Pollinator specialisation fails to explain rapid speciation in terrestrial orchids

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Abstract

Pollinator attraction is central to the reproductive biology and ecology of flowering plants, and pollinator specialisation has long been thought of as a driving force of species generation. Orchids are central to this idea, which dates back to Darwin's work on pollinator-driven floral evolution. However, most macroevolutionary evidence for the speciation hypothesis comes from studies of genera or tribes, leaving broad-scale patterns unresolved. Here, we reconstruct the evolution of pollination strategy in the species-rich terrestrial orchid subfamily Orchidoideae, and test whether speciation rate is shaped by pollinator specialisation. We identify numerous transitions among reward-based, deception-based, and autonomous pollinator attraction strategies, but along evolutionarily constrained pathways. Curiously, we find that speciation rates are not significantly impacted by pollinator specialisation, a result that is robust to differences both in methodological approaches and definitions of specialisation. Despite shaping ecological interactions and microevolutionary divergence, pollinator specialisation does not influence rates of speciation on macroevolutionary scales. Our findings support a growing view that the origins of plant biodiversity involve complex interactions between traits, ecological opportunities, and environmental contexts, rather than by single force in isolation.

Main

Orchids are one of the most speciose angiosperm families, with some ~29,500 species found in nearly every terrestrial ecosystem [1–3]. They are also among the most celebrated, having held the attention of evolutionary biologists since Darwin's early work on their relationships with pollinators [4]. Darwin hypothesised that the adaptation of floral traits in response to pollinator interactions could drive reproductive isolation, leading to speciation into numerous forms. A great deal of orchid variation, especially floral morphology, is linked to their extraordinary variety of specialised pollination strategies [5–8]. These range from mutualistic interactions involving nectar, oils, or sleep-site rewards, to deceptive strategies like food mimicry, brood-site deception, and sexual deception [5,9], and attract diverse pollinators, predominantly insects. One iconic example of the latter is the sexually deceptive *Ophrys*, which trick male bees and wasps into pollination by having flowers that resemble female insects, and by mimicking female pheromones [10,11]. However, whether the divergences associated with specialised pollination adaptations scale to shape broad macroevolutionary patterns remains uncertain.

Pollinator specialisation has long been proposed as a mechanism for driving diversification, particularly in genera where small shifts in floral traits may lead to rapid reproductive isolation [12,13]. Population divergence is expected to follow transitions between pollinators and pollination strategies, as floral traits, pollinator behaviour, and patterns of pollen movement are altered by coevolutionary interactions [14–17]. If these microevolutionary processes repeatedly generate divergence, leading to speciation, their accumulation through time will accelerate diversification at broader taxonomic scales [18,19]. This model remains influential in investigations into the origins of diversity in orchids [20–24], other flowering plant clades [25–27], and across angiosperms more broadly [28–30]. However, evidence for this pattern across larger orchid groups is mixed. Some studies suggest a link between pollinator specialisation and speciation rates [12,13], while others suggest other factors, such as geographical shifts and environmental change [2,31–34], and adaptive traits such as epiphytism and CAM photosynthesis (in Epidendroids [20,21]) are more powerful predictors of diversification.

However, pollinator specialisation is not a single or consistently defined concept. At broad macroevolutionary scales, specialisation is often considered in terms of pollination strategies such as sex deception and pollen reward [5,6,13,20,35]. Similarly, specialisation can be expressed through pollination syndromes, which are shared suites of floral traits that are associated with particular pollinator groups, such as Hymenoptera and Lepidoptera [17,36–38]. At the finest ecological scales, specialisation presents as highly specific interactions between plants and pollinators, including one-to-one species relationships [6,39–41]. These highly-specific relationships characterise a large portion of orchid species [5]. Perhaps the best known is *Angraecum sesquipedale*, which inspired Darwin’s famous prediction of a hawkmoth with a very long tongue, whose existence was later confirmed [42]. These different concepts of specialisation capture distinct evolutionary processes and consequently, evaluating the role of specialisation in orchid diversification requires testing different definitions.

One challenge in identifying the correlates of orchid diversification is data limitations. Phylogenies of large lineages (family or sub-family) that are well sampled at the species level, critical for analyses characterising diversification rate heterogeneity [43], have only recently become available [2,31]. As a result, most previous research into pollinator evolution has focussed on smaller lineages, such as individual genera or tribes [12,13]. It is worth noting that the taxonomic sampling still remains incomplete, especially compared to animal clades such as the mammals and birds, for which complete phylogenies are available [44,45]. However, methods for detecting rate-heterogeneity and trait-dependent diversification can accommodate incomplete and even imbalanced phylogenetic sampling [43,46–50]. This allows for inference of broad-scale patterns when data are sparse, provided taxonomic coverage is representative and sampling biases are modelled appropriately. Similarly, data on pollinator variation has historically had poor coverage across the diversity of orchid lineages, a problem that is also encountered in other families [51–53]. However, a recent compilation of pollinator data has been published which samples the majority of orchid genera [5]. This compilation records different levels of specialisation including pollinator strategy, pollinator identity such as Lepidoptera, as well as numbers of pollinators for orchids, at species level. The taxonomic coverage at species level is ~10%, which is similar to datasets for other plant families [26,51,54], and is comparable to datasets successfully used to explore pollinator-dependent diversification, in orchids [20] and other families [26]. Although there are taxonomic gaps, there is now the framework for investigating the macroevolution of pollination in the orchids on the largest scales.

