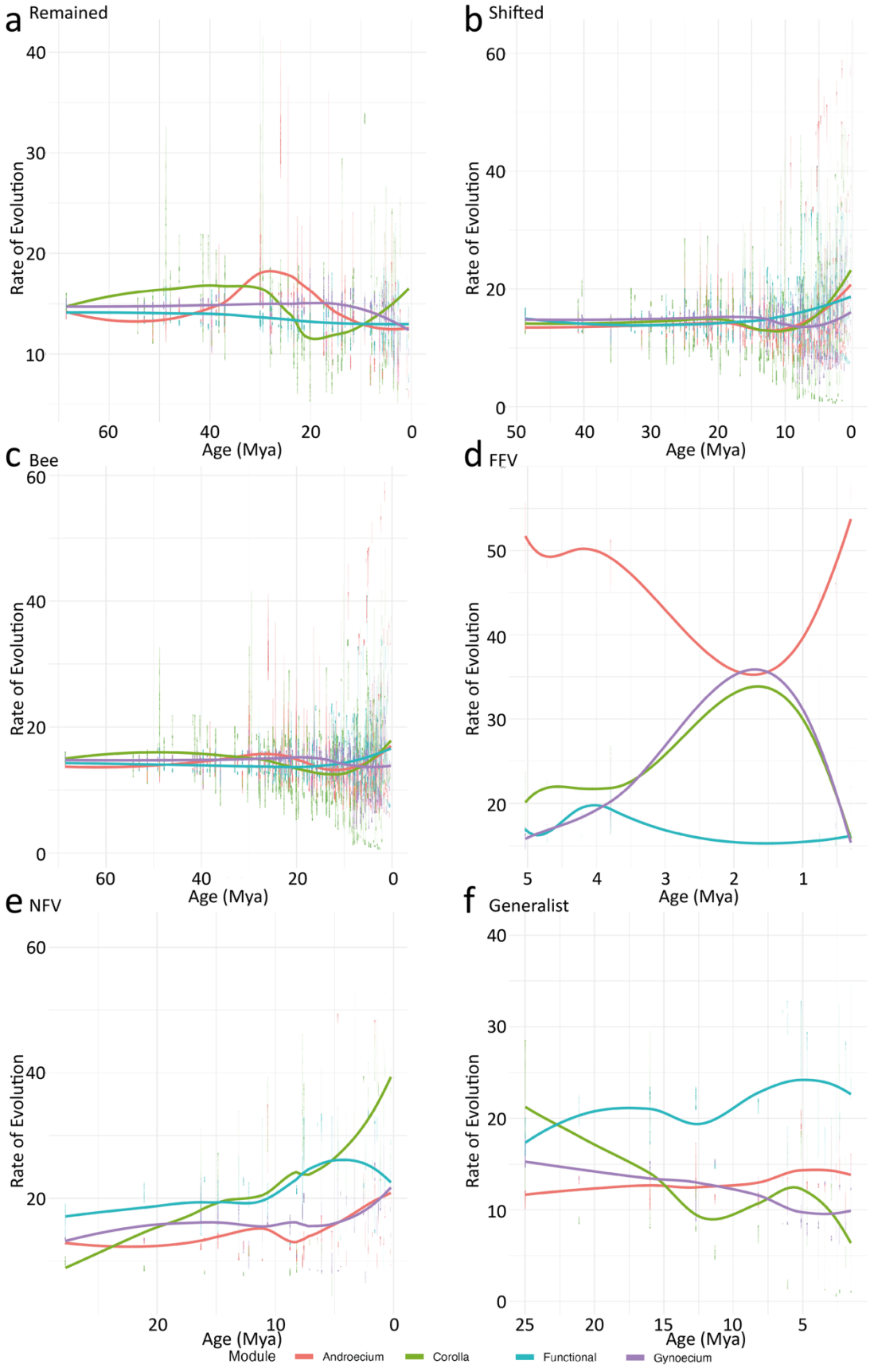

Figure 5: Timing of rate shifts across the different pollination syndromes and floral modules.(a) timing of rate shifts in tribes which remained bee-pollinated (non-shifted tribes); (b) timing of rate shifts in tribes which contain species that shifted pollinators (shifted tribes); (c) timing of rate shifts in bee-pollinated species; (d) timing of rate shifts of "food-body-foraging vertebrates" (FFV); (e) timing of rate shifts of "nectar-foraging vertebrates" (NFV); (f) timing of rate shifts of "generalists". colored dots represent the observed data; colored lines were fitted using the LOESS (Locally Estimated Scatterplot Smoothing) method.

Floral organ modules evolved under different evolutionary rates depending on pollination syndromes

In general, we found rates of flower evolution to vary across the floral phenotype, and among Melastomataceae lineages. Also, we could show that the highest rates of evolution can be found close to the tips of the tree. The entire phenotype evolved roughly tenfold more slowly compared to the individual floral organ modules with a maximum rate of evolution (MRE) of 6.78 (Tab. 2). Comparing the evolutionary rates of the different floral organ modules, we found that the corolla evolved the fastest (MRE = 62.88, Tab. 2), which differs from the median rate of evolution (MedRE), where the gynoecium evolved the fastest (MedRE = 14.90, Tab. 3). The median rate of evolution for the whole phenotype was 2.93 (for p values see Tab. S4).

Table 2: Range of rates of module evolution. Gen = "Generalist", NFV = "nectar-foraging vertebrate"; FFV = "food-body-foraging vertebrate", "Shifted Tribes" = tribes containing species that shifted pollinators, "Remained Tribes" = tribes which remained purely bee pollinated, "Bee Shifted" = bee-pollinated species of shifted tribes.

Range	Corolla	Androecium	Gynoecium	Functional	Phenotype
Total Data	0.53 - 62.88	5.61 - 58.84	3.87 - 34.52	6.99 - 40.86	0.76 - 6.78
Bee	0.53 - 55.72	5.61 - 58.84	3.87 - 30.12	7.14 - 40.86	0.76 - 5.92
Gen	0.56 - 40.65	8.52 - 20.93	6.24 - 17.78	12.48 - 34.60	1.23 - 3.49
NFV	4.40 - 62.88	8.12 - 49.35	6.51 - 30.99	6.99 - 33.43	1.28 - 6.09
FFV	15.00 - 36.72	41.85 - 58.05	14.52 - 34.51	14.69 - 22.13	2.85 - 6.78
Bee Shifted	0.53 - 58.88	6.34 - 58.84	3.87 - 30.12	6.99 - 40.86	0.76 - 5.92
Shifted Tribes	0.53 - 62.88	6.34 - 58.84	3.87 - 34.51	6.99 - 40.56	0.76 - 6.78
Remained Tribes	5.08 - 41.57	5.61 - 41.16	6.92 - 19.55	7.14 - 23.13	1.36 - 3.84

When comparing tribes which contain species that shifted pollinators (shifted tribes) to tribes which remained bee pollinated (non-shifted tribes), we found that shifted tribes evolved faster (MRE phenotype = 6.78) than tribes which remained bee pollinated (MRE phenotype = 3.84). Individual floral organ modules also evolved faster in shifted tribes (Tab. 2). Additionally, within shifted tribes, all four floral organ modules had some rates higher than 25, while within bee tribes, only corolla- and functional traits showed rates of evolution above 25 (Fig. 6). Importantly, these differences only apply to maximal rates of evolution, while median rates of evolution were similar across shifted and bee-only tribes (Fig. S7, Tab. S2, S3 for p values see Tab. S6, S7).

Table 3: Median rates of module evolution. Gen = "Generalist", NFV = "nectar-foraging vertebrate"; FFV = "food-body-foraging vertebrate", "Shifted Tribes" = tribes containing species that shifted pollinators, "Remained Tribes" = tribes which remained purely bee pollinated, "Bee Shifted" = bee-pollinated species of shifted tribes.

Media	Corolla	Androecium	Gynoecium	Functional	Phenotype
Total Data	14.31	13.52	14.90	13.67	2.93
Bee	14.02	13.54	14.89	13.55	2.93
Gen	11.88	11.99	12.87	20.04	2.53
NFV	28.19	13.16	17.37	20.65	3.41
FFV	21.14	49.78	18.34	16.97	3.60
Bee Shifted	13.66	13.25	14.82	13.63	2.91
Shifted Tribes	14.02	13.17	14.86	13.95	2.92
Remained Tribes	14.51	14.01	14.95	13.40	2.94

Bee-pollinated species of shifted tribes showed similar patterns of trait rates as species from shifted tribes (Fig. 6) but with lower frequencies of rates higher than 25 and a slightly slower evolving overall phenotype (MRE phenotype = 5.92, Tab. 2)

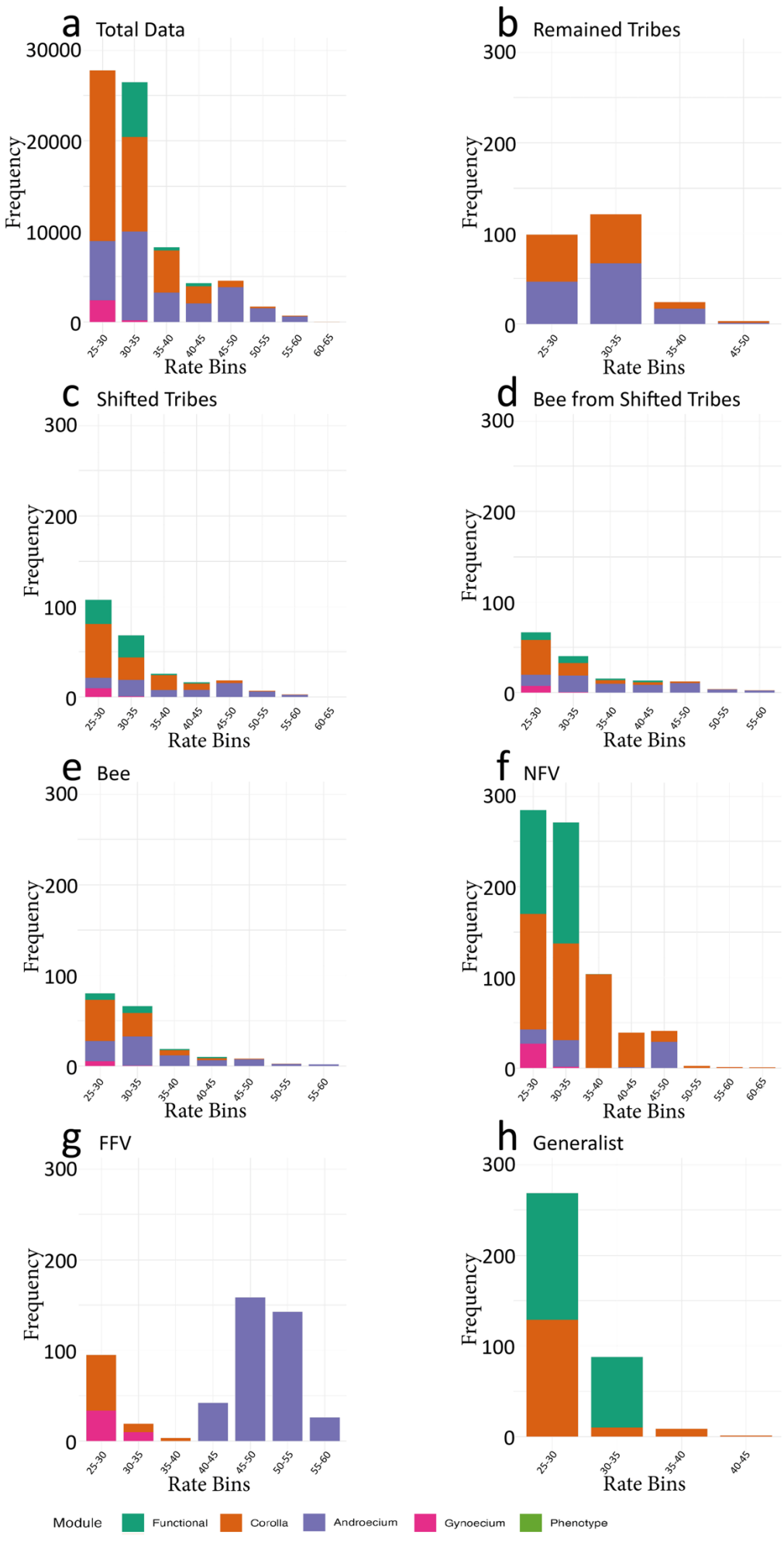
a Total Data
b Remained Tribes
c Shifted Tribes
d Bee from Shifted Tribes
e Bee
f NFV
g FFV
h Generalist
Frequency
Rate Bins
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
Module
Functional
Corolla
Androecium
Gynoecium
Phenotype

Figure 6: Upper bins (rates > 25) of frequency plots of rates of module evolution. (a) frequency plot using total data; (b) frequency plot using only tribes which remained bee-pollinated (= tribes which remained purely bee pollinated,); (c) frequency plot using shifted tribes (= tribes containing species that shifted pollinators); (d) frequency plot using bee-pollinated species from shifted tribes (= bee-pollinated species of shifted tribes); (e) frequency plot using only bee-pollinated species; (f) frequency plot using only "nectar-foraging vertebrate" (NFV) species; (g) frequency plot using only "food-body-foraging vertebrate" species; (h) frequency plot using only "generalist" species.

When comparing rates of trait evolution among pollination syndromes, we found that the FFV syndrome evolved the fastest (MRE phenotype = 6.78), followed by the NFV (MRE phenotype = 6.09), the "buzz-bee" (MRE phenotype = 5.92) and the "generalist" (MRE phenotype = 3.49, Tab 2) syndrome. In contrast to the "generalist" and FFV syndrome, the "buzz-bee" and the NFV syndrome showed high rates of evolution (> 25) in all four floral organ modules (Fig. 6). When considering the median rate of evolution, the results remained the same when comparing the rate for the entire phenotype (MedRE phenotype FFV = 3.60, MedRE phenotype NFV = 3.60, MedRE phenotype Bee = 2.93, MedRE phenotype Gen = 2.53), but we did not find a consistent ranking of modules across syndromes (Fig. S7, Tab. 3, for p values see Tab. S7, S8, S9, S10).

When comparing bee-pollinated species from shifted tribes to bee-pollinated species from tribes which remained bee pollinated, we found that bee-pollinated species from shifted tribes evolved faster (MRE phenotype = 5.92) than bee pollinated species from tribes which remained bee pollinated (MRE phenotype = 3.84, Tab. 2). The corolla showed the highest rate of evolution (MRE shifted = 58.88, MRE remained = 41.57, Tab. 2) and median rates of evolution were comparable among bee-pollinated species from shifted and non-shifted tribes (Fig. 6, S7, Tab. 3).

Discussion

Our study shows that flowers may evolve in a modular manner in response to selection by different functional pollinator groups, and that these different modules may not only differ in how much, but also in when they diversify. The increased rates of floral evolution observed toward the tips of the Melastomataceae phylogeny align with recent shifts to novel pollinators in the group's evolutionary history. These shifts have likely contributed to an increase in floral disparity. Higher evolutionary rates in montane, shifted clades could suggest adaptive diversification in response to new environments or pollinators. Together, our findings underscore the role of changes in selective regimes, such as those driven by pollinator transitions, in promoting the evolution of divergent floral phenotypes. Given that macroevolutionary studies on angiosperm floral disparity suggest that disparification often occurred early in clade histories (Mahler et al. 2010, López-Martínez et al. 2023), it is plausible

that those early bursts of trait divergence were similarly driven by shifts in selection pressures, such as pollinator changes or environmental transitions.

Disparity

In concordance with Kopper et al. (2024), who found that the ancestral "buzz-bee" syndrome had the highest disparity, we found that the "buzz-bee" syndrome had the highest partial disparity across all four floral organ modules (Fig. 1, Tab. 1). Given that species exhibiting the "buzz-bee" syndrome occur across the whole elevational distribution of Melastomataceae, and hence in a wide range of environmental niches (Kopper et al. 2025), it is plausible that these species experienced varied selective forces in the course of their evolutionary history, leading to high disparity in all four floral modules over time. Specifically, divergent selection in response to locally variable bee pollinator communities along elevational gradients may have contributed to this high floral disparity in the absence of pollinator shifts (Macior 1971, Vogel 1975, Renner 1989, Dellinger et al. 2019b, Wenzell et al. 2023, Kopper et al. 2024).

