

Perspective

Opposing effects of plant traits on diversification

Bruce Anderson,^{1,*} John Pannell,² Sylvain Billiard,³ Concetta Burgarella,⁴ Hugo de Boer,⁵ Mathilde Dufay,⁶ Andrew J. Helmstetter,⁷ Marcos Méndez,⁸ Sarah P. Otto,⁹ Denis Roze,¹⁰ Hervé Sauquet,^{11,12} Daniel Schoen,¹³ Jürg Schönenberger,¹⁴ Mario Vallejo-Marin,¹⁵ Rosana Zenil-Ferguson,¹⁶ Jos Käfer,^{17,*} and Sylvain Glémin^{15,18,*}

SUMMARY

Species diversity can vary dramatically across lineages due to differences in speciation and extinction rates. Here, we explore the effects of several plant traits on diversification, finding that most traits have opposing effects on diversification. For example, outcrossing may increase the efficacy of selection and adaptation but also decrease mate availability, two processes with contrasting effects on lineage persistence. Such opposing trait effects can manifest as differences in diversification rates that depend on ecological context, spatiotemporal scale, and associations with other traits. The complexity of pathways linking traits to diversification suggests that the mechanistic underpinnings behind their correlations may be difficult to interpret with any certainty, and context dependence means that the effects of specific traits on diversification are likely to differ across multiple lineages and timescales. This calls for taxonomically and context-controlled approaches to studies that correlate traits and diversification.

INTRODUCTION

Unevenness in diversification rates is a striking feature across the Tree of Life, with sister clades often differing enormously in their diversity.^{1,2} Why this should be is an abiding question of evolutionary biology.^{3–5} Neutral processes of lineage splitting and extinction can account for some variation in inter-clade diversity.^{6–8} But because many phylogenies are more uneven than predicted by these neutral processes,^{9–13} non-random macro-evolutionary processes are still frequently invoked (reviewed in ref.¹⁴) as the cause of this additional variation. By accelerating speciation rates or by reducing extinction rates, the evolution of phenotypic “key innovations” may increase diversification beyond the background rates of neutral processes.^{15–18} In this context, reproductive traits may be particularly important, given their central role in regulating gene flow, demographic persistence, and the maintenance of genetic diversity within populations—all of which are expected to affect diversification rates. For instance, the convergent origin of zygomorphy^{19–21} or the possession of floral spurs^{22,23} provides well-studied illustrations of apparent key innovations by plants, with positive effects on diversification. On the other hand, some traits might slow speciation or accelerate extinction. For instance, the evolution of wind pollination might slow down speciation rates if it maintains more inter-population gene flow.^{24,25} The general characterization of traits according to whether they positively or negatively affect diversification rates thus remains a key goal of studies relating to organismal diversity,⁵ but there is little evidence to suggest that any particular traits have consistent effects on diversification.¹⁴

Variation in how phenotypic traits affect diversification may be complex for at least two reasons. First, a particular trait might directly affect diversification in different ways; in some instances increasing diversification and in other instances decreasing it. The contrast between selfing and self-incompatibility provides a revealing illustration of this duality. Transitions toward self-fertilization constitute the most frequent evolutionary transition known to have taken place in angiosperms.²⁶ But as a general strategy, selfing may lead to an evolutionary dead-end^{27–30} because genetic drift and reduced effective recombination rates permit the accumulation of weakly deleterious mutations and a reduced capacity to adapt to environmental change.^{30,31} At the same time, populations of selfing species tend to be more divergent, likely due to their smaller effective sizes and lower rates of pollen movement among them,^{30,32,33} and this reduction in gene flow may have a positive effect on speciation rates. In a study that appears to support both of these

¹Department of Botany & Zoology, Stellenbosch Univ., Cape Town, South Africa

²Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland

³University Lille, CNRS, UMR 8198 – Evo-Eco-Paleo, 59000 Lille, France

⁴Department of Organismal Biology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

⁵Natural History Museum, University of Oslo, 0318 Oslo, Norway

⁶CEFE, University Montpellier, CNRS, University Paul Valéry Montpellier 3, Ephe, IRD, Montpellier, France

⁷FRB – CESAB, 5 Rue de l'École de Médecine, 34000 Montpellier, France

⁸Area of Biodiversity and Conservation, Universidad Rey Juan Carlos, Madrid, Spain

⁹Department of Zoology, University of British Columbia, Vancouver, BC, Canada

¹⁰IRL 3614, CNRS, Sorbonne Université, Station Biologique de Roscoff, 29688 Roscoff Cedex, France

¹¹National Herbarium of New South Wales, Royal Botanic Gardens and Domain Trust, Sydney, NSW 2000, Australia

¹²Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia

¹³Department of Biology, McGill University, Montreal, QC, Canada

¹⁴Department of Botany and Biodiversity Research, University of Vienna,

Continued



expectations simultaneously, Goldberg et al.³⁴ raised the possibility that transitions from self-incompatibility (SI) to self-compatibility (SC) might act in opposite directions on the two components of diversification, with extinction coming to dominate over speciation.