The subfamily Orchidoideae (~5,000 species) are an ideal study system for this hypothesis. Unlike their sister subfamily Epidendroideae, in which rapid diversification is associated with epiphytism and Crassulacean acid metabolism (CAM) photosynthesis [20,21], Orchidoideae are predominantly terrestrial; are extremely widespread, especially in the extratropical regions [31]; and are characterised by exceptional diversity in pollination strategies, having evolved both reward-based and deceptive strategies [5]. Furthermore, many genera, such as *Gymnadenia*, *Herminium*, *Ophrys* and *Orchis* were the subjects of Darwin's early observations underlying his coevolutionary hypothesis [4]. Understanding the evolution of pollination strategy diversity, and impacts on diversification dynamics, is critical for fundamental evolutionary knowledge, but also for conservation. As global pollinator populations continue to decline [55,56], the diversity of pollinator-dependent orchids is threatened [57]. By understanding how past evolutionary dynamics have, or have not, changed in response to pollinator shifts, we may improve our ability to predict how orchids, many of which are threatened with extinction [3], will fare in the future.

Here, we investigate the evolutionary dynamics of pollination strategy in terrestrial orchids. Using a large phylogeny [31] and a curated dataset of pollination [5], we reconstruct the evolution of pollination strategies, and test for associations between diversification rates and different types of specialisation at the levels of pollination strategy, taxonomic identity of pollinators, pollinator number and functional specificity. To do this, we use character-free diversification methods that are designed to accommodate incomplete and imbalanced taxonomic sampling [46,47]. Our results reveal frequent but constrained transitions among pollination strategies, but no consistent relationship between specialisation and speciation rate. These findings suggest that while pollinator specialisation contributes to ecological and reproductive diversity, it plays a limited role in shaping macroevolutionary patterns of diversification across terrestrial orchids.

Results

Evolutionary model and ancestral states

We investigated the evolutionary gains and losses of pollination strategies in a taxonomically comprehensive sample of Orchidoideae, comprising 485 species in 88 genera (~10% of species and ~43% of genera [1]). We reconstructed ancestral states after comparing support for six different transition models (equal rates (ER), symmetrical (SYM) and all-rates-different (ARD)), all with and without hidden rates [58]. The best-supported model was ARD without hidden states (AIC weight ~ 1), indicating variation and asymmetry in transition rates among strategies. Ancestral states of the deepest nodes were relatively weak; the highest supported root state is lipid reward (root state probability 0.47), and there is weaker support for other strategies. The earliest branches are predominantly reward-based, with nectar reward in branches leading to tribes Diurideae, Codonorchideae and Cranichideae and lipid reward in branches leading to tribe Orchideae. Transition counts among pollination strategies were highly asymmetric (Supp Mat), with the most frequent from nectar reward to autonomous selfing (median # transitions = 51), followed by from food deception to nectar reward (median n = 30), then autonomous selfing to food deception (median n = 19). Transitions into sex deception were rare, with low counts from food deception (median n = 3) and autonomous selfing (median n = 9). Notably, lipid reward appears evolutionarily constrained, with transitions away from it (median n = 12) but none toward it, consistent with its early origin and subsequent persistence within Orchideae.