The only floral organ module which was more diverse than a randomly chosen set of traits was the corolla in the "nectar-foraging-vertebrate" syndrome (Fig. 2). Species of the NFV syndrome have evolved urceolata, campanulate or tube-like corolla shapes likely to improve the fit between flowers and pollinators (Dellinger et al. 2019, Kopper et al. 2024). Bee-pollinated species possess flowers with flat or solanum like displays, while "generalist" species present only flat flowers. Flowers of the FFV vertebrate syndrome, on the other hand are usually bowl-shaped. This wide range of different corolla shapes of the "nectar-foraging-vertebrate" syndrome is reflected by the significantly higher disparity of the corolla compared to a randomly chosen set of traits from the whole flower of the same size. Another driver for the high diversity observed in the corolla could be *corolla color*, as we can see high variation in this trait across syndromes (Fig. 3, S2, S3).

The high morphological diversity and evolutionary lability within the buzz-bee pollination syndrome may have facilitated complex trait adaptations during evolutionary shifts to non-buzzing bee pollinators (Wagner & Altenberg 1996, Opedal 2019), suggesting a high degree of floral evolvability in Melastomataceae (Dellinger et al. 2019b). This raises the question of whether the comparatively more constrained floral phenotypes observed in other buzz-pollinated lineages, such as *Solanum*, may result from limited floral evolvability, thereby precluding transitions away from the buzz-pollination syndrome. Exploring this hypothesis across a broader range of buzz-pollinated plant clades, particularly those that include non-buzz-pollinated relatives, such as *Vaccinium*, could provide important insights into how evolvability shapes the evolutionary trajectories of pollination systems.

Trait variation and single rates

We found strong positive correlation between androecial and total floral disparity across pollination syndrome (Tab. S3), which indicates that disparity is driven by the androecium (Chartier et al. 2017). This finding is further supported by our observation that eight of the ten traits with the highest evolutionary rates are associated with the androecium module (Fig. S4). Traits which are important to delimit syndromes (e.g., *pollen expulsion mechanism, reward type* Kopper et al. 2024), show slow rates of evolution (Fig. S4), since they are stable within syndromes. On the other hand, traits which are involved in pollen removal, pollen placement and relative orientation of anther pore to stigma show higher rates of evolution (Fig. S4). These traits might be highly important while adapting to slightly different selective pressures imposed by a wide range of different buzzing bees (Macior 1971, Dellinger et al., 2019b, Wenzell et al., 2023, Kopper et al. 2024), but also when adapting to a range of different vertebrate pollinators (i.e., different hummingbirds, bats, Dellinger et al. 2019). Thus, the high evolutionary lability of traits related to pore and stigma orientation in Melastomataceae is in line with previous studies reporting increased evolvability of pollinator-fit traits such as herkogamy (Opedal et al. 2019).

Timing of rate shifts across syndromes

Across the phylogenetic tree of Melastomataceae, we found differential increase and decrease of rates of trait evolution at different times in their history (Fig. 4). Specifically, lineages did not show elevated rates of evolution in more than one module at a given time (Fig. 4). Floral organ modules tended to evolve in a specific sequence, depending on whether lineages include species which have shifted pollinators or remained with the ancestral "buzz-bee" syndrome (Fig. 5, S6). In tribes which remained fully bee-pollinated, the corolla was the first module to show elevated rates (> 15) of evolution (around 60 Mya) with a continuous increase until about 30 Mya, while the androecium showed a marked increase in androecial evolutionary rates starting at around 40 Mya, and decreasing after corolla rates around 20 Mya (Fig. 5). These findings suggest that the high androecial disparity of Melastomataceae evolved independently of pollinator shifts. The drivers behind the accelerated rates of androecial evolution in bee-pollinated tribes approximately 30 Mya remain unclear, but it is plausible that lineages diversified into varied ecosystems at the time, with selection favoring adaptations to geographically variable bee-pollinator communities (Macior 1971, Classen et al. 2015, Ollerton 2017). Buzzing bees reached their peak diversity between 56 – 34 Mya (Engel 2000, Danforth

et al. 2006, Cardinal & Danforth 2013), making a scenario plausible where increased niche opportunity drove morphological divergence.

In contrast, among bee-pollinated species of shifted clades, we observed moderate rates of evolution for all organ modules, and increases in evolutionary rate of all modules starting at around 15 Mya, with particularly strong increases in corolla and androecium rates within the past 10 My. On the one hand, these results suggest that rapid evolution of corolla and androecial traits may have facilitated adaptation to altered montane bee pollinator communities upon mountain colonization within the past 15 My (Kopper et al. 2025). In agreement with this finding, we could recently show that montane Melastomataceae have larger flowers than their lowland relatives (Kopper et al. 2025), potentially indicative of increased investment into attracting the relatively scarce montane bee pollinators (Dafni et al. 1997, Goodwillie et al. 2010). Similarly, we found montane Melastomataceae to have larger stamen pores as a potential mechanism of maximizing pollen export with montane bee pollinators (Kopper et al. 2025). On the other hand, the increased rates of corolla and androecial evolution within the past 15 My among bee-pollinated species in shifted tribes may also point towards a high floral evolvability which facilitated shifts to non-vibrating pollinators such as vertebrates and other insects later in the evolutionary history of the family.

The temporal sequence of evolutionary rates in bee-pollinated species follows closely the patterns we can see in bee-pollinated species of shifted tribes and in tribes containing species which shifted pollinators, because many of the bee-species are included in tribes which shifted pollinators (194 species in shifted tribes of 273 bee-pollinated species). In the NFV-syndrome, functional traits showed elevated rates of trait evolution before corolla, androecium and gynoecium rates started to increase, highlighting the importance of functional trait changes (e.g. *pollen expulsion mechanism*, *reward type*) which go along with pollinator shifts (Smith & Kriebel 2018, Dellinger et al., 2019, Kopper et al. 2024). Also, we can see that corolla, androecium and gynoecium modules evolved in a correlated manner but at different rates (corolla faster than androecium and gynoecium; Fig. 5, S6), showing that depending on pollination syndrome, floral organ modules can differ in their degree of evolutionary inter-dependence. In the FFV syndrome we can see a correlated increase in evolutionary rates of the corolla and the gynoecium module, while androecium traits decrease, highlighting that different combinations of modules can evolve in a correlated fashion depending on pollination syndromes. Additionally, we can see that the evolutionary rate of androecium traits dropped rapidly, starting from a very high level (higher 50), indicating high selective pressure on androecium traits prior to pollinator shifts, which relaxed during the initial phase of pollinator shifts roughly 5 Mya (Dellinger et al. 2021, Kopper et al. 2024). Thus, it is plausible that rapid

trait changes in the androecium where a prerequisite for pollinator shifts from bees to food-body-foraging vertebrates. On the other hand, the rapid increase of evolutionary rates of the androecium around 2 Mya suggests that further adaptations where needed. Additionally, at the same time, gynoecium and corolla module rates dropped rapidly, indicating that these modules had reached an optimum. In the "generalist" syndrome we can see that rates of corolla trait evolution started at a high level (higher 20) and constantly decreased until about 10 Mya, suggesting that divergent selection on corolla traits were high prior to shifts to generalist pollinators. Rates of functional trait evolution, on the other hand, increased in the course of shifts to generalist pollinators (Fig. 5, S6).

We could show that depending on pollination syndrome, floral organ modules can differ in their degree of interdependency. The correlated, staggered increase in trait evolution rates of the androecium and the corolla in shifted tribes, NFV-lineages and bee-pollinated species of shifted tribes suggests that these modules evolve in a correlated fashion when species occur in environments conducive to pollinator shifts (Kopper et al. 2025). On the other hand, functional and gynoecium modules evolved independently from each other and from androecium traits, a pattern also shown in other studies (Wagner & Altenberg 1996, Hansen 2003, Ordano et al. 2008, Claverie et al. 2013, Felice & Goswami 2018, Larouche et al. 2018). Functional traits like *reward type* and *pollen expulsion mechanism* seemed to evolve prior to the rest of the floral modules and decreased towards the present in lineages where pollinator shifts have occurred. This makes sense given that these traits are highly different among, but stable within pollination syndromes (Dellinger et al. 2019b, Kopper et al. 2024).

Rates of floral module evolution

In general, we found rates of flower evolution to vary across the floral phenotype, and among Melastomataceae lineages. Shifted tribes evolved faster than tribes which remained bee pollinated (Tab. 2). Per definition, pollinator shifts represent changes in selection regime and are hence expected to go along with trait changes (Schiestl & Johnson 2013, Gervasi & Schiestl 2017, Schiestl et al. 2018, Mackin et al. 2021), resulting in higher rates of floral evolution (Simpson 1944, Pagel 1994, Lee et al. 2013). Individual floral organ modules evolved also faster in shifted tribes and all four floral organ modules had some rates higher than 25, while within tribes which remained bee pollinated, only corolla and androecium traits showed rates of evolution above 25 (Fig. 6). These findings suggest that in tribes where pollinator shifts occurred, all four floral modules were subject to strong disruptive selective pressure, as evidenced by the elevated evolutionary rate of the entire phenotype and high rates of evolution across the floral modules. In contrast, only corolla and androecium traits experienced such

pressure in tribes that retained bee pollination. A high rate of evolution suggests that disruptive selection might have acted more strongly on these modules, as the rate of evolution is defined by the number of changes per unit time along a branch of a phylogenetic tree (O'Meara 2016). Also, we found that depending on pollination syndrome, different floral organ modules are under selection. In the "buzz-bee" syndrome, the androecium in particular was under strong selection, reflected by its elevated rates of evolution (Tab. 2). The high rates of androecium trait evolution might come from adaptations to subtle divergent selection in response to locally variable bee pollinator communities (Macior 1971, Dellinger et al., 2019b, Wenzell et al., 2023, Kopper et al. 2024), or the utilization of a wide range of bee pollinators, differing in size and/or behavior (Vogel 1975, Renner 1989, Dellinger et al. 2019, Kopper et al. 2024). In the NFV syndrome, the corolla evolved the fastest (highest overall rate of evolution; Tab. 2), which may reflect adaptations of corolla shape and color, likely to improve pollinator attraction and fit (Dellinger et al. 2019, Kopper et al. 2024). In the FFV syndrome, the androecium module evolved the fastest (Tab. 2), which could be explained by the drastic adaptations in stamen morphology (Dellinger et al. 2014). Species of this syndrome evolved stamen appendages rich in sucrose, which serve as food-bodies for pollinating birds and, in addition, are functional in the pollen release mechanism (bellows-mechanism, Dellinger et al. 2014). In the "generalist" syndrome, the corolla module evolved the fastest (Tab. 2), which is reflected by short petals, displaying a flat, reflexed or upright, very reduced corolla (Kopper et al. 2024). In bee-pollinated species of shifted tribes, all four modules experienced increased selective pressure while in species from tribes which remained entirely bee pollinated, only the corolla and androecium modules were showed elevated rates of evolution (Fig. 6, Tab. 2). Bee-pollinated species of shifted tribes occupy wider environmental niches (both warmer and colder and wetter) compared to species of tribes which remained bee-pollinated (Kopper et al. 2025). This greater abiotic environmental variability experienced by bee-pollinated species of shifted tribes may also expose them to more varied bee pollinator communities, hence resulting in more varied floral phenotypes (Cruden 1972, Warren et al. 1988, Primack & Inouye 1993, Dellinger et al. 2021, Kopper et al. 2025).

We found the highest rate of evolution in tribes containing species which have shifted pollinators, and our results show that these high rates are found both among bee-pollinated and shifted species. The overall high disparity found within the "buzz-bee" syndrome has evolved both at deeper times (in non-shifted tribes) and more recently (among bee-pollinated species in shifted tribes). Accordingly, we could show that the highest rates of evolution can be found close to the tips of the tree (especially in shifted tribes). Since pollinator shifts happened late in the evolutionary history of Melastomataceae, this marked increase in rate shifts towards the tips

of the tree makes sense. On the other hand, the increased rates of corolla and androecium evolution in non-shifted tribes at deeper time is somewhat reminiscent of patterns reported for other lineages, indicating higher rates of evolution early in a lineage's evolutionary history (Mahler et al. 2010). Such patterns have been interpreted as increased opportunities to explore new niches early on, which decline with time as niches become filled (Mahler et al. 2010). While such a pattern may also apply to entirely bee-pollinated tribes, most species in our sample are from shifted tribes which occur in geologically relatively young mountains. Thus, with mountain colonization by Melastomataceae falling within the mid to late Miocene (15-5 Mya), new niche opportunities likely arose late in the Melastomataceae evolutionary history (crown node age 68.5 – 75.2 my; based on the ML topology of Penneys et al. 2022), explaining the increased rates of evolution towards the tips.

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Competing interests

None declared.

Author contributions

CK, JS and ASD conceived the study. CK compiled the data matrix and carried out the statistical analyses. CK wrote the manuscript. All authors contributed to finalizing the manuscript. JS and ASD contributed equally to this work and joint senior authors.

ORCID

Agnes S. Dellinger https://orcid.org/0000-0003-1394-3414

Constantin Kopper https://orcid.org/0000-0002-5567-1720

Jürg Schönenberger https://orcid.org/0000-0001-6791-2731

Data availability

All datasets, R-codes and the floral trait matrix have been deposited in the open access repository phaidra (https://phaidra.univie.ac.at/o:2143351).

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Research

Mountain colonization precedes shifts away from bee pollination in Melastomataceae

Constantin Kopper, Jürg Schönenberger* and Agnes S. Dellinger*

Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, Vienna, 1030, Austria

Author for correspondence:
Constantin Kopper
Email: constantin.kopper@univie.ac.at

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Summary

- Shifts among different groups of pollinators are central in the evolution of flowering plants, yet mechanisms underlying pollinator shifts remain unclear. Environment-induced reduction in pollinator availability and hence efficiency may destabilize ancestral plant–pollinator interactions and trigger shifts to new, more efficient pollinators, but formal tests remain scarce.
- We used a series of phylogenetic comparative methods on 333 species of the pantropical family Melastomataceae to test whether elevation, latitude and climatic variables explain pollinator shifts and the distribution of floral traits governing pollen release.
- We find that shifts away from bee pollination to generalist insect and vertebrate pollination associate with occurrence in cooler and wetter mountain environments. Also, we show that mountain colonization repeatedly preceded and, hence, likely triggered shifts away from bee pollination. Furthermore, our results suggest that the evolution of floral traits (larger petals and pore sizes) facilitating pollen transfer by bees may have been critical for the initial colonization of mountains by bee-pollinated species.
- By identifying environments conducive to pollinator shifts, our results do not only provide a much-needed hypothesis for mechanisms underlying the evolution of different pollination systems but also confirm their validity through empirical testing. Whether environment-induced evolutionary pollinator shifts are the norm across angiosperms remains to be explored.

Introduction

Mutualistic interactions, such as between plants and their animal pollinators, are increasingly considered important for the evolution and maintenance of biodiversity (Schemske *et al.*, 2009). Such interactions are unevenly distributed across the globe, with more diverse interaction systems commonly found at lower latitudes (Sinnott-Armstrong *et al.*, 2021). Considering pollination, there are, for instance, more functional groups of pollinators in the tropics than in temperate zones (Endress, 1996; Ollerton, 2017). Similarly, the importance and availability of different pollinator groups change across elevational gradients (Cruden, 1972; Warren *et al.*, 1988; Primack & Inouye, 1993; Dellinger *et al.*, 2021). Globally, diverse and abundant insect pollinator assemblages at low elevations are narrowed to fly-dominated pollinator assemblages at high elevations (Arroyo *et al.*, 1982; Warren *et al.*, 1988; Primack & Inouye, 1993; Lefebvre *et al.*, 2018; Adedoja *et al.*, 2020; McCabe & Cobb, 2021). In the Neotropics, these elevational turnovers in pollinator assemblages have been shown to lead to a dominance of vertebrate (particularly hummingbird) pollination in montane cloud forests (Dellinger *et al.*, 2023). These latitudinal and elevational patterns indicate a critical role of the abiotic climatic environment in the evolution and distribution of plant–pollinator interactions, but large-scale ecogeographic assessments of pollination strategies within plant lineages are currently scarce.

Differences in the abiotic environmental tolerances of different groups of pollinators (Ollerton, 2017; Lefebvre *et al.*, 2018) may impact their pollination efficiency and have been proposed as main drivers of evolutionary pollinator shifts (Cruden, 1972; Arroyo *et al.*, 1982; Krömer *et al.*, 2006; Dellinger *et al.*, 2021). For example, the flower visitation activity of ectothermic insect pollinators, such as most bees, depends on warm ambient temperatures and dry conditions (Cruden, 1972; Brito & Sazima, 2012; McCallum *et al.*, 2013; Classen *et al.*, 2015, 2020; Cozien *et al.*, 2019). By contrast, not only many groups of endothermic vertebrate pollinators (e.g. birds and bats) but also flies and bumblebees may forage under cool and wet environmental conditions common in mountain ecosystems (Bruggemann, 1958; McCallum *et al.*, 2013; Sun *et al.*, 2014; Woodard, 2017; Oyen & Dillon, 2018; Lawson & Rands, 2019). Thus, while ectothermic insects may be highly efficient pollinators in warm and dry environments, their relative pollination efficiency may be reduced (i.e. through reduced visitation rates) compared with vertebrates, flies and bumblebees under cool and moist conditions (Cruden, 1972; Dellinger *et al.*, 2021). In

*These authors contributed equally to this work and joint senior authors.