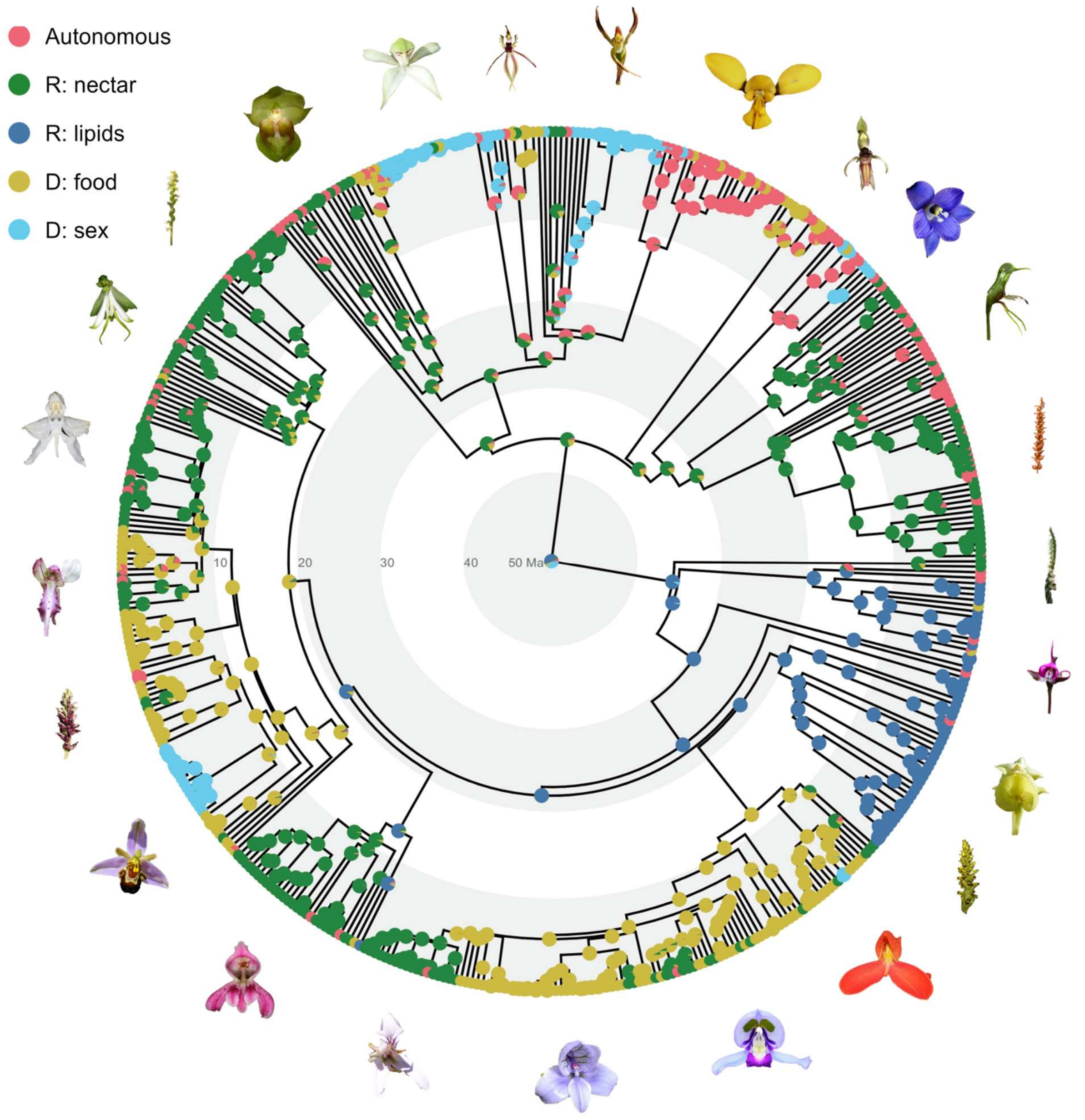

Figure 1. Phylogenetic distribution of pollinator attraction strategies in Orchidoideae. *Tips and nodes are coloured by pollination strategy: autonomous selfing/agamospermy (red), nectar reward (green), lipid reward (dark blue), food deception (yellow), and sex deception (light blue). Pie charts at internal nodes represent posterior probabilities of ancestral states based on 300 stochastic character maps estimated with an all-rates-different transition model, using corHMM* [58]*. The root state is uncertain, with highest support for a lipid reward strategy. Flower photographs are provided under Creative Commons licences, sourced primarily from Flickr and Wikimedia Commons (details in Supp Mat), and are positioned approximately at their respective species or genera in the tree, but spaced for visual clarity.*

Directional transitions and trait lability

Given the uncertainty in ancestral states, we further explored transition rates using an ARD reversible-jump MCMC framework, which, unlike corHMM models, incorporates rate uncertainty and infers support for whether transitions actually occurred [59]. Results suggest that certain strategies, especially food deception and sex deception, are relatively labile and have transitioned to several other states. Autonomous selfing is also evolutionary labile and transitions to food and sex deception. In contrast, reward-based strategies, particularly lipid, are more constrained. Lipid reward only significantly transitions to autonomous selfing, and no strategies transition to lipid reward at a significant rate. Nectar reward similarly shows low lability, with significant transitions only to autonomous selfing, although several strategies transition to nectar reward.

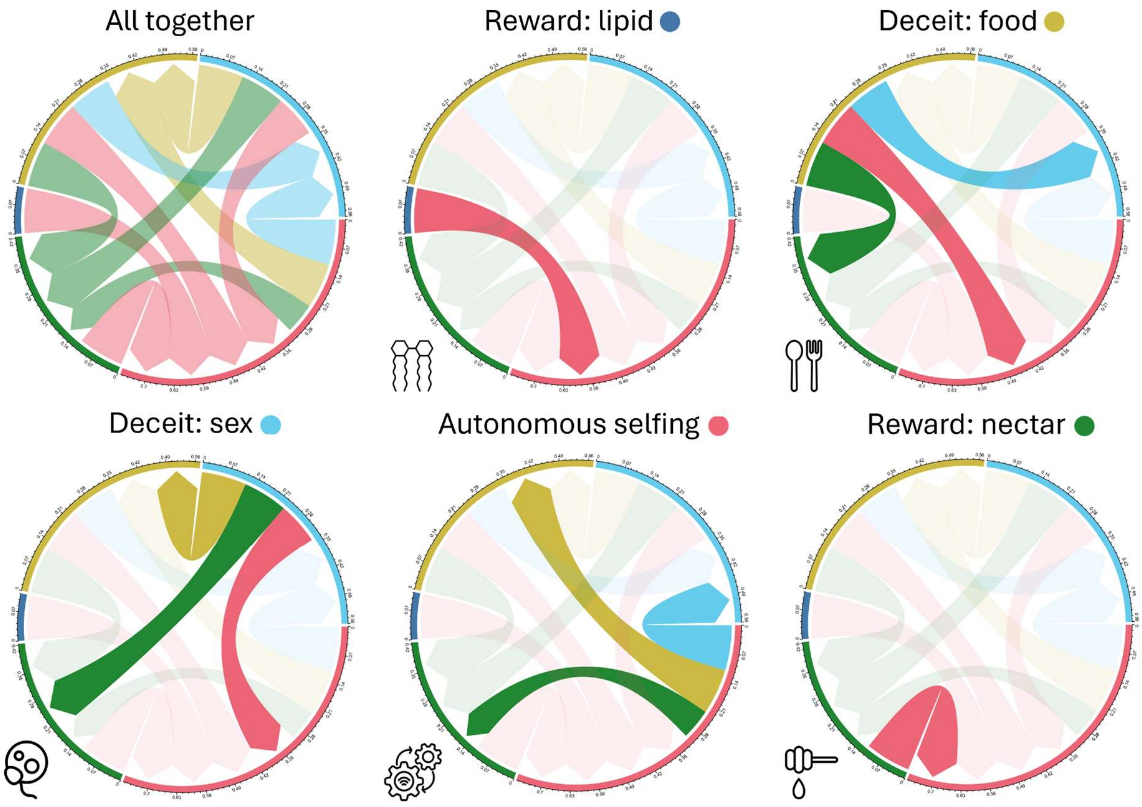

Figure 2. The complex evolution of diverse pollination strategies in Orchidoideae. *Reward-based strategies are less evolutionarily labile than deception-based strategies, and autonomous selfing is a common macroevolutionary destination. (Top left) A chord diagram showing all significantly non-zero pairwise transition rates among pollination strategies, estimated using an all-rates-different model with reversible-jump MCMC. Chord widths are proportional to posterior median transition rates. Following this are individual plots of directionality of transitions from each focal strategy, individually. Icons are provided under Creative Commons licences, sourced from The Noun Project (details in Supp Mat).*

Speciation rates are not strongly shaped by pollination strategy

We tested whether pollinator attraction strategies are associated with differences in tip-speciation rate estimated with two character-independent diversification methods (BAMM [46] MiSSE [47]) (Fig. 3). Rates are heterogeneous across both methods, but associations with strategies are not significant. Species with the lipid reward strategy exhibited the lowest average tip speciation rates with narrow variance (BAMM mean = 0.25, SD = 0.07; MiSSE mean = 1.23, SD = 0.86). In contrast, sex-deceptive species had the highest average speciation rates but with broad variance (BAMM mean = 2.98, SD = 1.85; MiSSE mean = 3.18, SD = 2.16). Species with autonomous selfing, food deception, and nectar reward have intermediate mean speciation rates, broad variances, and heavily overlapping distributions (Fig. 3). The STRAPP test using BAMM rates was insignificant, when treating the small number of polymorphic combinations as distinct states (e.g., autonomous selfing with nectar reward) (Kruskal-Wallis $p = 0.091$). Similarly, a PGLS on MiSSE speciation rates also did not differ significantly (for most comparisons, $p > 0.44$; one marginally significant effect at $p = 0.048$ that is unlikely to be robust given multiple comparisons and very low explanatory power, $R^2 = 0.02$). We also found no significant impacts after removing polymorphic species (BAMM Kruskal-Wallis $p = 0.11$; MiSSE $p > 0.15$, in all cases), when binarising each strategy (BAMM Mann-Whitney $p > 0.05$; MiSSE $p > 0.05$, in all cases), and when categorising species broadly, comparing autonomous selfing versus reward-based versus deception-based strategies (BAMM Kruskal-Wallis $p = 0.14$; MiSSE $p > 0.91$, in all cases) (Supp Mat). The bimodal distribution of BAMM speciation rates among sex-deceptive species is shaped by monotypic and small genera such as *Leporella* and *Spiculaea*.

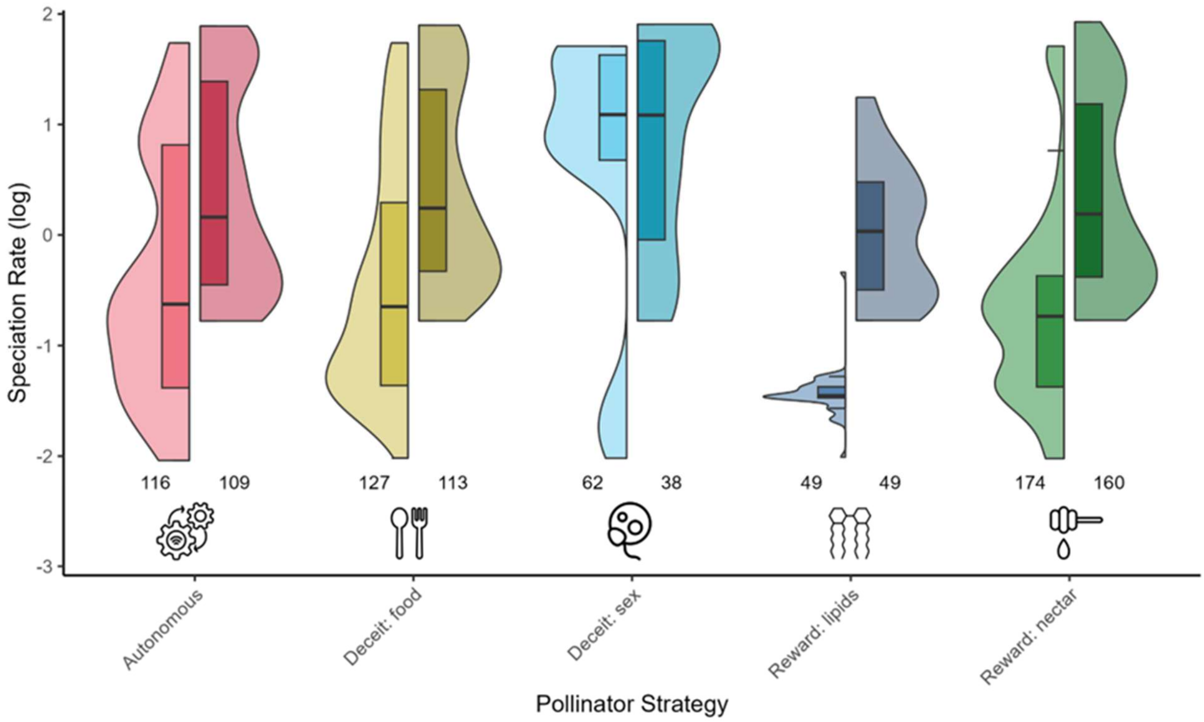

Figure 3. Speciation rates vary across pollination strategies but differences are not statistically significant. *Half-violin plots show the distribution of log-transformed tip speciation rates estimated using BAMM (left) and MiSSE (right), grouped by pollination strategy. Speciation rates differ due to different underlying assumptions between the methods, especially regarding accounting for incomplete sampling. Sample sizes are indicated below half-violins. Sample sizes differ because we pruned some short branches before analysis with MiSSE, to avoid issues associated with formal SSE models. While species with sex deception tend to have higher median rates and those with lipid rewards lower, statistical tests indicate that differences among strategies are not significant ($p > 0.05$), when background rate heterogeneity and effects of shared ancestry are accounted for. Icons are provided under Creative Commons licences, sourced from The Noun Project (details in Supp Mat).*