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theory, such environment-induced reductions in pollination efficiency could lead to the destabilization of formerly successful ancestral pollination systems and allow for shifts to new pollinators that are more efficient in a given environment (Thomson & Wilson, 2008). In the tropics, for example, we may expect consistent shifts between bee and vertebrate pollination in

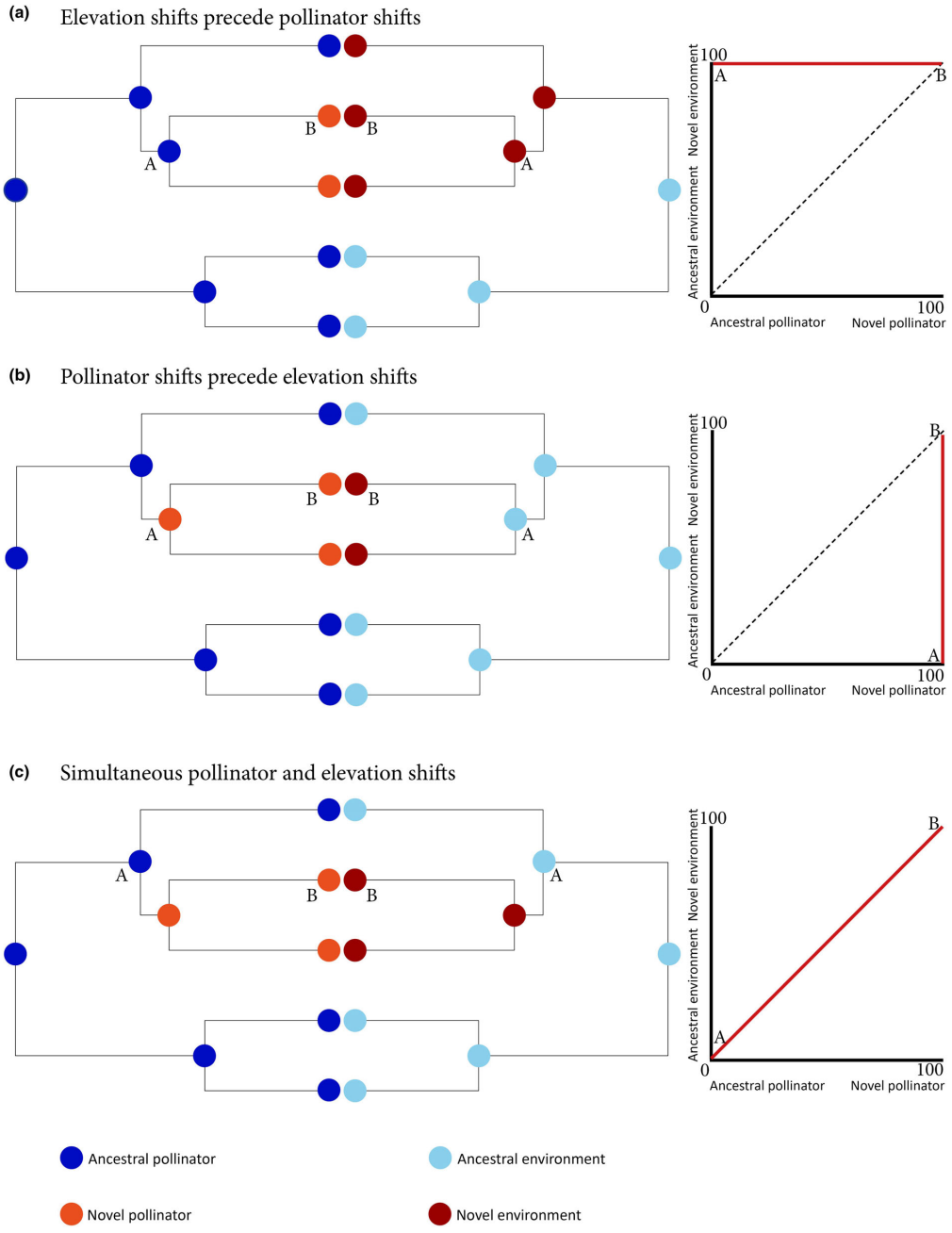

Fig. 1 Schematic of the three different scenarios on the potentially interlinked nature of evolutionary pollinator shifts (left cladograms) and shifts into novel environments (right cladograms) and correlation plots for ancestral shift probability (right panels), where the *x*-axis represents the ancestral node probability of having shifted pollinators and the *y*-axis represents the ancestral node probability of having shifted environments. Dashed lines represent a 1 : 1 correlation, and red lines indicate expected values for ancestral nodes. (a) If environment shifts precede pollinator shifts, we expect shifts to novel environments to occur with a high probability at older nodes in a lineage's evolutionary history than pollinator shifts, and we would hence expect the observed values to lie above the 1 : 1 line in a correlation plot, with a high intercept indicating a high probability of having shifted to novel environments while still being pollinated by ancestral pollinators. (b) If pollinator shifts precede environmental shifts, we expect pollinator shifts to occur with a high probability at older nodes in the evolutionary history of a lineage than environment shifts, resulting in ancestral node values falling below the 1 : 1 correlation line and intercepts being negative, indicating a higher probability of having shifted pollinators while still being in the ancestral environment. (c) If pollinator and environment shifts occur more or less simultaneously and hence at similar nodes in the phylogeny, we expect empirical node values to fall around the 1 : 1 line in correlation plots, indicating joint shifts in environment and pollination system.

lineages that have colonized mountains, with bee-pollinated species at lower and vertebrate-pollinated species at higher elevations (cloud forests; Cruden, 1972, Dellinger *et al.*, 2021).

Although the ecogeographic patterning of pollination systems suggests reciprocity between the abiotic environment and evolutionary pollinator shifts (Arroyo *et al.*, 1982; Lefebvre *et al.*, 2018; McCabe & Cobb, 2021; Dellinger *et al.*, 2023), their interlinked nature has rarely been explored macroevolutionarily (but see Hamilton & Wessinger, 2022, Dellinger *et al.*, 2024). Combining information on pollination systems and the abiotic environment and mapping both on molecular phylogenies of plant clades allow us to trace the sequence of evolutionary events (i.e. pollinator shifts and environment shifts), and hence identify their relative timing and potential for reciprocal effects. Specifically, we may envision three scenarios for links between environment and pollinator shifts (Fig. 1). First, if environment-induced reductions in the efficiency of ancestral pollinators are indeed a common theme in evolutionary pollinator shifts, we would expect shifts into novel environments (with unfavorable conditions for ancestral pollinators) to precede pollinator shifts (Fig. 1a). In such a scenario, plant species would first colonize novel environments (i.e. mountains), where reductions in the pollination efficiency of ancestral pollinators (i.e. bees) may induce shifts to novel, more efficient pollinators (i.e. birds). Support for such a scenario comes from the North American genus *Penstemon*, where bee- and hummingbird-pollinated sister pairs occur in significantly different habitats compared with more distantly related bee-pollinated species, suggesting that shifts into novel environments preceded pollinator shifts (Hamilton & Wessinger, 2022). Similarly, in the Melastomataceae tribe Merianieae, biogeographic range expansion into the Andes preceded shifts from bee to vertebrate pollination (Dellinger *et al.*, 2024). Alternatively (Fig. 1b), shifts to novel pollinators may represent a critical precondition for range expansions into novel environments, and hence precede environmental shifts. Finally (Fig. 1c), pollinator shifts and environment shifts may occur more or less simultaneously (i.e. species are changing pollinators as they are expanding ranges) and should result in similar macroevolutionary patterns of correlated evolution. Empirical support for either of the latter hypotheses is currently lacking.

The two studies finding support for the scenario that environment shifts preceded pollinator shifts (Hamilton & Wessinger, 2022; Dellinger *et al.*, 2024) also show that species with the ancestral pollination system were not only able to initially colonize novel environments but also persist there. Hence, the question arises why colonization of novel environments sometimes triggers pollinator shifts, and sometimes does not. Adaptive floral trait evolution might play a critical role in retaining ancestral pollinators even under unfavorable conditions (Galen, 1989; Bradshaw & Schemske, 2003; Smith & Kriebel, 2018; Dellinger *et al.*, 2019a). Especially when ancestral pollinators occur in lower abundance in the newly colonized environment (i.e. fewer bee pollinators in mountains, Cruden, 1972, Dellinger *et al.*, 2021), the evolution of traits maximizing pollen transfer even with the ancestral pollinators might be advantageous (Thomson *et al.*, 2000). Such traits include investment in overall floral display (e.g. flower size), which is positively correlated with pollination rates across angiosperms (Dafni *et al.*, 1997; Goodwillie *et al.*, 2010). Traits regulating pollen dispensing (e.g. tempo and mode of anther dehiscence and pollen release), as well as the amount of pollen released at each pollinator visit, may also change with changing pollinator availability (Castellanos *et al.*, 2006). Specifically, pollen may be dispensed in larger doses if the overall probability of pollinator visits is low or if pollinators 'waste' large proportions of the received pollen (e.g. bees actively collect pollen as fodder for their brood; Castellanos *et al.*, 2006). Although highly relevant for understanding the mechanisms behind evolutionary pollinator shifts, these predictions stem from the study of few selected species only and have not incorporated the impact of different abiotic environmental contexts. It thus remains unclear whether trait modifications to maximize pollen transfer might generally represent an alternative to pollinator shifts when lineages colonize environments where their ancestral pollinators are rare. In such a scenario, we may expect selective pressure to maximize pollen transfer by increasing floral display and reducing pollen dosing traits (Dafni *et al.*, 1997; Thomson *et al.*, 2000; Castellanos *et al.*, 2006; Goodwillie *et al.*, 2010).

We here use the species-rich, pantropical family Melastomataceae (5858 species in 173 genera and 23 tribes; Penneys *et al.*, 2022) as a model to test whether environment shifts precede, follow or go hand-in-hand with pollinator shifts. The family is well suited for this assessment since it provides the required evolutionary replicates of environment and pollinator shifts. Specifically, our data encompass at least 11 independent environment shifts (across 128 out of 333 sampled species) from lowland rainforests to montane cloud forests, and at least 21 independent pollinator shifts (across 53 species from seven tribes) from ancestral buzz pollination by bees (most common, *c.*

96% of species) to nonbuzz pollination systems by vertebrates (i.e. birds, bats and rodents; Lumer, 1980, Dellinger *et al.*, 2014, 2019b) or generalist insect pollinators (i.e. flies, wasps, beetles and butterflies; Kriebel & Zumbado, 2014, Brito *et al.*, 2016). Melastomataceae are further characterized by poricidal anthers, whereby anthers only dehisce through a small apical pore (Endress, 1996). This stamen morphology effectively restricts the amount of pollen released at each pollinator visit (i.e. Larson & Barrett, 1999; Dellinger *et al.*, 2019c), and across buzz-pollinated lineages, pore size has been hypothesized as an important parameter controlling the amount of pollen released, with smaller pores strongly restricting pollen release (i.e. Kemp & Vallejo-Marín, 2021; Boucher-Bergstedt *et al.*, 2025). In addition, Melastomataceae vary in the structure of their anther walls, which are either smooth or more or less corrugated, the latter potentially hindering pollen flow and further modulating pollen release (Dellinger *et al.*, 2019c).

Comparing the elevational distribution and pollination systems (bee-pollinated vs shifted) of 333 Melastomataceae species, we here first assess whether pollinator shifts do indeed associate with occurrence in cool and wet mountain environments across the family. Second, we reconstruct the evolutionary history of pollinator shifts and elevation shifts to test whether colonization of novel environments (mountains in our case) repeatedly preceded, and hence likely triggered, shifts away from bee pollination (Fig. 1a). If mountain colonization indeed likely preceded pollinator shifts, we expect to find shifts to mountains at older nodes in the Melastomataceae phylogeny than pollinator shifts so that closely related bee-pollinated and shifted species both occur in mountains. If, instead, pollinator shifts represent a prerequisite for the colonization of mountain environments (Fig. 1b), we expect all bee-pollinated species (regardless of whether they belong to tribes encompassing shifts or not) to occur at lower elevations and in warmer and drier, bee-friendly habitats than shifted species. If, lastly, pollinator and environment shifts happened simultaneously (Fig. 1c), we expect pollinator shifts to mostly coincide with elevation shifts along the same nodes of the phylogeny and show correlated evolution. Given that we find strongest support for Scenario 1 (environment shifts preceding pollinator shifts), we further explore whether adaptive trait evolution potentially facilitating pollinator attraction and pollen transfer (larger pores and smooth anthers) may explain how some bee-pollinated species could retain bee pollination in montane environments despite the reduced abundance of bee pollinators.

Materials and Methods

We ran all statistical analyses in R (R Core Team, 2022); please find detailed methods in Supporting Information Notes S1.

Species selection, pollination mode and phylogenetic hypothesis

Our analyses were based on a dataset of 333 species (*c.* 6% of Melastomataceae), for 206 of which floral visitors were empirically documented (Dellinger *et al.*, 2022). For the remaining 127, we have predicted pollinators in a previous study using machine learning-based classification methods (random forest analyses), where we trained initial models on 44 floral traits and 252 species with documented pollinators. Then, we used these trained and validated (high prediction accuracy) models to predict pollinators for the remaining species (Kopper *et al.*, 2024). Of the 333 species used in this study, 53 species across seven tribes have shifted pollinators. These shifted species can be summarized into three pollination syndromes: 'generalist' (flies, wasps, beetles, butterflies and bees), 'nectar-foraging vertebrate' (hummingbirds, flower piercers, lizards, rodents and bats) and ' food-body-foraging vertebrate' (passerine birds and parrots; Dellinger *et al.*, 2022, Kopper *et al.*, 2024). For most analyses, we merged these three pollination syndromes (all derived from the ancestral 'buzz-bee' syndrome) into 'shifted' since we wanted to test whether there are general associations between shifts and environmental conditions. Also, this grouping was necessary to assure sufficient evolutionary replication.

We used the most recent dated molecular phylogeny for Melastomataceae (Reginato *et al.*, 2022; 2454 tips, crown node age: 68.5–75.2 million years (Myr)) and pruned the maximum clade credibility (MCC) tree to the 333 species in our data matrix (see Notes S1). Throughout the manuscript, we use the term *tribe* to refer to the 23 taxonomic tribes recognized in Melastomataceae (Penneys *et al.*, 2022), while we use the term *clade* for groups of closely related species *within* a tribe that all share a common ancestor, that is differentiating *pollinator-shifted* and *nonshifted clades* within *tribes*. We define a shifted clade as a group of species whose last common ancestor and all of its descendants have shifted pollinators. Because we observe few or no reversals back to bee pollination, species descending from a lineage where a shift has occurred typically retain the new pollination system. Thus, a pollinator shift at the ancestral node of a *shifted clade* usually results in a clade composed entirely of species with shifted pollination systems. Note that *nonshifted clades* can exist within shifted *tribes* and be sister to *shifted clades*. *Nonshifted clades* are composed only of bee-pollinated species and do not contain any descendants with a pollinator shift.

GBIF occurrence data, elevation and climatic variables

We screened the initial plant species list (n = 336) using *Taxonstand* (U.TAXONSTAND v.2.4; Zhang & Qian, 2023) to correct spelling mistakes and synonyms. Next, we submitted the list to GBIF (Global Biodiversity Information Facility) to search for occurrence data for each species (RGBIF v.3.7.9; Chamberlain *et al.*, 2024; 120 923 occurrence points). We excluded records lacking coordinates and submitted the resulting dataset to standard cleaning procedures (COORDINATECLEANER v.3.0.1; Zizka *et al.*, 2019) to remove records located in country centroids, the sea, around GBIF headquarters, duplicates or records with equal longitude and latitude, leaving 88 932 records across 307 species. Since *Taxonstand* did not correct all misspelled names and synonyms, we manually corrected names, adding eight species (1158 occurrence points). Furthermore, 21 rare species had been excluded by our strict filtering settings; for these species, we

searched occurrences with more relaxed settings, adding 4538 records and 18 species (three species were not found). We combined these datasets (resulting in 333 species and 94 628 occurrence points) and performed a final round of manual cleaning to ensure that all obtained records correspond to the known distribution ranges of the species. Specifically, for each species, we plotted range maps (MAPTOOLS v.1.1.4; Bivand & Lewin-Koh, 2023), compared these maps against the distributions documented in Plants of the World Online (https://powo.science.kew.org/; Fig. S1b) and removed records outside of the distribution range of the species (resulting in 90 498 records, 333 species and 22 tribes). Next, we subsampled occurrences so that each species was only represented by one occurrence in a 1 km grid. This left us with a final dataset of 71 122 occurrence records across 22 tribes and 333 species (Table S1), with a median number of 74 occurrence points per species (range: 1–3037). Across pollination strategies, bee-pollinated species are represented by 63 911 records (90%) and shifted species are represented by 7211 records (10%; 4404 records of 'generalist', 2162 records of 'nectar-foraging vertebrate' and 645 records of 'food-body-foraging vertebrate' syndrome).