Pollinator specificity and identity are not associated with diversification rates

We tested whether speciation rates are shaped by pollinator number, functional pollination specificity (FPS, defined as the number of pollinator families multiplied by the number of pollinator orders [5]), broadly-discretised specialisation level (generalist versus specialist versus autonomous), and dominant pollinator order (Hymenoptera and Lepidoptera). Across all tests, we found no significant association with speciation rates (Fig. 4). Pollinator number and FPS (Fig. 4a) showed wide variation across species but did not correlate with tip speciation rates. Neither significantly predicted speciation (BAMM pollinator number Spearman $p = 0.81$, MiSSE $p = 0.99$; BAMM FPS $p = 0.80$; MiSSE $p = 0.69$), including when excluding autonomous selfing species with zero pollinators (BAMM number $p = 0.65$, MiSSE $p = 0.94$; BAMM FPS $p = 0.76$, MiSSE *p* = 0.73). Similarly, speciation rates did not differ significantly among autonomous selfing, generalist, and specialist species (BAMM Kruskal-Wallis, *p* = 0.82; MiSSE *p* > 0.89, in all cases) (Fig. 4b). Nor is there a significant impact of pollination by Hymenoptera (BAMM Mann-Whitney, *p* = 0.84; MiSSE *p* = 0.93) (Fig. 4c), or Lepidoptera (BAMM Mann-Whitney, *p* = 0.87; MiSSE *p* = 0.87) (Fig. 4d).

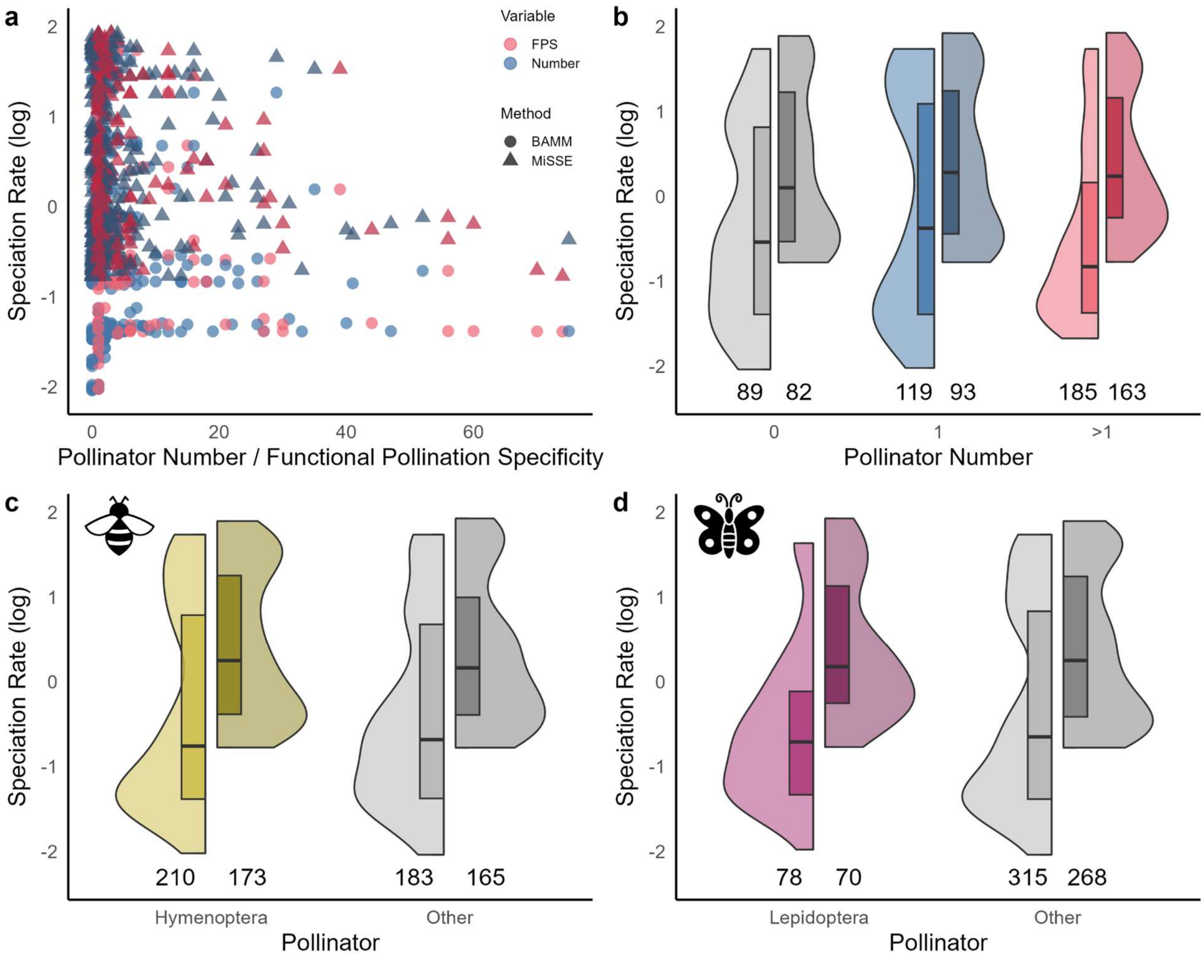

Figure 4: Speciation rates are not significantly influenced by pollinator specialisation. *(a) Scatterplot showing no correlation between speciation rate and either pollinator number or functional pollinator specificity. (b) Violin plots comparing generalist and specialist species (autonomous selfing species are not shown but were included in statistical tests). Speciation rates by Hymenoptera versus others (c), and Lepidoptera versus others (d). Statistical tests for all comparisons were non-significant (p > 0.05). Icons are provided under Creative Commons licences, sourced from The Noun Project (details in Supplementary Information).*

Discussion

Orchids have long captured the attention of evolutionary biologists and horticulturalists with their remarkable floral adaptations [1,2,4,5,20,22]. The idea that pollination drives orchid diversification dates back to Darwin, who linked orchid variation to interactions with insect pollinators [4]. Later work expanded this to a broader theory of pollinator-driven speciation across angiosperms [13,60], which remains a powerful hypothesis. In our study, by analysing the most comprehensive available phylogeny of any orchid subfamily [31] and pollinator dataset [5], we find no strong link between speciation and pollinator specialisation, a result that is robust to methodological differences. Our results contradict long-standing expectations that pollinator-mediated microevolutionary divergences accumulate to shape macroevolution [18,19]. We suggest that other factors, such as environmental forces and complex ecological interactions, may play a larger role, the latter of which is a hypothesis gaining strength in different taxa [5,20,31,61–65].