To investigate the environmental context of pollinator shifts, we chose to focus on elevation, temperature and precipitation since all of these variables can significantly impact the flower visitation activity and abundance of bee pollinators and may hence be conducive to pollinator shifts (Cruden, 1972; Dellinger *et al.*, 2023). Accordingly, we downloaded layers for mean annual temperature (bio1), mean annual precipitation (bio12, Notes S2) and elevation from CHELSA v.2.1 (http://chelsa-climate.org/) at 1-km resolution (Karger *et al.*, 2023). We extracted the respective value for each occurrence record and calculated the median value per species for analyses in a phylogenetic context. The final dataset is available in the online repository phaidra (https://phaidra.univie.ac.at/o:2096899). Note that elevation and mean annual temperature are strongly correlated ($r > -0.8$); we hence only included one at a time in our models (to avoid bias due to autocorrelation; Fig. S1a).

Do shifts away from bee pollination associate with montane environments?

To assess whether high-elevation occurrence is linked to non-bee pollination in Melastomataceae, we used boxplots to visualize the elevational distribution of bee-pollinated and shifted species. We also plotted elevation across latitude for the different pollination systems using GGPLOT2 (v.3.5.1; Wickham, 2016). We applied phylogenetic ANOVAs (PHYTOOLS, v.2.1.1; Revell, 2024) to test whether pollination systems differ in elevation. Next, to explore the impact of elevation, latitude and/or annual precipitation on pollination system, we fitted binary phylogenetic generalized linear mixed models (binaryPGLMM, *binaryPGLMM* and APE v.5.8; Paradis & Schliep, 2019). We built an initial binaryPGLMM, including interaction effects among elevation, latitude and precipitation. We used stepwise model selection to retain only significant parameters and interaction effects (elevation and latitude in our case) and plotted the expected value of the response variable to evaluate model fit (mu; Fig. S2a).

Since median values do not capture the range of environmental conditions that species with wider distribution ranges may encounter, we additionally performed a random sampling approach of the occurrence data to include environmental variables for single occurrence points per species. Specifically, 100 times, we randomly sampled a single occurrence point per species and reran binaryPGLMMs to evaluate the effect of elevation on pollination system. We calculated the percentage of runs where P values were smaller than 0.05 (indicating significant differences in the elevational distribution among pollination systems).

We used Whittaker biomes (Whittaker, 1975; Ricklefs, 2008; PLOTBIOMES Ștefan & Levin, 2018) and the chi-squared statistics to further explore whether bee-pollinated and shifted species occur in different environments. We plotted standardized residuals of chi-squared tests using correlation plots (CORRPLOT v.0.92; Wei & Simko, 2024), with standardized residuals higher than $\pm$ 2 indicating a strong contribution of the respective biome to pollination system distribution.

Did niche expansion into mountain habitats precede pollinator shifts?

To explore the three different scenarios of evolutionary pollinator and environment shifts (Fig. 1), we ran ancestral state reconstructions for both pollination system and elevation (which we determined as most important factor explaining the distribution of pollination systems). We chose to use the four different pollination syndromes ('bee', 'nectar-foraging vertebrate', 'food-body foraging vertebrate' and 'generalist') for the initial reconstruction of pollination systems since shifts into each syndrome require different modifications in floral morphology, and reconstructing them all together as 'shifted' might artificially inflate shift proportions at internal nodes. To make reconstructions comparable with elevation (a quantitative trait), we chose to categorize elevation into four broad categories: below 500, 500–1000, 1000–1500 m and *c.* 1500 m. This categorization is based on the observation that most pollinator shifts happen above 1000 m (Fig. 2). To ascertain that this categorization accurately mirrors elevation–pollination relationships as reported before, we repeated binaryPGLMMs using pollination system as binary response and categorized elevation as predictor.

For ancestral character estimation for pollination system, we ran three different MuSSE models (SYM, ARD and Constraint) using the function *make.musse* (DIVERSITREE v.0.10.0 FitzJohn, 2012) following Kopper *et al.* (2024). The constraints for transition rates were determined based on our knowledge of the family (Dellinger *et al.*, 2022; see Notes S1). For elevation, we ran two different MuSSE models (SYM and ARD). We compared model fit using Akaike information criterion (AIC) scores (Table S2) and chose the model with the lowest score for either trait (SYM for pollination system and ARD for elevation). We also reconstructed elevation evolution as a continuous trait; results of these analyses can be found in the Supporting Information.

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For visualizing evolutionary patterns on the phylogeny and calculating summary statistics, we binarized the pollination system (bee-pollinated/shifted) and elevation (lowland/montane). Since elevation is a continuous variable and binarization is challenging, we employed three different binarization schemes: > 500 m – montane; > 1000 m – montane; and > 1500 m – montane.

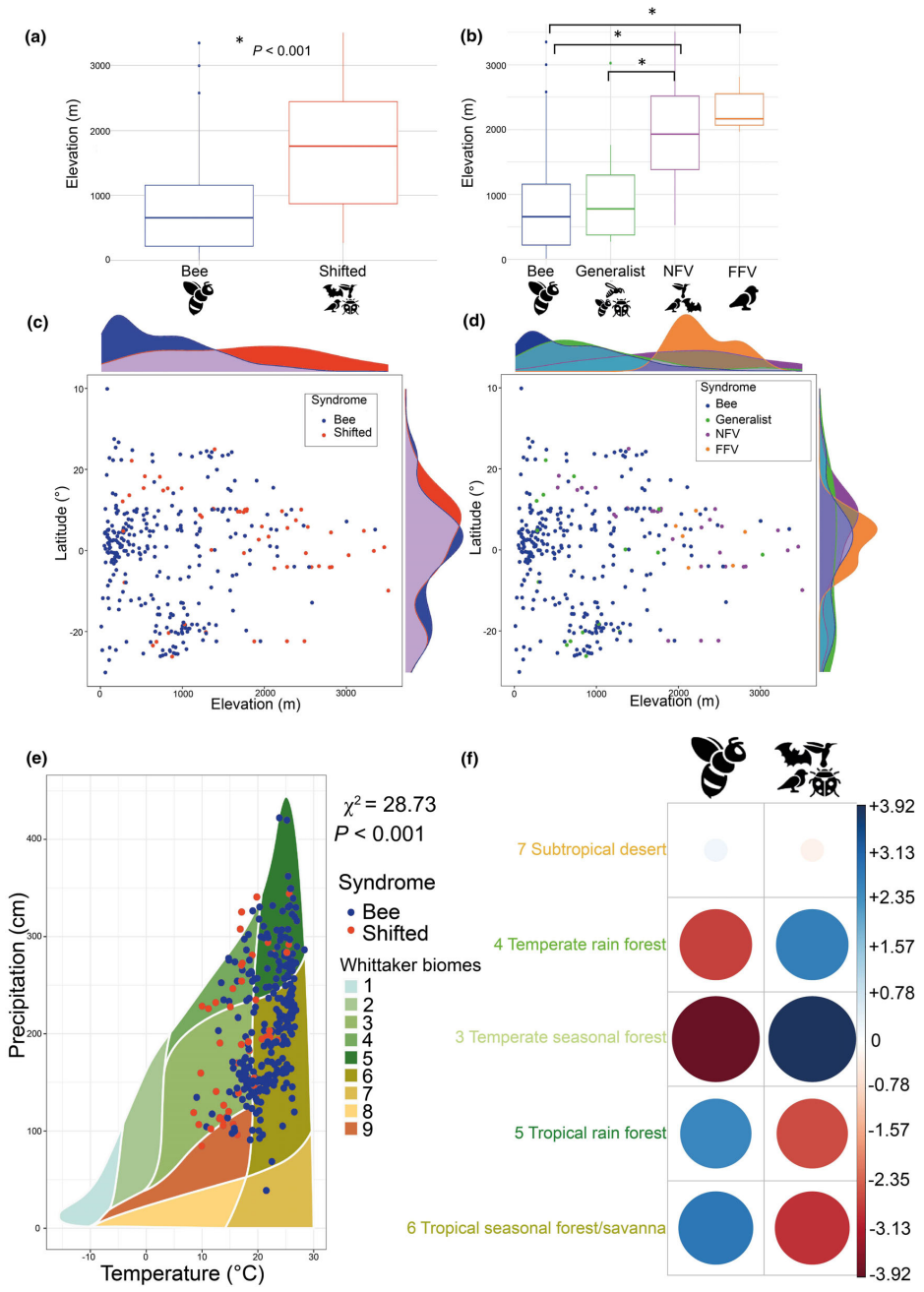

Fig. 2 Melastomataceae species with shifted pollination systems occur at significantly higher elevations and in different biomes than bee-pollinated species. (a) Bee-pollinated species (blue) are more common below 1000 m while species that shifted pollinators (red) are more common above 1500 m. (b) Comparing all four pollination syndromes reveals that species that shifted to generalist pollination (green) are most common below 1000 m (like bee-pollinated species (blue)), while all species that shifted to vertebrate pollination are significantly more common above 1500 m; boxes in (a) and (b) represent the interquartile ranges; bars inside the boxes represent the median values; whiskers represent the range and the dots represent outliers. Asterisk indicates significant differences. *P* values were obtained using phylogenetic ANOVA. (c, d) picture the elevation–latitude relation, showing that at higher latitudes, species that shifted away from bee pollination may occur at lower elevations. In (c), the comparison between bee-pollinated and shifted species is shown (compare a), while (d) represents all four pollination syndromes (compare b). Density plots on top of (c, d) represent the interpolated elevational distribution; density plots on the right side of (c, d) represent the interpolated latitudinal distribution. (e) Whittaker biomes (Whittaker, 1975) are shown, with differently colored surfaces indicating different biomes (circumscribed by temperature (*x*-axis) and precipitation (*y*-axis)), and dots indicating species' means in temperature and precipitation. Bee-pollinated species (blue dots) most commonly occur in tropical seasonal forests and savannas (Biome 6) and tropical rain forests (Biome 5), while shifted species (red dots) commonly occur in temperate rain forests (Biome 4) and temperate seasonal forests (Biome 3). (f) Plot of absolute standardized residuals of the chi-squared test on the differences in biome occupation between bee-pollinated and shifted species, with blue indicating a positive relationship, and red indicating a negative relationship. Size of the circles indicates the magnitude of the relationship; faded and small circles indicate a weak relationship. Numbers represent Whittaker biomes: 1, Tundra; 2, Boreal forest; 3, Temperate seasonal forest; 4, Temperate rain forest; 5, Tropical rain forest; 6, Tropical seasonal forest/savanna; 7, Subtropical desert; 8, Temperate grassland/desert; 9, Woodland/shrubland; Melastomataceae are absent from Biomes 1, 2 and 8.

This approach allowed us to evaluate whether choosing different elevational thresholds for lowland/montane biased our results. We next extracted the reconstructed probability of having shifted pollinators and elevation, as well as node ages for all nodes descending from tips with pollinator shifts, until reaching 100% probability of being bee-pollinated (ancestral condition). If only the tip showed 100% probability of having shifted, we used the tip values of this species. If sister species shared a lower node with 100% probability of having shifted, we used the value of the last shared node for this clade with 100% probability of having shifted. We plotted the extracted probabilities for each shifted clade (pollinator shift probability on the *x*-axis and elevation shift probability on the *y*-axis) for three different elevation binarizations. We further tested for correlated evolution between pollinator and elevation shifts using the function *fitCorrelationTest* (corHMM v.2.8; Beaulieu *et al.*, 2013) using the four elevation categories and the four pollination syndromes.

To further explore the link between elevation and pollination system evolution, we tested three different hypotheses using Ornstein–Uhlenbeck models (OU models; l1ou, v.1.43; Khabbazian *et al.*, 2016). First, we assigned separate elevationary optima for tribes containing species that have shifted pollinators and tribes that remained bee-pollinated. If this model came out as the best fitting, it would further support the hypothesis that elevation shifts (whole tribes) precede pollinator shifts (within tribes; Fig. 1a). Second, we assigned separate optima for species that shifted pollinators and those that remained bee-pollinated. If this model came out as the best fitting, it would support the hypothesis that pollinator shifts are a prerequisite for elevation shifts (Fig. 1b). Third, to explore the elevational relation of different pollination systems, we assigned two separate elevational optima, one for bee-pollinated species and species that shifted to the 'generalist' syndrome and one separate optima for species that either shifted to the 'nectar-foraging vertebrate' or to the 'food-body-foraging vertebrate' syndrome. We used *estimate_convergent_regimes* (l1ou, v.1.43; Khabbazian *et al.*, 2016) to test for convergent elevational shifts across all models.

Do bee-pollinated species from shifted tribes occur in environments conducive to pollinator shifts?

To investigate whether bee-pollinated relatives of shifted species are indeed more likely to occur in environments conducive to pollinator shifts (cool and wet mountain habitats, in our case) than bee-pollinated species from nonshifted tribes, we analyzed the environmental associations between bee-pollinated species only (n = 280). Using phylogenetic *t*-tests and Whittaker biomes, we tested whether bee-pollinated species from tribes containing shifts generally occur at higher elevations and cooler and wetter environments than bee-pollinated species from tribes lacking shifts.

Are bee-pollinated species in mountains confined to more bee-friendly environments?

To understand whether pollinator shifts may have allowed Melastomataceae to expand their niches into even harsher environments compared with species with the ancestral pollination system, we subset the full dataset to only contain species growing above 1000 m (the general threshold for pollinator shifts, n = 128). We built a binaryPGLMM to test whether mean annual temperature and precipitation can determine the probability of being bee-pollinated or shifted among these montane species, with the expectation that shifted species may occur in even colder and wetter environments than their bee-pollinated relatives (McCallum *et al.*, 2013; Classen *et al.*, 2015, 2020; Cozien *et al.*, 2019). We used stepwise selection to select the parameters with significant effects on the pollination system and plotted the expected value of the response variable to assess model fit (Fig. S2b).

Do montane bee-pollinated species have larger flowers and traits facilitating pollen dispersal?