Transition patterns reflect ecological lability and evolutionary constraint

Pollination strategies in terrestrial orchids are evolutionarily labile, showing frequent transitions among strategies and ecological flexibility. However, transitions are asymmetric, suggesting that floral evolution is constrained [66–68]. Some strategies, especially food deception, nectar reward, and autonomous selfing, are common evolutionary destinations. In contrast, lipid reward and sexual deception states are rarely gained. These patterns likely reflect a combination of developmental constraints, physiological trade-offs, and ecological selection pressures [69,70]. Reward-based strategies such as nectar and lipid production often involve substantial honest investment in pollinator attraction, and may be more advantageous in environments with reliable pollinator communities. Once lost, these strategies may be difficult to regain, especially if alternatives such as deception or selfing provide adequate reproductive success. In contrast, deceptive strategies may offer a lower-cost method of dishonest pollinator attraction [41,69], able to evolve without the same level of investment into nectar or lipid production. The relative rarity of sexual deception may reflect the greater ecological specificity involved when flowers mimic female bees [10,11], while the infrequent gains of lipid reward are explained by historical contingency. It may have been ancestral in the entire subfamily and remains stable within tribe Orchideae.

Transitions to autonomous selfing are common, although the specific origin strategies differ by method. Given that up to 88% of orchids are self-compatible [5], autonomous selfing may be a readily accessible and low-cost strategy when pollinators are scarce or unreliable. Asymmetric transitions may therefore reflect both physiological costs of floral evolution and ecological selection for reproductive success. However, it must be noted that this pattern could also arise from data scarcity. As Ackerman et al. [5] acknowledge, a substantial proportion of species classified as autonomously selfing were scored based on indirect evidence, such as high fruit set without observed pollinators, rather than experimental exclusion trials. In fact, only 44% of the species in the total dataset assigned to autonomous selfing or agamospermy were supported by experimental data. This introduces the possibility that the prevalence of autonomous selfing may be overestimated, especially when recorded from fieldwork or short-term studies, where pollinators may simply go undetected. Given that many orchids are visited infrequently and require extended observation to confirm pollination, caution is warranted in interpreting the evolutionary accessibility or prevalence of this particular strategy.

Pollination specialisation is not a universal driver of diversification

Our results challenge long-standing hypotheses that pollination specialisation, assessed under different definitions covering broad and finer scales, promotes orchid speciation. This is difficult to reconcile with decades of theory on pollination-mediated divergences, but supports findings that orchid macroevolution is shaped by other forces beyond pollination [50]. While important examples of rapid speciation through pollinator shifts have been documented in iconic genera such as *Ophrys* [12] and *Disa* [13,35], these appear clade-specific rather than universal processes. In our results, this clade-specific pattern is observed comparing speciation rates in larger sex deceptive genera against monotypic and depauperate genera, such as *Leporella* and *Spiculaea*. While some deceptive species certainly do speciate more rapidly, this is not a universal pattern [35].

The absence of a consistent effect of pollinator specialisation on speciation is consistent with patterns reported elsewhere in Orchidaceae, using much sparser data. Using a sparse phylogenomic reconstruction (sampling ~0.68% of species), Givnish et al. [20] found that although deceit pollination increased overall richness of orchids, it was not associated with accelerated diversification. Similarly, they did not detect a significant effect of either pollination by Hymenoptera or Lepidoptera. Givnish et al. [20] instead found strong impacts of pollinia, epiphytism, CAM photosynthesis and tropical distributions. Similarly, Gravendeel et al. [21] reported no association between pollinator specialisation and species richness across orchid subfamilies, instead finding a stronger impact of epiphytism in some genera.

One explanation for this pattern may lie in the demographic context of orchid reproduction. As Tremblay et al. [22] describe, many orchids are pollen-limited and have skewed reproductive success. A few individuals achieve pollination while the majority do not. In this context, pollinator specialisation may constrain reproductive assurance, thereby reducing the potential for long-term speciation. Furthermore, even flexible or rapidly evolving pollination strategies may not increase the probability of speciation, especially if gene flow remains limited or inconsistent. Another potential explanation lies in abiotic forces, which can shape macroevolutionary dynamics beyond the effect of adaptive traits [61]. Recently, Thompson et al. [31] demonstrated that speciation in Orchidoideae is associated with global cooling throughout the Cenozoic, and Guo et al. [32] found an impact of sea level on *Paphiopedilum* diversification. Similarly, Pérez-Escobar et al. [34] found that Neotropical epiphytic orchid diversification was associated with the abiotic forces of Andean uplift and geographic shifts, rather than different pollinators. Subsequent work by Pérez-Escobar et al. [71] strengthened this by showing that environmental sex determination (ESD) is associated with increased diversification in Neotropical epiphytic orchids, but ESD itself is triggered to evolve by habitat fragmentation and climatic instability. Therefore, the impact of reproductive traits on diversification is likely to be context-dependent, shaped by interactions with abiotic forces and lineage-specific constraints.