To explore whether montane bee-pollinated species exhibit floral traits potentially increasing attractiveness to pollinators (larger petals) and facilitating pollen release with the scarce montane bee pollinators (smooth stamen walls and larger pores), we compared

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floral traits across bee-pollinated species. Again, we split the dataset into lowland (< 1000 m) and montane (> 1000 m) species. We used phylogenetic linear mixed models (phylolm Ho & Ane, 2014) to test for the impact of elevation, mean annual precipitation, latitude and the interaction between latitude and elevation on petal length and pore size, and corresponding

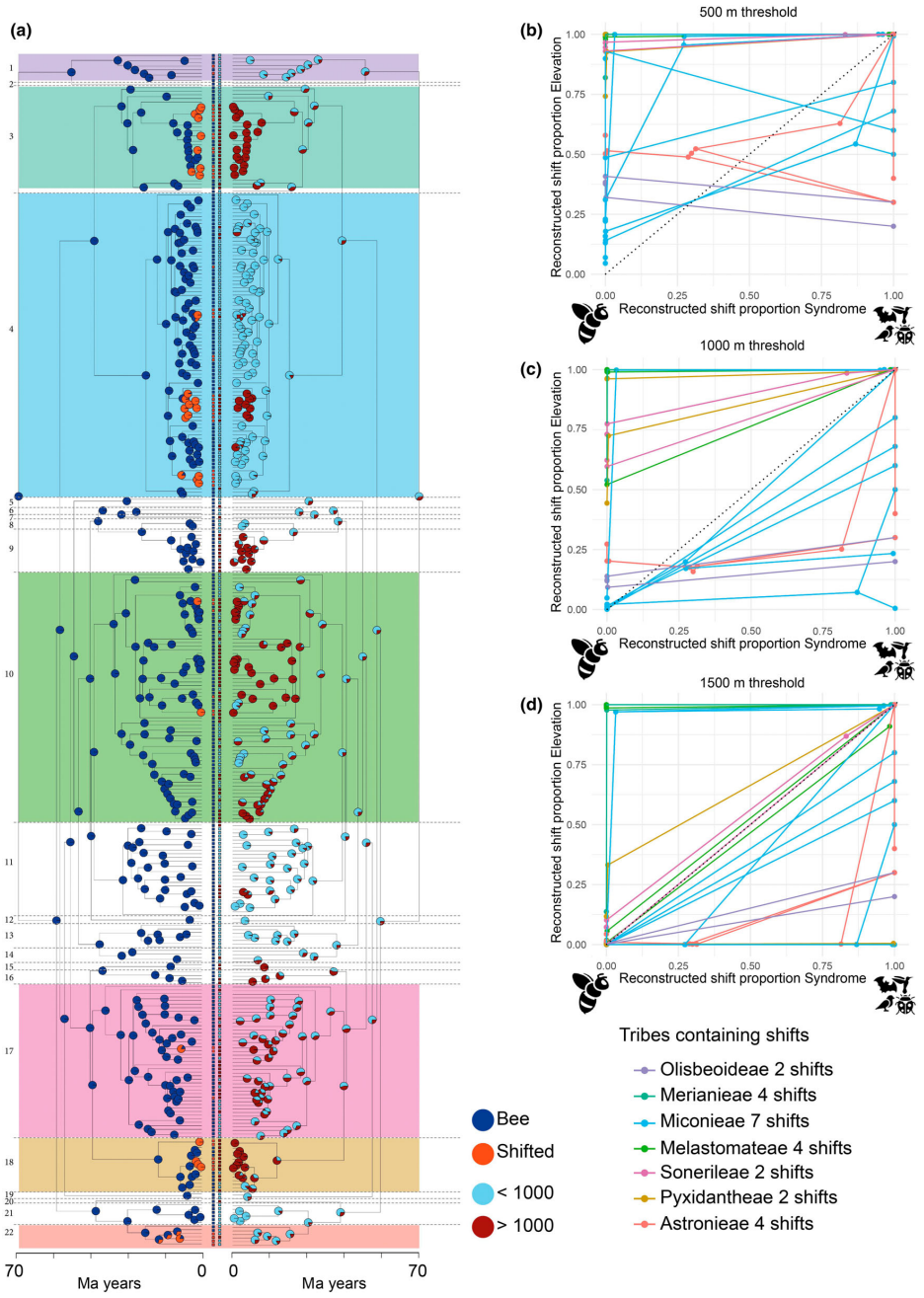

Fig. 3 Ancestral character estimations of pollination systems (bee or shifted) and elevation showing that, generally, elevation shifts happened independently from pollinator shifts and that pollinator shifts usually happened after shifts to higher elevations. (a) The two dated phylogenetic trees show the reconstruction of pollination syndromes (left) using a MuSSE (SYM) model with nodes and tips colored as a binary trait (bee as blue and shifted as orange) and the reconstruction of elevation (right) based off a MuSSE (ARD) model, with nodes and tips colored in a binary manner with reconstructions < 1000 m summarized as 'lowland' and > 1000 m as 'montane'. Pie charts at nodes represent probabilities of being bee-pollinated (blue) or shifted (orange) and of being lowland (light blue) or montane (dark red); tip states for pollination system and elevation are also shown as pies. The seven tribes where pollinator shifts have occurred (1, 3, 4, 10, 17, 18 and 22) are highlighted with colors and correspond to colors used in Table 1 and (b–d). (b–d) The reconstructed shift proportions of pollination syndrome on the *x*-axis and elevation on the *y*-axis of tribes containing pollinator shifts ('shifted tribes') are shown; dots along the lines represent ancestral nodes descending from shifted tips (100% shift probability) to nodes with 0% shift probability (also compare Table 1); colors correspond to tribes as indicated in (a). All tribes are represented by at least two lines of the same color since all tribes contain at least two independent pollinator shifts. Following theoretical expectations from Fig. 1, a grouping of ancestral nodes along the *y*-axis and lines above the 1 : 1 ratio supports elevation shifts preceding pollinator shifts, a grouping of ancestral nodes along the *x*-axis and lines below the 1 : 1 ratio supports pollinator shifts preceding elevation shifts, while a grouping of nodes close to 0 and lines following the 1 : 1 ratio supports correlated evolution between elevation and pollinator shifts. (b) When all species occurring above 500 m were considered 'montane', 25 out of 25 ancestral nodes lie on the *y*-axis and lines group above the 1 : 1 ratio. (c) When all species occurring above 1000 m were considered 'montane' (compare (a)), 19 out of 25 ancestral nodes lie on the *y*-axis and six nodes lie close to 0. Thirteen out of 25 lines fall above the 1 : 1 ratio. (d) When choosing 1500 m as the cutoff for being 'montane', 10 ancestral nodes lie on the *y*-axis, seven ancestral nodes lie close to 0 and 8 ancestral nodes lie along the *x*-axis, and 10 out of 25 lines fall above the 1 : 1 ratio. Numbers on the very left in (a) indicate tribes: 1, Olisbeoideae; 2, Kibessieae; 3, Merianieae; 4, Miconieae; 5, Eriocnemeae; 6, Trioleneae; 7, Rupestreae; 8, Rhexieae; 9, Microlicieae; 10, Melastomateae; 11, Marcetieae; 12, Dinophoreae; 13, Dissochaeteae; 14, Cambessedesieae; 15, Stanmarkieae; 16, Cyphostyleae; 17, Sonerileae; 18, Pyxidantheae; 19, Bertolonieae; 20, Lithobieae; 21, Henrietteeae; 22, Astronieae; tribes containing species which shifted pollinators: 1, 3, 4, 10, 17, 18 and 22.

binaryPGLMMs for the structure of stamens (smooth or ruminate). We used trait values from Kopper *et al.* (2024). Pore size was determined using the total pore area ($A = \pi \times a \times b$), where '*a*' represents half the pore height and '*b*' represents half the pore width. We log-transformed petal length and pore size to improve PGLMM fit and used stepwise model selection to identify significant variables.

Results

Shifts away from bee pollination are associated with cool montane environments

Following our expectations, species which have shifted away from ancestral bee pollination occurred at significantly higher elevations than bee-pollinated species (Fig. 2a). This pattern was mostly consistent when lumping all shifted species into one category (Fig. 2a) and when retaining the four distinct pollination syndromes (Fig. 2b). Species from the 'nectar-foraging vertebrate' and the 'passerine' syndromes generally occur above 1000 m and increase in abundance with increasing elevation (Fig. 2a, b). Species from the 'generalist' insect pollination syndrome can also occur at lower elevations (at *c.* 500 m), but with a peak in abundance at slightly higher elevations than bee-pollinated species (Fig. 2d). Bee-pollinated species were found along the whole elevational gradient, but their abundance decreased markedly with increasing elevation (Fig. 2c,d).

Using binaryPGLMMs, we found that the distribution of pollination systems was best explained by elevation and latitude ($P_{elevation}$ = 1.2e−08, $P_{latitude}$ = 0.02; Tables S3, S4; Figs 1a–d, S2a), but not precipitation. Species that shifted pollinators occurred at higher elevations around the equator than at higher latitudes (Fig. 2c,d). These results were consistent when resampling the dataset to include one randomly chosen occurrence per species, with 100% of resampling runs finding shifted species to have a significantly higher probability of occurring at high elevations than bee-pollinated species.

Further exploring the association between pollination system and environment through Whittaker biomes, we found bee-pollinated species in significantly different biomes than species which shifted pollinators (χ^2 = 28.73, P = 8.87e−06; Fig. 2e,f). Bee-pollinated Melastomataceae species grow more commonly in warm tropical rain forests and tropical seasonal forests, while shifted Melastomataceae species grow more commonly in cooler temperate rain forests and temperate seasonal forests. This differentiation in biome occupation among pollination systems is mostly driven by temperature rather than precipitation (Fig. 2e). As elevation and temperature are highly correlated (Fig. S1a), these results are in line with results from binaryPGLMMs identifying elevation as the most important explanatory variable in differentiating bee pollination from shifted pollination systems in Melastomataceae.

Colonization of mountain habitats precedes pollinator shifts

To investigate the interplay of evolutionary pollinator shifts and niche expansion into novel mountain environments, we reconstructed the evolutionary history of pollinator shifts and elevation. Across reconstruction methods (treating elevation as categorical or continuous trait), the root of Melastomataceae was reconstructed with a high probability as occurring in lowland conditions with bee pollination as the ancestral pollination system. Mountain colonization occurred repeatedly across Melastomataceae, both in tribes containing pollinator shifts and in purely bee-pollinated tribes (Figs 3a, S3a). Bee-pollinated lineages sister to species/lineages that have shifted pollinators were generally found in montane environments, supporting the hypothesis that shifts in elevation preceded pollinator shifts (Figs 1a, 3a, S3). In the tribes Olisbeoideae (two pollinator shifts) and Astronieae (three out of four pollinator shifts), pollinator shifts have occurred at relatively low elevations (around or below 500 m), while the five other tribes containing pollinator shifts (Melastomatoideae – four shifts, Merianieae – four shifts, Miconieae – seven shifts, Pyxidantheae – two shifts and

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Table 1 Reconstructed shift probability of pollination system and elevation at different nodes of tribes, which contain species that shifted pollinators.

Tribe	Node ID	Node Age	Prob Shifted	Prob < 500 m	Prob 500 – 1000 m	Prob 1000 – 1500 m	Prob > 1500 m
	TribeNode	50.23	< 0.01	**0.40**	0.30	0.23	0.07
Olisbeoideae	ShiftedNode	26.47	< 0.01	**0.59**	0.27	0.14	< 0.01
	TribeNode	31.49	< 0.01	0.32	**0.34**	0.30	0.04
	ShiftedNode	12.14	< 0.01	< 0.01	< 0.01	0.02	**0.98**
	Shift	4.03	0.99	< 0.01	< 0.01	< 0.01	**0.99**
	Shift	1.79	0.99	< 0.01	< 0.01	< 0.01	**0.99**
	Shift	3.08	0.99	< 0.01	< 0.01	< 0.01	**0.99**
Merianieae	Shift	5.29	0.98	< 0.01	< 0.01	< 0.01	**0.99**
	TribeNode	18.20	< 0.01	**0.69**	0.22	0.09	< 0.01
	ShiftedNode	4.42	< 0.01	0.07	**0.92**	0.01	< 0.01
	ShiftedNode	4.89	0.27	0.05	**0.76**	0.20	< 0.01
	Shift	4.07	< 0.01	**0.51**	0.48	< 0.01	< 0.01
	ShiftedNode	5.35	< 0.01	**0.86**	0.14	< 0.01	< 0.01
	ShiftedNode	8.60	0.03	< 0.01	< 0.01	0.03	**0.97**
	Shift	7.93	0.96	< 0.01	< 0.01	< 0.01	**0.99**
Miconieae	ShiftedNode	9.08	0.87	0.46	**0.47**	0.07	< 0.01
	TribeNode	34.46	< 0.01	0.27	**0.36**	0.35	0.02
	ShiftedNode	5.66	< 0.01	0.01	0.47	**0.51**	< 0.01
	ShiftedNode	2.35	< 0.01	0.02	0.02	**0.95**	0.01
	Shift	3.08	0.98	< 0.01	< 0.01	0.09	**0.91**
	ShiftedNode	4.80	< 0.01	< 0.01	< 0.01	< 0.01	**0.99**
	ShiftedNode	16.34	< 0.01	< 0.01	0.01	0.05	**0.94**
Melastomateae	Shift	1.77	0.99	< 0.01	< 0.01	< 0.01	**0.99**
	TribeNode	37.49	< 0.01	0.29	**0.32**	0.31	0.07
	ShiftedNode	9.20	0.83	< 0.01	0.01	0.12	**0.87**
Sonerileae	ShiftedNode	10.25	< 0.01	0.07	0.33	**0.60**	< 0.01
	TribeNode	17.79	0.01	0.07	0.20	**0.39**	0.33
	ShiftedNode	2.319	0.99	< 0.01	< 0.01	< 0.01	**0.99**
Pyxidantheae	ShiftedNode	5.97	< 0.01	< 0.01	0.04	**0.96**	< 0.01
	TribeNode	22.60	0.01	**0.49**	0.31	0.19	< 0.01
Astronieae	ShiftedNode	14.19	0.31	**0.48**	0.34	0.18	< 0.01

Tribe crown nodes (TribeNode) are usually located at lower elevations than nodes of shifted clades within a tribe (ShiftedNode). Nodes that show a high probability of having shifted pollinators (Shift) are usually located at higher elevations than ancestral nodes of a tribe (TribeNode) and nodes of shifted clades within a tribe (ShiftedNodes). Bold values with highlighted cell margins represent maximum values for elevation shifts. Colors correspond to tribes as used in Fig. 3. Prob Shifted gives the probability at a certain node of having shifted. Prob < 500, 500–1000, 1000–1500 and > 1500 m give the probabilities at a certain node of being in each respective elevation bin. Different colors in writing are further used to emphasize differences in nodes considered – light blue for tribe crown nodes (TribeNode), dark blue for nodes of clades encompassing pollinator shifts (ShiftedNode), and grey for nodes within clades containing pollinator shifts, with these nodes also having a high probability of having shifted (Shift) away from bee pollination.

Sonerileae –two shifts) showed shifts at or above 1000 m (Table 1).

Extracting reconstructed node values for elevation and pollination for all lineages, including pollinator shifts, we explored whether pollinator shifts likely occurred within montane environments (Fig. 1a), preceded mountain colonization (Fig. 1b) or went hand in hand with mountain colonization (Fig. 1c). We found strongest support for the first scenario (Fig. 1a), which shifts into mountain environments preceded shifts in pollinators. When the cutoff for 'montane' is set to 500 m, 25 of 25 lineages had intercepts above the 1 : 1 line, indicating a higher probability of having shifted to montane habitats while still being bee-pollinated. Also, lines mostly group above the 1 : 1 line (Fig. 3b), indicating that shifts to mountains preceded pollinator shifts. When the cutoff is 1000 m, 19 of 25 lineages still had intercepts along the *y*-axis, with 6 close to 0, suggesting a low probability of having shifted to mountains or pollinators, and 13 out of 25 lineages grouped above the 1 : 1 line (Fig. 3c). When

the cutoff for being montane is 1500 m, the majority of lineages (10) had intercepts above the 1 : 1 line, 7 were close to 0, and 8 were following the *x*-axis, indicating a higher probability of having shifted pollinators while still occurring below 1500 m. Additionally, 10 out of 25 lineages grouped above the 1 : 1 line (Fig. 3d). In support of the pattern that shifts to montane environments precede pollinator shifts, the last common ancestors of tribes (tribe crown nodes) where pollinator shifts have occurred (Table 1; TribeNode, likely bee-pollinated) are commonly located below 1000 m, while within these tribes, the last common (likely bee-pollinated) ancestors of shifted clades (Table 1; ShiftedNode) are commonly located at a higher elevation than tribe crown nodes. These findings again indicate that mountains were mostly colonized by species that were bee-pollinated and pollinator shifts happened later in mountain environments.

We found no evidence for correlated evolution of elevation and pollination system (AIC dependent model = 1103.698, AIC independent model = 1011.816), again supporting the hypothesis that shifts in elevation and pollination system happened independently.

These results were confirmed when reconstructing elevation as a continuous trait, with 19 out of the 25 shifts across all tribes showing pollinator shifts in mountain environments and continued expansion into higher elevations in shifted clades (Fig. S3b). OU models further confirmed these findings, with Hypothesis 1 (separate elevational optima for entire tribes with shifted species, and hence also encompassing closely related bee-pollinated species) resulting as best fit (Fig. S4a–c; Table S5).

Bee-pollinated species of shifted tribes occur in environments conducive to pollinator shifts

To further establish whether bee-pollinated species from shifted tribes are more likely to occur in environments conducive to pollinator shifts (i.e. mountains) than bee-pollinated species from nonshifted tribes, we next focused on bee-pollinated species only (n = 280). We found that bee-pollinated species from tribes containing shifted lineages occur at significantly higher elevations than bee-pollinated species from nonshifted tribes (phylogenetic mean difference = 772.71, P = 1.1e−57; Fig. 4a; Table S6). Species from tribes lacking pollinator shifts did not commonly reach the elevational threshold (> 1000 m) generally triggering pollinator shifts. In line with this, we found bee-pollinated species of tribes containing pollinator shifts in significantly different biomes (cooler temperate rainforests) than bee-pollinated species from nonshifted tribes (tropical rain forests and tropical seasonal forests; χ^2 = 13.51, P = 0.009; Fig. 4b).

To explore in more detail whether bee-pollinated species from shifted and nonshifted tribes occur in different environments at different elevations, we split all bee-pollinated species into species occurring below the general elevational threshold for pollinator shifts (< 1000 m, n = 190) and above (> 1000 m, n = 90). Below 1000 m, bee-pollinated species from shifted tribes occurred in warmer (phylogenetic mean difference = 23.25, P = 1.9e−176; Table S7) but wetter (phylogenetic mean difference = 2175.14, P = 4.5e−107; Table S8) niches than bee-pollinated species from nonshifted tribes (Fig. 4c,e). Above 1000 m, however, bee-pollinated species from shifted tribes occurred in colder (phylogenetic mean difference = 17.73, P = 1.3e−37; Table S9) and wetter (phylogenetic mean difference = 1853.53, P = 4.2e−43; Table S10) environments than bee-pollinated species from nonshifted tribes (Fig. 4d,f). These results suggest that bee-pollinated species from shifted tribes explore a wider range of climatic niche space (generally wetter, but both warmer and colder environments), making it more likely for these bee-pollinated species to encounter environmental conditions selecting for pollinator shifts.

Bee-pollinated species occur in more bee-friendly environments than shifted relatives

To investigate why mountain environments did not always trigger pollinator shifts, but a substantial proportion of Melastomataceae species retained ancestral bee pollination in mountains, we next tested whether bee-pollinated montane species are confined to more bee-friendly (warmer and drier) environments than their vertebrate-pollinated relatives. Subsetting the dataset to only include montane species (> 1000 m, n = 128), we tested how elevation, latitude and precipitation affect the pollination system using binaryPGLMMs. We only retained mean annual temperature after model selection (Tables S11, S12) and found that bee-pollinated montane species occurred in significantly warmer, but not drier, mountain environments than shifted species (P = 4.6−e05; Figs S2b, S5a–d).

Montane bee-pollinated species have larger flowers and pores than lowland species

Among bee-pollinated species, we tested whether floral traits potentially increasing attractiveness to pollinators (larger flowers) and facilitating pollen dispersal (smooth anther walls and enlarged pores) are more common among species occurring under environmental conditions unfavorable to bee pollination (i.e. high elevations and high precipitation). Indeed, we found that montane bee-pollinated species have larger flowers (Fig. 5a; residual variance = 0.38, $P_{elevation}$ = 3.8e−05 and $P_{latitude}$ = 0.01; Tables S13, S14) and pores (Fig. 5b; residual variance = 0.83, $P_{elevation}$ = 0.01 and $P_{latitude}$ = 0.002; Tables S15, S16), with the best-fit PGLMM, including elevation and the interaction between elevation and latitude, but not precipitation (Tables S14, S16; Figs 5a,b, S6–S8). At higher latitudes, where elevation zones are naturally depressed, increases in flower and pore size start at lower elevations (Fig. S8). Importantly, we also found that pore size and petal size are uncorrelated (R^2 = 0.15), indicating independent adaptive evolution of both traits. We did not find any effect of elevation, latitude or precipitation on the distribution of smooth vs corrugated stamen walls (Table S17; Figs 5c, S2c).

Discussion

Our assessment of the environmental context of pollinator shifts in the large flowering plant family Melastomataceae revealed that

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evolutionary shifts away from bee pollination have repeatedly been triggered when bee-pollinated species colonized cool and wet mountain environments (Figs 1a, 3). These shifts were often followed by subsequent niche expansion into even colder and wetter niches. In mountains, bee-pollinated relatives of shifted species occur in significantly colder and wetter environments than bee-pollinated species of tribes lacking shifts. This difference in niche occupation among bee-pollinated species indicates that these cool and wet environments are likely conducive to evolutionary shifts away from bee pollination. Our results hence

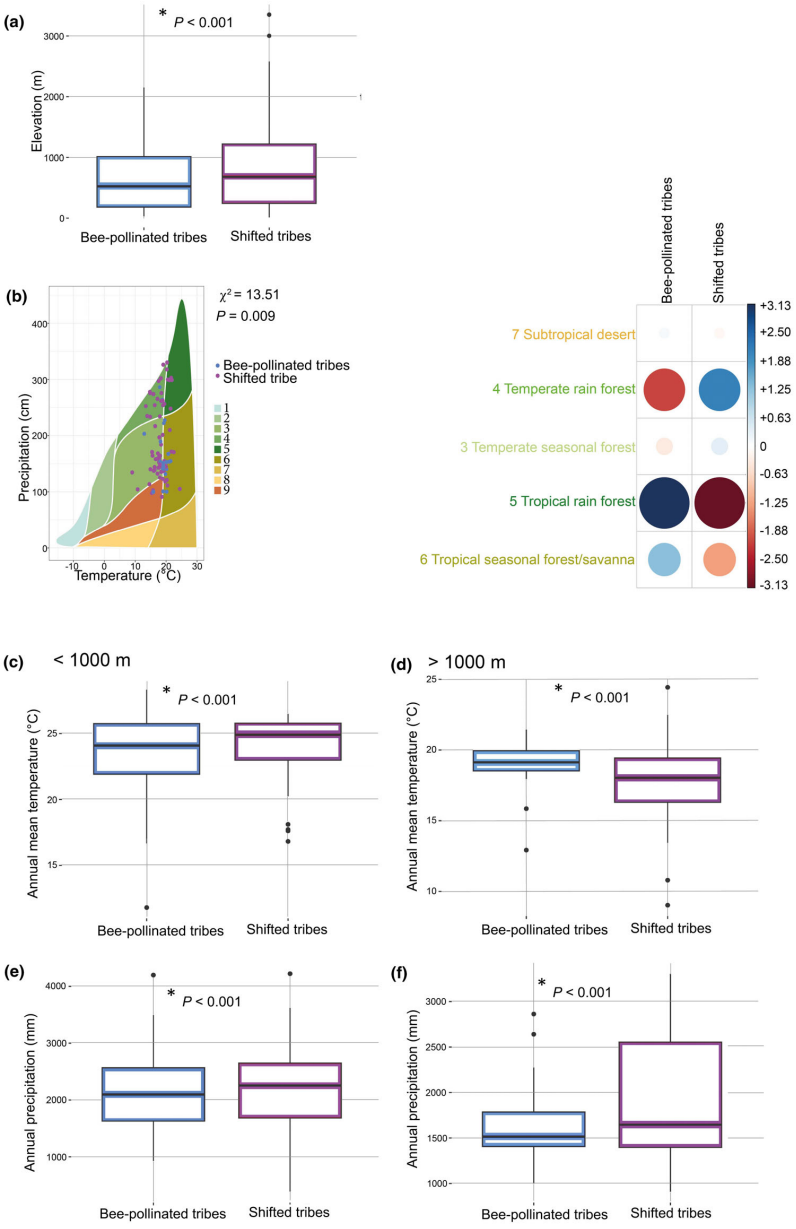

Fig. 4 Bee-pollinated species from tribes that contain pollinator shifts occur at higher elevations and under colder and wetter conditions than bee-pollinated species from tribes without pollinator shifts. (a) Bee-pollinated species from entirely bee-pollinated tribes (blue) occur, on average, at 653 m, while bee-pollinated species from tribes containing pollinator shifts (pink) occur, on average, at 820 m. (b) Comparison of Whittaker biome (Whittaker, 1975) occupation of bee-pollinated species from shifted tribes (pink) and from nonshifted tribes (blue), showing that species from entirely bee-pollinated tribes mostly occur in tropical rainforests (Biome 5) and savannahs (Biome 6), while bee-pollinated species from shifted tribes are more common in temperate rainforests (Biome 4). Plot of standardized residuals of the chi-squared test. Blue indicates a positive correlation, while red indicates a negative correlation. Size of the circles indicates the magnitude of the correlation; faded and small circles indicate a weak correlation. Colored surfaces indicate different biomes, defined by temperature (*x*-axis) and precipitation (*y*-axis). (c) Comparison of the annual mean temperature (°C) of bee-pollinated species from entirely bee-pollinated tribes and shifted tribes below 1000 m, showing that entirely bee-pollinated tribes occupy warmer niches in lowlands. (d) Comparison of the annual mean temperature (°C) of bee-pollinated species from entirely bee-pollinated tribes and shifted tribes above 1000 m, showing that entirely bee-pollinated tribes occupy colder niches in mountains. (e) Comparison of the annual precipitation (mm) of bee-pollinated species from entirely bee-pollinated tribes and shifted tribes below 1000 m, showing that entirely bee-pollinated tribes occupy drier niches in lowlands. (f) Comparison of the annual precipitation (mm) of bee-pollinated species from entirely bee-pollinated tribes and shifted tribes above 1000 m, showing that entirely bee-pollinated tribes occupy drier niches in mountains. Boxes represent the interquartile ranges; bars inside the boxes represent the median values; whiskers represent the range; dots represent outliers. * indicate significant differences. *P* values were obtained using phylogenetic *t*-test. Whittaker biomes: 1, Tundra; 2, Boreal forest; 3, Temperate seasonal forest; 4, Temperate rain forest; 5, Tropical rain forest; 6, Tropical seasonal forest/savanna; 7, Subtropical desert; 8, Temperate grassland/desert; 9, Woodland/shrubland.

support the rarely tested hypothesis that certain environmental conditions will consistently trigger pollinator shifts (Stebbins, 1990). Adaptive evolution of flower traits potentially facilitating pollinator attraction (flower size) and pollen dispersal (pore size) may further be critical in enabling some species to retain bee pollination even in environments conducive to pollinator shifts.

Shifts away from bee pollination associate with cool montane environments

Across Melastomataceae, species which shifted away from ancestral bee pollination mostly occur in montane environments above 1000 m (Figs 2, 3, S9). This pattern matches well with expectations from empirical studies documenting that the harsher climatic conditions prevalent in mountains significantly reduce the abundance and diversity of bee pollinators (Cruden, 1972; Brito & Sazima, 2012; McCallum *et al.*, 2013; Classen *et al.*, 2015, 2020; Cozien *et al.*, 2019). Our results hence support a pollinator-shift scenario proposed by Thomson & Wilson (2008), which predicts that reductions of bee pollinator visitation rates to flowers may disrupt the stable interaction between plants and their ancestral pollinators (buzzing bees in Melastomataceae) and open the opportunity for pollinator shifts.

The mountain-pollinator-shift association reported by us is not only replicated across Melastomataceae but also found across a large sample of Neotropical plant clades, where bee-pollinated species commonly occur at low elevations and vertebrate-pollinated relatives occur in montane environments, such as the Andes mountains (e.g. Bromeliaceae, Gesneriaceae, *Psychotria*, *Passiflora*, Loranthaceae and *Salvia*; Dellinger *et al.*, 2023). While the lack of detailed assessments of the relative timing of mountain colonization and pollinator shifts in these clades currently impedes inference of causality, the increased abundance of vertebrate (particularly hummingbird) pollination in tropical mountains points toward an overwhelming role of the abiotic environment in triggering pollinator shifts (i.e. 'environments conducive to evolutionary pollinator shifts', compare to the idea of 'hummingbird habitats' by Stebbins, 1990). Importantly, such 'environments conducive to evolutionary pollinator shifts' might be clade-specific or depend on the respective biogeographic context. For example, lower elevations associate with shifts to hummingbird pollination in temperate *Penstemon* (while bee-pollinated species occur at higher elevations, Hamilton & Wessinger, 2022). Also, in some clades, pollinator shifts seemingly occur in the absence of environment shifts (i.e. Melastomataceae tribe Astroniaeae; Figs 3b, S3b). The latter is true also for the Neotropical section of the genus *Costus*, where pollinator shifts are unrelated to elevation (Kay & Grossenbacher, 2022). In such cases, alternative mechanisms, such as competition for pollinators or major mutations in floral traits involved in pollinator attraction (i.e. color and scent), may lead to pollinator shifts even without ecogeographic divergence (Bradshaw & Schemske, 2003; Hoballah *et al.*, 2007; Muchhala & Potts, 2007; Peakall *et al.*, 2010; Xu *et al.*, 2011; Binaghi *et al.*, 2023). Investigating the link between pollinator shifts and environment shifts across more plant clades and biogeographic contexts in the future is necessary to establish whether environment-induced reduction in the abundance and efficiency of ancestral pollinators is indeed a common driver of evolutionary pollinator shifts.

Another aspect worth investigating in more detail is whether each functional group of pollinators has its respective 'environment conducive to shifts' (i.e. where its abundance and efficiency is significantly reduced in comparison with other groups). We here merged all species belonging to shifted pollination syndromes ('generalist', 'nectar-foraging vertebrate', 'food-body-foraging vertebrate') to broadly evaluate whether shifts away from bee pollination are consistently triggered by the same environmental variables. Among the shifted melastome groups, however, 'generalist' syndrome Melastomataceae are noteworthy as occurring at lower elevations (even at lower latitudes) than vertebrate-pollinated species (Fig. 2c,d). This trend may be due to undersampling of comparatively inconspicuous generalist Miconieae in the Andes, with numerous recent reports of generalist pollination in *Miconia* in the tropical Andes (Valderrama *et al.*, 2022, Angulo *et al.*, unpublished). Alternatively, there may

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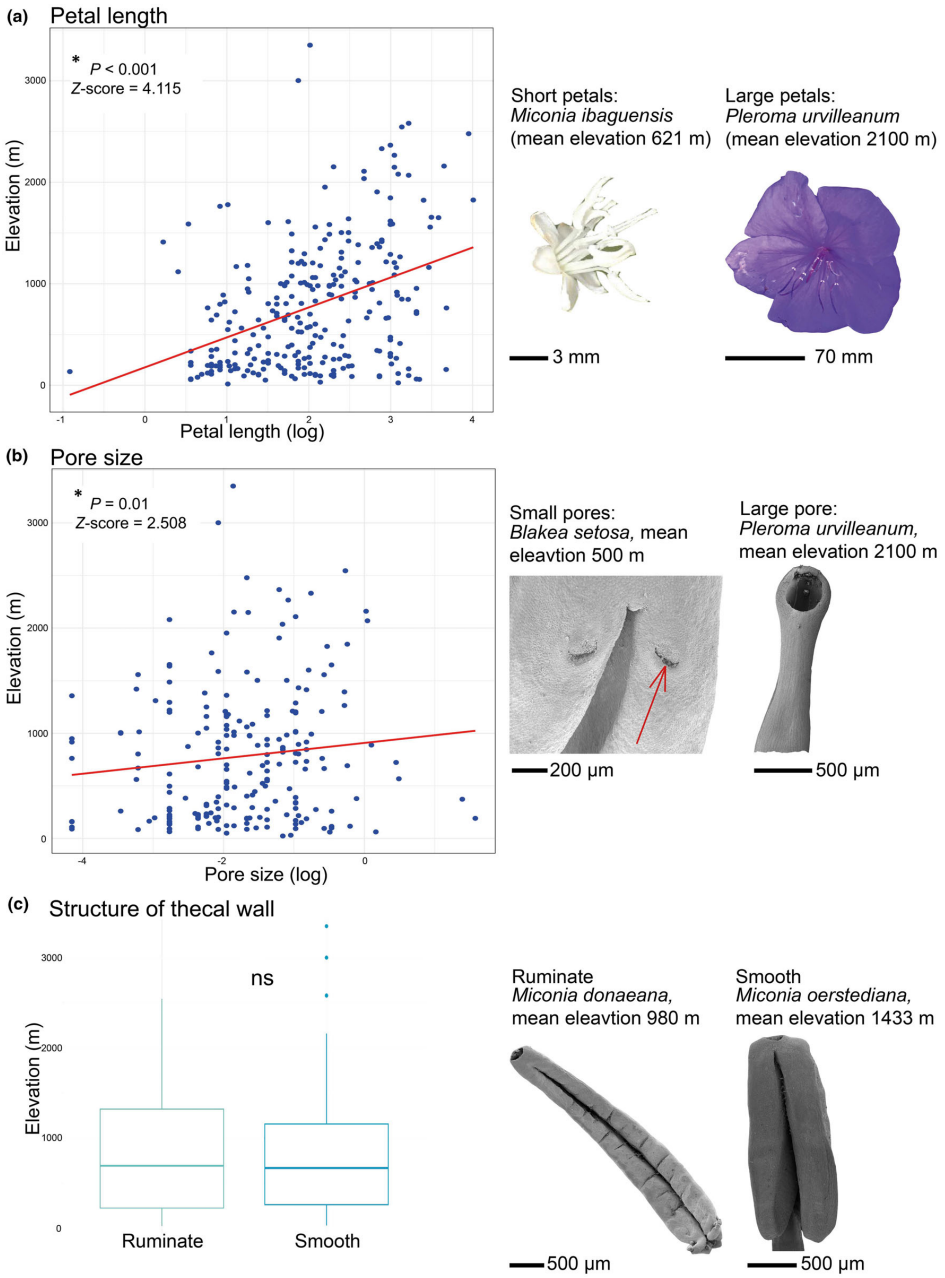

Fig. 5 Montane bee-pollinated Melastomataceae evolved larger flowers and pores (potentially facilitating pollen dispersal) than lowland bee-pollinated Melastomataceae, but thecal wall structures showed no association with elevation. (a) Scatterplot of petal size on the *x*-axis and elevation on the *y*-axis of bee-pollinated species. (b) Scatterplot of pore size (total pore area: $\pi \times$ ½ pore height $\times$ ½ pore width) on the *x*-axis and elevation on the *y*-axis. Red line is the regression line of the model (c) Boxplot of elevation data comparing ruminate and smooth anther wall structures of bee-pollinated species, with no significant difference among the two groups (ns). Boxes represent the interquartile ranges; bars inside the boxes represent the median values; whiskers represent the range and dots represent outliers. *P* values and *Z*-scores were obtained from the phylogenetic generalized linear mixed models (PGLMM), the raw data are shown in plots.

be conditions currently unknown that favor transitions to generalist flowers in Melastomataceae, which, being smaller in size with lower amounts of nectar (Kopper *et al.*, 2024), may be cheaper to produce than vertebrate-adapted flowers.