Our results are robust to differences and uncertainty of diversification rate estimation methods [72,73], and to the incomplete and uneven taxonomic sampling that currently characterises phylogenies for plant groups [74–77]. Our methods were designed to explicitly accommodate imbalanced sampling and have successfully explored pollinator-dependent diversification under similar levels of sampling (~12% of cactus species, [26]), and much weaker (~0.68% of orchid species in ~24% of genera, [20]). Our analysis includes 485 species from 88 genera (~10% of species, ~43% of Orchidoideae genera [1]), with some unevenness in sampling, notably 99 species of *Disa*. But this is not necessarily a drawback, as *Disa* is a model genus for pollination biology because it captures a wide range of pollination strategies [13,35,78]. Although BAMM and MiSSE differ in their exact estimated tip speciation rates and agree only on broad patterns (Supplementary Information), they converge in their associations with pollinators. This concordance between methods, and their different treatments of missing data, suggests that our result is not a statistical artefact resulting from incomplete data.

The complexity of triggering rapid diversification

Our results highlight that rapid diversification in orchids likely arises from complex interactions among ecological, geographic, and evolutionary factors, rather than from single forces like pollination strategy. This contributes to the growing appreciation showing that triggers of diversification are rarely explained by simplistic models [49,61,62,79]. While pollination strategy in terrestrial orchids is certainly a powerful driver of ecological differentiation [22], it does not consistently trigger increased diversification. Recent work in another diverse family also found this pattern. It was previously thought that pollinator divergence was a driver of cactus diversification [26], but by extensively sampling biotic and abiotic variables, Thompson et al. [61] found that pollinator divergence is not among the strongest predictors. Subsequent work found that while pollinators shape cactus floral morphology, neither pollinator nor floral morphology influences diversification rates, and it is the rate of floral evolution that drives speciation [31,80]. Across cacti, diversification was shaped by a complex combination of interacting abiotic and biotic forces [61,80], and it is likely that a similar pattern drives orchid evolution [2,20,31,65].

The traditional narrative that key traits can act in isolation as direct drivers of speciation is being overhauled, and the multifactorial nature of evolution is becoming clearer. Bouchenak-Khelladi et al. [63] offer a useful framework for interpreting these patterns. They argue that adaptive radiations require not only adaptive traits but also the optimal ecological conditions, which they delimit as “backgrounds”, “triggers”, and “modulators”. In orchids, pollination strategy may serve as a modulator, i.e., a context-dependent trigger that facilitates divergence when coupled with ecological opportunity or environmental change, but it cannot drive radiation in isolation. Bouchenak-Khelladi et al. [63] also distinguish "polymorphic" traits, which are labile and variable and can promote diversification by allowing species to partition ecological niches or undergo reproductive isolation, from "simple" traits, which are conserved and necessary for survival. Polymorphic traits do not necessarily trigger radiations, but they can facilitate or maintain one once it begins. The recovered lability of pollination strategies, and the different ways in which they mediate interactions with the environment and other organisms, may fit this description of polymorphic traits. Unless matched with extrinsic triggers or ecological shifts, pollinator strategy alone may not lead to accelerated diversification of terrestrial orchids [34,61]. Further work should begin to integrate all forces hypothesised to be important simultaneously within an analytical framework, to understand the conditions in which pollinator transitions do or do not accelerate orchid diversification, as recently suggested [5,65].

Materials and methods

Pollinator strategy data

Data on pollinator attraction strategy were sourced from a recently published and comprehensive database of orchid reproductive biology [5]. These data were assembled from an exhaustive search of literature published since Darwin [4], and scored attraction strategies as deceit-based (sex and food deceit) or reward-based (fragrance, nectar, oil, and sleeping site). Taxonomy was corrected against the World Checklist of Vascular Plants [81] using the R package *rWCVP* [82]. The majority of species names were exactly matched to species in WCVP, and the few "fuzzy matches" were checked manually. Species names with multiple matches were resolved automatically by keeping the accepted name where exactly one was accepted and keeping a synonym (as opposed to invalid or illegitimate names) when exactly one was a synonym. Finally, synonyms were corrected to their accepted name, and duplicates that resulted from the correction steps were removed. Species were categorised based on autonomous selfing/agagamospermy (119 species), nectar reward (174 species), lipid reward (50 species), sleep site reward (four species), brood-site deception (two species), food deception (131 species) and sex deception (65 species). A small number of species (50) were scored for more than one category, which were either the result of more than one category being operational or of conflicting source reports.

Phylogenetic framework

The cleaned dataset was matched with species from a recently published molecular phylogeny [31], and species not sampled in the dataset were pruned for analysis, ultimately leaving 485 species. This phylogeny was chosen because it is currently the most taxonomically comprehensive phylogeny for subfamily Orchidoideae, with 1,475 of ~5,000 species sampled. It was constructed using the supermatrix approach, sampling up to nine commonly-sequenced nucleotide loci publicly available in GenBank, and it was calibrated against geological time with RelTime [83], using robust phylogenomic estimates implemented as secondary calibrations [20,83], given the lack of an extensive fossil record for Orchidaceae [2].