In addition, environments conducive to pollinator shifts may not always be geographical, but also seasonal, with different seasons favoring different reproductive/pollination strategies. For example, Brito *et al.* (2017) found that pollinator-dependent bee-pollinated Melastomataceae flowered during the warmer rainy season and pollinator-independent (autogamous self-pollinating and apomictic) species flowered during the colder dry season in the Brazilian Atlantic Forest. We could thus envision scenarios in Melastomataceae where divergence in flowering times (i.e. due to pollinator competition) from the warm 'bee-friendly' to the cold 'shift' season could trigger pollinator shifts independent of elevation. Such seasonality-linked pollinator shifts have been reported for South African orchids (Peter & Johnson, 2014) and are also known from Asia and Africa, where vertebrate-pollinated species tend to flower in the cold and rainy season (Janeček *et al.*, 2015; Funamoto, 2019). Again, these season-shift links underscore the importance of the abiotic environment in understanding the evolution of pollinator shifts.

Colonization of mountain habitats precedes pollinator shifts

Our finding that entire Melastomataceae clades shift into mountains typically before pollinator shifts occur (Fig. 3; Table 1) supports the idea that niche expansion into environments conducive to pollinator shifts preceded pollinator shifts. Most species with empirically verified pollinator shifts in our sample occur in geologically relatively young mountain ranges with final phases of mountain uplift during the mid/late Miocene (15–5 Million years ago; tropical Andes (Boschman & Condamine, 2022), New Guinea (Roycroft *et al.*, 2022), East African Mountains (Dellinger *et al.*, 2022; Dagallier *et al.*, 2024; but also ancient Western Ghats, Johnson *et al.*, 2022)). Accordingly, our reconstruction of the elevational history of Melastomataceae shows repeated colonization of these uplifting mountains within the past 20–30 Myr, followed by shifts away from bee pollination commonly only within the past 10 Myr (Table 1). A scenario thus seems plausible where bee-pollinated ancestors of present-day Melastomataceae colonized the newly forming mountain ranges from lowland rainforests. With the generally reduced availability of bee pollinators in these mountain environments (Arroyo *et al.*, 1982; Classen *et al.*, 2020), successive niche filling might then have intensified competition for the already limited resource of montane bee pollinators, spurring evolutionary shifts to vertebrate pollination and generalists (Armbruster, 1985). Clearly, the interpretation of our analyses is affected by how elevation is defined, with clearest support for environment shifts preceding pollinator shifts when classifying species > 500 m as montane (which captures latitudinal differences, generalist species and island effects). Since the general trend that tribe crown nodes occur at lower elevations than shifted nodes (Table 1) holds irrespective of binarizing elevation, we are confident that environment shifts are indeed a precursor to pollinator shifts in most Melastomataceae lineages.

Another result revealed by our analyses is that lineages containing species which shifted pollinators diversified into even colder environments and higher elevations than their bee-pollinated ancestors and relatives (Fig. 3). Thus, while mountains likely provided the initial environments conducive to pollination shifts, shifts to efficient high-elevation pollinators, such as hummingbirds, sunbirds and white-eyes, allowed for subsequent niche expansion to even higher elevations in tropical mountains. At least in Neotropical mountains, this expansion into higher elevations may also represent 'niche tracking' of the newly acquired hummingbird pollinators, which concomitantly diversified in the cold and wet cloud forests during the Miocene (Barreto *et al.*, 2023).

Bee-pollinated species of shifted tribes occur in environments conducive to pollinator shifts

We found that bee-pollinated species of tribes containing pollinator shifts generally occur in wetter niches compared with bee-pollinated species from tribes without pollinator shifts (Fig. 4f). Bee-pollinated montane species from shifted tribes are hence more likely to be exposed to environments conducive to pollinator shifts (i.e. cold and wet conditions reducing bee flower visitation activity) than species from nonshifted tribes. Bee-pollinated species from tribes lacking pollinator shifts seem to have only colonized warmer and drier subniches in mountains (i.e. rain-sheltered slopes). Whether these niche differences among bee-pollinated species from shifted and nonshifted tribes are caused by physiological constraints (i.e. limited temperature tolerance; Eller *et al.*, 2020), genetic constraints (i.e. inability to evolve certain traits, Bradshaw, 1991), biogeographic history (i.e. ancestral presence in drier mountains), dispersal limitations (i.e. remaining on drier slopes; Polato *et al.*, 2018) or lower adaptability to montane bee pollinators (to be described later), remains to be explored.

Montane bee-pollinated species occur in more bee-friendly environments than shifted relatives

Contrasting bee-pollinated and shifted species found above 1000 m, we found that bee-pollinated species grow in warmer environments than their shifted relatives (Fig. S5b). These warmer montane environments may be more 'bee friendly', featuring higher bee diversity and abundance and thus bee pollinator availability (Brito & Sazima, 2012; Dellinger *et al.*, 2021). Melastomataceae species colonizing such 'bee-friendly' environments in tropical mountains might thus not have experienced the radical reduction in bee pollinator abundance otherwise triggering pollinator shifts, thus maintaining the stable buzz pollination system.

An aspect that we could not assess in this study due to a lack of detailed bee pollinator data is how turnovers in the functional (i.e. body size) and taxonomic composition of bee pollinator assemblages across elevation affect pollinator shifts. Bee body size, for example, generally determines the thermoregulation capacity of bees, with larger bees being able to maintain high body temperatures more efficiently even in low temperature environments (Hodkinson, 2005). Accordingly, bee body size commonly increases along elevational gradients and often is paralleled by a

narrowing in bee taxonomic diversity to relatively large-bodied genera (e.g. *Bombus*, *Eufriesea* and *Centris*; McCabe *et al.*, 2019). Furthermore, some bee families, such as buzz-pollinating Euglossini, show marked latitudinal differences, with large-bodied genera, such as *Eulaema*, *Eufriesea* and *Exaerete*, common both in the Andes and Amazonian lowlands, but small-bodied *Euglossa* species are more common in Central America (Roubik & Hanson, 2004; Kay & Grossenbacher, 2022). Collecting more detailed data on Melastomataceae bee pollinator communities will be essential in the future to test whether montane bee-pollinated Melastomataceae are generally adapted to pollination by larger buzzing bees than lowland bee-pollinated Melastomataceae. It is possible that shifts to larger bee pollinators facilitated mountain colonization of Melastomataceae and that bee-pollinated species from shifted clades are generally adapted to bees with more extreme (i.e. cold) environmental tolerances.

Montane bee-pollinated species have larger flowers and anther pores than lowland species

Besides pollinator shifts, adaptive trait evolution may mitigate negative effects of reduced pollinator availability. Accordingly, among montane Melastomataceae, we found an increase in petal length and anther pore size (Fig. 5). Larger floral display is known to increase pollinator attraction (Dafni *et al.*, 1997; Goodwillie *et al.*, 2010) and may increase a species' probability of being visited. In addition, species may adapt their pollen dosing to the abundance and efficiency of their pollinators and release pollen in larger portions if pollinator visits are rare (Castellanos *et al.*, 2006). Both mathematical models (Boucher-Bergstedt *et al.*, 2025) and experiments on the Melastomataceae species *Adelobotrys adscendens* (Sw.) Triana (Dellinger *et al.*, 2019a) suggest that larger pores may indeed release larger proportions of pollen. The increase in anther pore size with elevation in Melastomataceae may thus represent an adaptation to release larger quantities of pollen with the infrequent bee visits.

With the general increase in bee pollinator body size at higher elevations, we cannot rule out that the increase in flower and pore size is merely related to the larger pollinator size (Classen *et al.*, 2015) rather than a mechanism to optimize pollen dispersal. The fact that flower and pore size are uncorrelated, however, suggests that the two traits may underlie different selection pressures so that selection for relaxed pollen dosing may indeed play a significant role in montane bee-pollinated Melastomataceae. Clearly, extensive comparative experiments on floral biomechanics are required to clarify the adaptive potential of flower and pore size. If functional relationships are indeed revealed, the evolution of increased floral display and enlarged anther pores may have been critical to allow for the initial colonization of montane environments by Melastomataceae, as well as for the persistence of bee-pollinated species in tropical mountains.

Conclusion

Although evolutionary shifts among functional groups of pollinators are commonly recognized as a key process in angiosperm diversification, our current understanding of the mechanisms triggering pollinator shifts is limited. In this study, using the tropical plant family Melastomataceae as a model, we investigated the role of environmental factors in explaining pollinator shifts and found that climatic niche shifts (into mountain environments) acted as important precursors for evolutionary shifts away from bee pollination. Specifically, Melastomataceae showed a pattern common for Neotropical plants, where shifts away from bee pollination associate with occurrence in montane environments (Dellinger *et al.*, 2023). These macroevolutionary associations confirm empirical case studies (Cruden, 1972; Dellinger *et al.*, 2021), suggesting that the reduction in bee pollinator abundance induced by montane climatic conditions acts as an important trigger of pollinator shifts (Thomson & Wilson, 2008). Our results raise the question of whether climatic niche shifts followed by pollinator shifts are a common pattern in angiosperm diversification (Donoghue & Sanderson, 2015). More broadly, such staggered evolutionary events and strong linkage of ecogeographic patterns and biotic interactions may transcend beyond plant–pollinator interactions and be characteristic also for other types of plant–animal interactions, such as ant–plant mutualisms (Luo *et al.*, 2023) or seed dispersal (Salazar-Tortosa *et al.*, 2019). Investigating evolutionary dynamics between climatic niche shifts and shifts in biotic interactions across a diversity of interaction types and plant clades represents a major avenue for future research.

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Competing interests

None declared.

Author contributions

CK, JS and ASD conceived the study. CK compiled the data matrix. CK and ASD carried out the statistical analyses. CK wrote the manuscript. All authors contributed to finalizing the manuscript. JS and ASD contributed equally to this work and joint senior authors.

ORCID

Agnes S. Dellinger https://orcid.org/0000-0003-1394-3414
Constantin Kopper https://orcid.org/0000-0002-5567-1720
Jürg Schönenberger https://orcid.org/0000-0001-6791-2731

Data availability

All datasets, R-codes and the floral trait matrix have been deposited in the open access repository phaidra (https://phaidra.univie.ac.at/o:2096899).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Correlation plots of climatic variables and elevation and distribution maps of pollination system.

Fig. S2 Reconstruction of pollination systems and elevation.

Fig. S3 Model fit of binaryPGLMM models.

Fig. S4 Ornstein–Uhlenbeck models for elevational optima.

Fig. S5 Bee-pollinated species generally occur in warmer environments than vertebrate-pollinated.

Fig. S6 Model fit of PGLMM for petal length.

Fig. S7 Model fit of PGLMM for pore size.

Fig. S8 With increasing latitude, petal and pore size of bee-pollinated species start to increase already at lower elevation.

Fig. S9 Tip states of elevation and pollination system mapped on a phylogeny (Reginato *et al.*, 2022).

Notes S1 Methods SI.

Notes S2 Climatic variables.

Table S1 GBIF occurrence points of the 333 species included in this study.

Table S2 AIC of MuSSE models.

Table S3 BinaryPGLMM full model for pollination syndromes using elevation, latitude and annual precipitation.

Table S4 BinaryPGLMM reduced model for pollination system using elevation and latitude.

Table S5 pBIC of OU models testing for elevational optima.

Table S6 Phylogenetic *t*-test using elevation.

Table S7 Phylogenetic *t*-test for bee-pollinated species from shifted and nonshifted tribes below 1000 m using annual mean temperature.

Table S8 Phylogenetic *t*-test for bee-pollinated species from shifted and nonshifted tribes below 1000 m using annual precipitation.

Table S9 Phylogenetic *t*-test for bee-pollinated species from shifted and nonshifted tribes above 1000 m using annual mean temperature.

Table S10 Phylogenetic *t*-test for bee-pollinated species from shifted and nonshifted tribes above 1000 m using annual precipitation.

Table S11 BinaryPGLMM full model for pollination system above 1000 m using annual mean temperature and annual precipitation.

Table S12 BinaryPGLMM reduced model for pollination system above 1000 m using annual mean temperature.

Table S13 PGLMM full model for petal length of bee-pollinated species using elevation, latitude and annual precipitation.

Table S14 PGLMM reduced model for petal length of bee-pollinated species using elevation and latitude.

Table S15 PGLMM full model for pore size of bee-pollinated species using elevation, latitude and annual precipitation.

Table S16 PGLMM reduced model for pore size of bee-pollinated species using elevation and latitude.

Table S17 BinaryPGLMM full model for structure of thecal wall using elevation, latitude and annual precipitation.

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Concluding Discussion

Pollination syndromes have been criticized for their low predictive accuracy, especially when few, easy-to-score traits are used as basis for model development (Ollerton et al. 2009, Abrahamczyk et al. 2017). Particularly when employing pollination syndromes at large scales (i.e. across angiosperms), finding meaningful traits which can be scored across all species can be challenging, limiting researchers to relatively coarse, generalized traits (Dellinger 2020). By applying a Random Forest model using floral traits specific to Melastomataceae and with known functional relevance in the pollination process of the group (based on previous field studies, Renner 1989, Varassin et al. 2008, Brito et al. 2016, Dellinger et al. 2014, 2019, 2019a, 2019b), we could achieve a very high predictive accuracy (99.2% success rate). We could show that the concept of pollination syndromes can be a useful tool for studying patterns of flower macroevolution and disparity in a pollination biological context even when pollinators cannot be observed for all species.

The high disparity of the "buzz-bee" syndrome is remarkable as it is one of few examples of high floral disparity in the absence of pollinator shifts (also e.g. the *Gorteria diffusa* complex, Asteriaceae, Ellis & Johnson 2009). In contrast, several examples of low floral disparity in clades without pollinator shifts are well documented (e.g. *Solanum*, Solanaceae, Symon 1979; *Myrcia*, Myrtaceae, Vasconcelos et al. 2019). Notably, more than 50% of all known bee species (over 10,000) are capable of buzz pollination (Cardinal et al. 2018). As a result, Melastomataceae are exposed to a broad diversity of buzzing bees, providing ample opportunities for floral divergence driven by adaptation to different bee species, without necessitating a shift to a different functional pollinator group (Vogel 1975, Renner 1989). The high disparity of the "buzz-bee" syndrome is also reflected by the highest partial disparity among the four pollination syndromes across floral organ modules. Given that "buzz-bee" pollination syndromes occur across the entire elevational gradient of the Melastomataceae distribution and span a wide range of environmental niches (Kopper et al. 2025), these species have been subjected to diverse selective pressures (i.e., shifts in selection regimes) resulting in high disparity across all four floral modules over time. In particular, divergent selection driven by locally subtle variations in pollinator communities (e.g., along elevational gradients) has been proposed as a key mechanism underlying these patterns of high disparity (Macior 1971, Dellinger et al. 2019b, Wenzell et al. 2023) in the absence of pollinator shifts (Vogel 1975, Renner 1989, Kopper et al. 2024). While adaptation to distinct buzzing bees may help explain the high floral disparity observed in buzz-bee pollinated Melastomataceae, it does not account

for why other large, buzz-pollinated lineages, such as *Solanum* exhibit much lower floral disparity. A key feature distinguishing Melastomataceae from the low-disparity *Solanum*-type flower (Symon 1979, Endress, 1996) is the loosely arranged androecium in the former taxon (Melo et al. 2022). The tightly arranged anther cones of *Solanum*-type flowers are known to release larger amounts of pollen and place it more accurately than loosely arranged stamens (Vallejo-Marín & Vallejo 2021). This highly efficient pollination mechanism may have constrained *Solanum*-type flowers within a narrow phenotypic range. In contrast, the loosely arranged stamens of Melastomataceae may have facilitated pollen placement on different parts of bees' bodies, paired with refined pollen dosing, thereby promoting phenotypic differentiation.