Model selection, ancestral state reconstruction and transition rates

To identify the evolutionary mode of pollinator strategy, model selection was performed using hidden Markov transition models with the R package *corHMM* [58]. Six models were estimated: all rates different (ARD), equal rates (ER) and symmetrical (SYM), all with and without hidden rates, and were compared with Akaike information criterion weights (AICw). After initial investigation, the two states with very few species were removed (sleep site reward, n = 4; brood site deception = 2). Rare states are known to introduce artifacts to estimated transition rates [67,84,85] and increase uncertainty around ancestral states (Meade, personal communication), which they did here in initial discarded analyses. After identifying the best model based on AIC weight, 300 SIMMAPs were estimated to explore uncertainty of ancestral states [86]. To explore transition rates further, an ARD model was estimated in reversible-jump Markov chain Monte Carlo (MCMC) analyses using the MultiState module in BayesTraitsV3 (www.evolution.reading.ac.uk/). This rj-MCMC approach was chosen because it automatically allows rates to be zero if there is not enough evidence for their existence [59]. A hyperexponential prior was applied to each transition rate, with the mean of the exponential drawn from a uniform 0-100. 51 million generations were sampled every 5,000 after discarding the first 1 million as burn-in. Convergence was checked with the R package *coda* [87], ensuring effective sample sizes of >1,000. In both corHMM and rj-MCMC analyses, polymorphic species were included, allowing transitions to reflect the full range, and uncertainty, of pollination strategies observed.

Estimating speciation rate variation

To estimate speciation rates, we used two character-independent methods that differ in approach and assumptions, especially regarding the setting of incomplete sampling fractions. This follows recent recommendations that tip-rate patterns be evaluated with more than one method, since different estimators capture different aspects of diversification dynamics and can behave inconsistently across phylogenies [72]. Namely, we used Bayesian Analysis of Macroevolutionary Mixtures (BAMM, 36) and Missing State Speciation and Extinction (MiSSE, 37). BAMM is widely used and has been implemented on this phylogeny of Orchidoideae previously [31]; MiSSE is a newer model, but has been used to conduct *post hoc* tests for drivers of diversification in various clades, including in animals [88], plants [89], and fungi [90]. We solely considered variation in speciation rate because extinction rates inferred with BAMM are thought to be unreliable [91], whereas speciation rates are more accurate [92]. We opted to use character-independent methods instead of character-dependent SSE models because the pollinator data currently available for Orchidoideae are too sparse to accurately account for incomplete sampling of states [5], which is a crucial step for character-dependent SSE models [93]. Instead, BAMM and MiSSE account for incomplete sampling with clade-specific and global sampling fractions, respectively. Both of these tools have benefits and pitfalls: Clade-specific fractions allow for different lineages to have different sampling fractions, which is important when genetic sequencing has been biased to particular groups, as is very common in plants. However, BAMM has been criticised because it can distort likelihood calculations, potentially leading to spurious inferences of rate shifts [94]. Further criticism of BAMM beyond the issue of clade-specific fractions [91,95] has been defended statistically [92,96]. We believe that by implementing both approaches, in accordance with recent suggestions for studies of tip diversification rates (58), we can provide a good and complementary understanding of overall diversification patterns. We used the BAMM-estimated rates of Thompson et al [31], where clade-specific sampling fractions were implemented at genus level, and 9,000 samples of the Bayesian posterior were estimated, accounting for uncertainty in parameter estimation (detailed methodological procedure in [31]). Unlike BAMM, MiSSE, which is implemented in the R package *hisse* [49], is a maximum-likelihood model that treats diversification as a set of up to 26 different hidden states to capture rate heterogeneity across the phylogeny [47]. Using the generateMiSSEGreedyCombinations function, we generated 30 possible model structures, each of which differed in combinations of one to ten turnover (speciation rate plus extinction rate) parameters and one to three extinction fraction (extinction rate divided by speciation rate) parameters, as in [89]. Because short terminal branches can bias SSE models [49], some of which are present in our Orchidoideae tree due to recent radiations in orchids [2,31], we pruned the 200 shortest tips from the phylogeny before analysis. We ran MiSSE for each of these model structures using the function MiSSEGreedy, implementing a global sampling fraction of 25.5% [1] to account for incomplete sampling. Using these models, we reconstructed marginal ancestral states with the MarginalReconMiSSE function, then extracted model-averaged tip diversification rates using the command GetModelAveRates. These two methods, BAMM and MiSSE, provide complementary estimates of speciation rate that differ as a result of methodological differences, capturing uncertainty inherent to estimating diversification rates [72,73,93].

Assessing drivers of speciation

To assess drivers of speciation we performed tests on both the BAMM and MiSSE estimates, using slightly different approaches. We used Structured Rate Permutation on Phylogenies (STRAPP) tests [50] with BAMM-estimated speciation rates [46], and phylogenetic regressions with speciation rates estimated using the Missing State Speciation and Extinction (MiSSE) model [47]. STRAPP tests were implemented with the R package *BAMMtools* ([97]8) and phylogenetic regressions with the R package *phylolm* (79). Since both are character-independent methods, they relax the assumption that all rate variation is associated with the focal trait, similar to the hidden-rates extensions [49] of classic formal SSE models [48]. Different tests were performed: (1) binary analyses, which tested each pollination strategy individually while retaining polymorphic species, and included comparisons of generalists (pollinated by >1 species) versus specialists, Hymenoptera-pollinated versus others and Lepidoptera-pollinated versus others; (2) multistate analyses, which tested all attraction strategies simultaneously after removing polymorphic species, and included a three-state comparison grouping species broadly into autonomous, reward-based, and deception-based strategies; (3) count-based analyses, which tested the effects of pollinator number and functional pollinator specificity (FPS, defined as the multiplying counts of pollinator families by orders). STRAPP tests were performed by permuting rates across the tree, while maintaining the position of rate shifts [50]. Statistical differences were assessed with a Kruskal-Wallis test for the multi-state tests, a Mann-Whitney U test for the binary tests, and a Spearman's rank correlation coefficient for the pollinator count and FPS tests. Equivalent statistical tests of MiSSE-estimated speciation rates were performed using a PGLS approach in the R package *phylolm* (79).

Supplementary materials

Code and data used to generate these results is available at https://github.com/jamie-thompson/orchid_pollinators.

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