While we found the highest disparity in the ancestral "buzz-bee" syndrome, the highest rate of evolution was associated with tribes where some species have shifted pollinators. One explanation for this discrepancy between disparity and rate of evolution could be that the high disparity found in bee-pollinated species has likely accumulated slowly over time. In general, we found that rates of evolution vary across the floral phenotype, and among Melastomataceae lineages, with shifted tribes evolving faster than tribes which remained bee pollinated. Pollinator shifts represent changes in the selective regime and are typically accompanied by floral trait modifications (Schiestl & Johnson 2013, Gervasi & Schiestl 2017, Schiestl et al. 2018, Mackin et al. 2021), often resulting in accelerated rates of evolution (Simpson 1944, Pagel 1994, Lee et al. 2013). Our findings further suggest that all floral modules experienced strong disruptive selection in tribes where pollinator shifts have occurred, as reflected in the elevated evolutionary rates across the four floral modules of these tribes. Conversely, in tribes that remained bee-pollinated, only corolla traits and androecium traits appear to have been subject to such selective pressure. In the "buzz-bee" syndrome, the androecium evolved most rapidly, likely due to slightly divergent selection from locally variable bee communities (Macior 1971, Dellinger et al. 2019b, Wenzell et al. 2023, Kopper et al. 2024) or the use of a broad range of bees differing in size and behavior (Vogel 1975, Renner 1989, Dellinger et al. 2019, Kopper et al. 2024). In contrast, the "nectar-foraging vertebrate" syndrome showed the highest rates in the corolla, reflecting adaptations in corolla shape and color, likely to enhance pollinator attraction and fit (Dellinger et al. 2019, Kopper et al. 2024), like shown in other systems (Mucchala 2007, Muchhala & Thomson 2009, Campos et al. 2015). The "food-body-foraging vertebrate" syndrome also showed fast androecium evolution, likely explained by the origin of complex stamen structures that function both as rewards and pollen-release mechanisms (Dellinger et al. 2014). In the "generalist" syndrome, corolla traits evolved the fastest, resulting in various corolla shapes ranging from *Solanum*-like floral forms to highly reduced corollas

(Kopper et al. 2024). Bee-pollinated species from lineages where pollinator shifts occurred experienced elevated evolutionary rates across all floral modules, likely due to ecological changes at high elevations, including cooler and wetter climates and thus reduced insect availability (Cruden 1972, Warren et al. 1988, Primack & Inouye 1993, Dellinger et al. 2021, Kopper et al. 2025). Mountain colonization may have imposed strong selection to either adapt to montane bee pollinators (hence retaining the ancestral buzz-bee syndrome) or facilitated shifts to other pollination strategies. In contrast, bee-pollinated species from lineages that remained bee-pollinated faced fewer ecological shifts (Kopper et al. 2025), and minor adjustments in the corolla and androecium were sufficient to remain bee-pollinated.

We also found that the highest rates of floral evolution occur near the tips of the phylogeny. In contrast, literature often reports elevated evolutionary rates early in clade history, consistent with the idea that opportunities to explore novel niches tend to decline over time (Mahler et al. 2010). However, most species in our dataset are distributed across geologically young mountain systems that underwent their final uplift phases during the mid to late Miocene (15–5 mya), including the tropical Andes (Boschman & Condamine 2022), New Guinea (Roycroft et al. 2022), the East African Mountains (Dagallier et al. 2022), and the ancient Western Ghats (Johnson et al. 2022). These recent geological events likely provided new ecological opportunities for diversification in Melastomataceae relatively late in the clade's history, despite its high crown node age (68.5–75.2 mya, based on the ML topology of Penneys et al. 2022), potentially explaining the observed increase in evolutionary rates towards the present. The simultaneous increase in androecium and corolla evolution rates in shifted tribes and bee-pollinated species of shifted tribes suggests these modules evolve in a coordinated manner under conditions favoring pollinator shifts (Kopper et al. 2025). In contrast, functional and gynoecium modules tend to evolve more independently, consistent with previous findings (Wagner et al. 1996, Hansen 2003, Ordano et al. 2008, Claverie et al. 2013, Felice & Goswami 2018, Larouche et al. 2018). Functional traits tend to evolve earlier than other floral modules and decline toward the present in lineages with pollinator shifts, underscoring their role in distinguishing pollination syndromes (Dellinger et al. 2019b, Kopper et al. 2024). Overall, shifts in evolutionary rates in Melastomataceae often align with mountain uplift, supporting a link between pollinator shifts and the colonization of montane environments in Melastomataceae (Kopper et al. 2025).

The association between mountain environments and pollinator shifts that we report in Melastomataceae is not unique to this family, but mirrors patterns observed across a wide range of Neotropical plant clades. In these groups, bee-pollinated species are typically found at lower elevations, while their vertebrate-pollinated relatives tend to occupy montane habitats such as

the Andes (e.g., Bromeliaceae, Gesneriaceae, *Psychotria*, *Passiflora*, Loranthaceae, *Salvia*; Dellinger et al. 2023). Our finding that pollinator shifts begin to occur at around 1000 meters aligns well with empirical studies showing that the harsher climatic conditions at higher elevations, such as lower temperatures and more variable weather, significantly reduce bee abundance and diversity (Cruden 1972, Brito & Sazima 2012, McCallum et al. 2013, Classen et al. 2015, Cozien et al. 2019, Classen et al. 2020, Dellinger et al. 2021). These results support the pollinator-shift scenario proposed by Thomson & Wilson (2008), which suggests that declining visitation rates by ancestral bee pollinators can destabilize long-established plant–pollinator interactions, thereby facilitating shifts to alternative pollinator groups. We also found that shift into mountains typically occur before pollinator shifts, which supports the idea that niche expansion into novel environments preceded pollinator shifts. Our reconstruction of the elevational history of Melastomataceae revealed that repeated colonization of uplifting mountains within the past 20-30 my was followed by shifts away from bee pollination commonly only within the past 10 my. With the generally reduced availability of bee pollinators in mountain environments (Arroyo et al. 1982, Classen et al. 2020), successive niche filling might have intensified competition for the already reduced montane bee pollinators, facilitating shifts to vertebrate pollination and generalists (Armbruster 1985). While mountain environments likely initially triggered pollinator shifts, shifts to efficient high elevation pollinators such as hummingbirds, sunbirds and white-eyes allowed for subsequent niche expansion to even higher elevations in tropical mountains.

We further showed that, in addition to pollinator shifts, adaptive evolution of floral traits may help mitigate the negative effects of reduced ancestral pollinator availability. We observed that montane bee-pollinated Melastomataceae species tend to exhibit increased petal lengths and larger anther pore sizes. Larger floral displays are known to enhance pollinator attraction and may increase the likelihood of floral visitation (Dafni et al. 1997, Goodwillie et al. 2010). In addition, species may adjust their pollen release strategies in response to pollinator availability, releasing larger quantities of pollen per visit when pollinator encounters are infrequent (Castellanos et al. 2006). In this context, the observed increase in anther pore size with elevation in Melastomataceae may represent an adaptive response to the reduced frequency of bee visits in montane environments, ensuring pollen transfer during these rare interactions.

In conclusion, this thesis demonstrates that pollination syndromes, when based on ecologically meaningful and functionally relevant traits, can be powerful tools for investigating floral macroevolution. By combining trait-based machine learning approaches with phylogenetic comparative methods, we uncovered contrasting patterns of disparity and evolutionary rate across pollination strategies in Melastomataceae. High floral disparity in the ancestral “buzz-

bee" syndrome appears to result from long-term diversification under subtle, localized selective pressures, whereas recent pollinator shifts in montane environments are associated with rapid evolutionary change across the entire floral phenotype. These findings underscore the interplay between ecological opportunity, pollinator availability, and adaptive trait evolution in shaping the remarkable floral diversity of this clade and highlight how mountain uplift and pollinator turnover have jointly driven floral diversification in tropical ecosystems.

Abstract

Angiosperm flowers have a large spectrum of animal pollinators including among others bees, flies, birds, and bats. Shifts between different functional groups of pollinators (e.g., from bees to hummingbirds) are thought to be key drivers of floral morphological diversity (disparity). Different functional pollinator groups are selecting for different floral traits, with distinct trait combinations commonly summarized as pollination syndromes. While pollination syndromes are a useful tool also for predicting pollinators for species lacking observations, their accuracy remains debated, and they are rarely applied to test whether floral diversification indeed associates with pollinator shifts. While it is well-documented that pollinator shifts may lead to drastically different floral phenotypes, the evolutionary process of floral diversification, i.e., whether certain parts of the flower evolve and adapt more quickly than others, have remined unclear so far. Furthermore, the actual mechanisms leading to pollinator shifts had not been clarified until now. Across environmental gradients, the distribution of pollination syndromes changes, with repeated transitions from bee pollination in tropical lowlands to vertebrate pollination in tropical mountains. However, few studies have evaluated whether shifts into these novel mountain environments preceded pollinator shifts or were a consequence of them. Furthermore, it has remained unclear until now whether adaptive evolution of floral traits maximizing pollinator attraction and pollen transfer may enable species to retain ancestral pollinators even in environments conducive to pollinator shift.

It was the main goal of my PhD thesis to integrate results from comparative morphology, machine learning, multivariate statistical modelling and comparative phylogenetic methods to better understand the evolution of floral disparity and pollination syndromes. Using classification models trained and validated on 44 functional floral traits across 252 Melastomataceae species with empirical pollinator observations, I confirmed four well-differentiated pollination syndromes for the family: "buzz-bee", "nectar-foraging vertebrate", "food-body-foraging vertebrate", and "generalist". Multivariate statistics and phylogenetic comparative analyses revealed that the ancestral, most species-rich 'buzz-bee' syndrome is the most disparate one, indicating that high floral disparity can evolve in the absence of pollinator

shifts. Additionally, disparity differs among floral organ modules and pollination syndromes. Buzz-bee-pollinated species exhibit the highest disparity in androecium traits, whereas species which shifted pollinators show the greatest disparity in corolla traits. My results further revealed that in tribes where pollinator shifts occurred, all four floral modules were subject to strong disruptive selective pressure, as evidenced by the elevated evolutionary rate of the entire phenotype and high rates of evolution across the floral modules. In contrast, only the corolla and functional traits experienced such high evolutionary rates in tribes that retained bee pollination. Using reconstructions of the evolutionary history of 333 Melastomataceae species, I could show that mountain colonization preceded and repeatedly triggered shifts away from bee pollination toward vertebrate and generalist pollination across the Melastomataceae phylogeny. Also, I found that traits potentially facilitating pollen transfer, such as larger petal and pore sizes, were associated with montane environments, possibly enabling bee-pollinated species the initial colonization of mountains and, in some cases, retention of bee pollination in warmer montane areas. By identifying environments conducive to pollination shifts, my results do not only provide a much-needed hypothesis for mechanisms underlying the evolution of different pollination systems but also confirm its validity through empirical testing.

Zusammenfassung

Blütenpflanzen (Angiospermen) werden von einer Vielzahl tierischer Bestäuber besucht, darunter Bienen, Fliegen, Vögel und Fledermäuse. Wechsel zwischen verschiedenen funktionellen Bestäubergruppen (z. B. von Bienen zu Kolibris) gelten als zentrale Triebkräfte für die morphologische Vielfalt (Disparität) von Blüten. Verschiedene funktionelle Bestäubergruppen selektieren für unterschiedliche Blütenmerkmale, wobei bestimmte Merkmalskombinationen als Bestäubungssyndrome zusammengefasst werden. Obwohl Bestäubungssyndrome ein nützliches Instrument zur Vorhersage potenzieller Bestäuber bei Arten ohne direkte Beobachtungen darstellen, ist ihre Genauigkeit umstritten. Weiters werden Bestäubungssyndrome selten verwendet, um zu testen, ob die Diversifikation der Blüte tatsächlich mit Bestäuberwechseln einhergeht. Es ist gut dokumentiert, dass Bestäuberwechsel zu drastisch unterschiedlichen Blütenphänotypen führen können**,** jedoch bleibt unklar, wie der evolutionäre Prozess der Blütendiversifikation im Detail abläuft, also ob bestimmte Blütenteile schneller evolvieren und sich anpassen als andere. Zudem sind die zugrunde liegenden Mechanismen, die zu Bestäuberwechseln führen, bislang wenig verstanden. Entlang von Umweltgradienten verändert sich die Verteilung von Bestäubungssyndromen: Wiederholt lassen sich Übergänge von Bienenbestäubung in tropischen Tieflagen zu Wirbeltierbestäubung in tropischen Gebirgen beobachten. Es war jedoch unklar, ob diese Besiedlung tropischer Gebirge den Bestäuberwechseln vorausging oder deren Folge war. Ebenso war bis jetzt unklar, ob die adaptive Evolution floraler Merkmale zur Maximierung von Bestäuberanziehung und Pollenübertragung es Arten ermöglichen kann, ihre ursprünglichen Bestäuber auch in einer Umgebung zu behalten, die eigentlich einen Bestäuberwechsel begünstigen würden.

Ziel meiner Dissertation war es, Erkenntnisse aus vergleichender Morphologie, maschinellem Lernen, multivariater Statistik und phylogenetischen Vergleichsmethoden zu integrieren, um die Evolution der Blütenvielfalt und Bestäubungssyndromen besser zu verstehen. Mithilfe von Klassifikationsmodellen, die auf 44 funktionellen Blütenmerkmalen und empirischen Bestäuberbeobachtungen für 252 Arten der Familie Melastomataceae trainiert und validiert wurden, konnte ich vier klar unterscheidbare Bestäubungssyndrome identifizieren: „Vibrationsbestäubende Bienen“, „Nektarsammelnde Wirbeltiere“, „Futterkörpersammelnde Wirbeltiere“ und „Generalist“. Das evolutionär ursprüngliche, artenreichste „Vibrationsbestäubende Bienen“-Syndrom ist zugleich das mit der größten Disparität – ein Hinweis darauf, dass hohe Blütenvielfalt auch ohne Bestäuberwechsel entstehen kann. Zudem konnte ich zeigen, dass es Unterschiede in der Disparität zwischen Blütenorganmodulen und Bestäubungssyndromen gibt: Arten, die von vibrationsbestäubenden Bienen bestäubt werden, zeigen die größte Disparität bei Staubblattmerkmalen, während Arten, die ihre Bestäuber

wechselten, die höchste Disparität bei Kronblattmerkmalen aufweisen. Meine Ergebnisse zeigen außerdem, dass in Triben, in denen Bestäuberwechsel stattfanden, alle vier Blütenodule unter starker disruptiver Selektion standen, was sich in erhöhten Evolutionsraten des gesamten Phänotyps und über alle Blütenodule hinweg widerspiegelt. Im Gegensatz dazu waren in Triben, die Bienenbestäubung beibehielten, nur die Krone und funktionelle Merkmale diesem Selektionsdruck ausgesetzt. Mithilfe der Rekonstruktion der Evolutionsgeschichte von 333 Arten der Melastomataceae konnte ich weiters zeigen, dass Gebirgsbesiedlungen zeitlich vor den Bestäuberwechseln stattfanden und diese wiederholt auslösten – hin zu Wirbeltier- oder generalistischer Bestäubung. Darüber hinaus ergab sich, dass bestimmte Merkmale, die möglicherweise die Pollenübertragung erleichtern (z. B. größere Kronblätter und Poren), mit Berglebensräumen assoziiert sind. Diese könnten es bienenbestäubten Arten ermöglicht haben, zunächst in Gebirgsregionen einzuwandern und in wärmeren Gebirgsbereichen sogar ihre Bienenbestäubung beizubehalten. Indem ich Umweltbedingungen identifiziert habe, die Bestäuberwechsel begünstigen, liefern meine Ergebnisse nicht nur eine dringend benötigte Hypothese zu den zugrunde liegenden Mechanismen der Evolution unterschiedlicher Bestäubungssysteme, sondern bestätigen diese auch durch empirische Tests.

References not included in chapter 1 - 3

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