Secondly, a focal trait might affect diversification through indirect associations with other traits (e.g.³⁵). Thus, the effects of traits on diversification may be driven more strongly by the associations between suites of traits than by any single trait in isolation. The SI/SC comparison also offers a good example of possible associations with other traits, such as lifespan,³⁶ dispersal and colonization ability,³⁷ or ploidy levels,³⁵ all of which potentially affect diversification in opposite or synergistic ways.

Because diversification is the net effect of speciation and extinction, different environmental or ecological conditions and potential associations with other traits can alter their relative importance, leading to context-dependent effects of traits on diversification. Previous studies have suggested the importance of context-dependent trait effects on diversification (e.g.^{8,38–40}). However, in this perspective piece, we aim to identify and break down some of the mechanisms and reasons behind context-dependent trait effects on diversification. In doing so, we show that context dependence is frequently expected because most phenotypic traits appear to impact multiple processes, often with different and even opposing effects on diversification.⁴¹ We also show that context dependence is likely associated with most traits and that it is unsurprising that comparative analyses often fail to reveal consistent effects of traits on diversification.⁴⁰ We conclude that the complex, interactive effects between traits and ecology necessitate careful, hypothesis-driven approaches, which explicitly include ecological context when studying trait-associated diversification. The conceptual framework outlined in this paper provides a useful starting point to develop predictions for such studies.

OPPOSING EFFECTS ON DIVERSIFICATION ARE WIDESPREAD

To illustrate the complex ways in which traits may affect diversification, we identify four broad consequences of ecology and evolution which affect the process of diversification in [Figure 1](#): “Divergence” and “reproductive isolation” may often affect the probability of speciation, while “demographic buffering” and “evolutionary buffering” may affect a population’s persistence (see definitions in the glossary, [Box S1](#)). We refer to these as the four broad mechanisms that influence diversification rates. We then consider 13 commonly studied life history and reproductive traits and synthesize how they could affect diversification. Based on interpretation of theoretical and conceptual literature, we identified the possible ecological and evolutionary mechanisms by which a trait can affect the processes contributing speciation and persistence. We thus obtained a general conceptual framework summarized in [Table 1](#) (a condensed version of [Table S1](#)).

The table strikingly illustrates the potential for traits to affect diversification in opposing ways. This could occur because the same trait may have opposing effects on the broad mechanisms influencing diversification, depicted with both “up” and “down” arrows in [Table 1](#). A trait may also have opposing effects on diversification because its effect on a particular mechanism varies with context (also depicted with “up” and “down” arrows in [Table 1](#)).

As an example, the multiple and complex effects of selfing presented in the introduction are graphically presented in more detail in [Figure 2](#) (also see [Table 1](#)). In addition, the contrast between dioecy and hermaphroditism provides another revealing illustration of these opposing effects. Theory suggests that the evolution of dioecy from hermaphroditism may have both negative and positive effects on components of diversification. On the one hand, the obligate outcrossing that is associated with dioecy may increase the scope for sexual selection, the efficacy of selection, and consequently the probability of adaptive population divergence and the build-up of reproductive incompatibility.⁴² However, dioecy is also often associated with generalist pollinators or wind pollination,⁴³ which, in contrast may reduce divergence and reproductive isolation.³² Dioecy may also have opposing effects on persistence, exposing dioecious populations to greater extinction risk.⁴⁴ For instance, dioecious populations may be more prone to extinction because they lack the benefits of reproductive assurance via selfing,⁴⁵ or because sexual selection may lead to pollinator failure if pollinators preferentially visit the larger male floral displays.⁴⁶ In contrast, dioecy and associated outcrossing may have a longer term evolutionary effect by promoting adaptation to changing environments.⁴⁷ Given the opposing effects that dioecy may have on diversification, we may either expect dioecy to have no general effects on diversification, or we may expect conflicting results across studies

Rennweg 14, 1030 Vienna, Austria

¹⁵Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

¹⁶Department of Biology, University of Kentucky, Lexington, KY 40506-0225, USA

¹⁷Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR 5558, 69622 Villeurbanne, France

¹⁸CNRS, Ecosystèmes Biodiversité Evolution (Université de Rennes), 35000 Rennes, France

*Correspondence: banderso.bruce@gmail.com (B.A.), jos.kafer@cnrs.fr (J.K.), sylvain.glemin@univ-rennes1.fr (S.G.)

<https://doi.org/10.1016/j.isci.2023.106362>

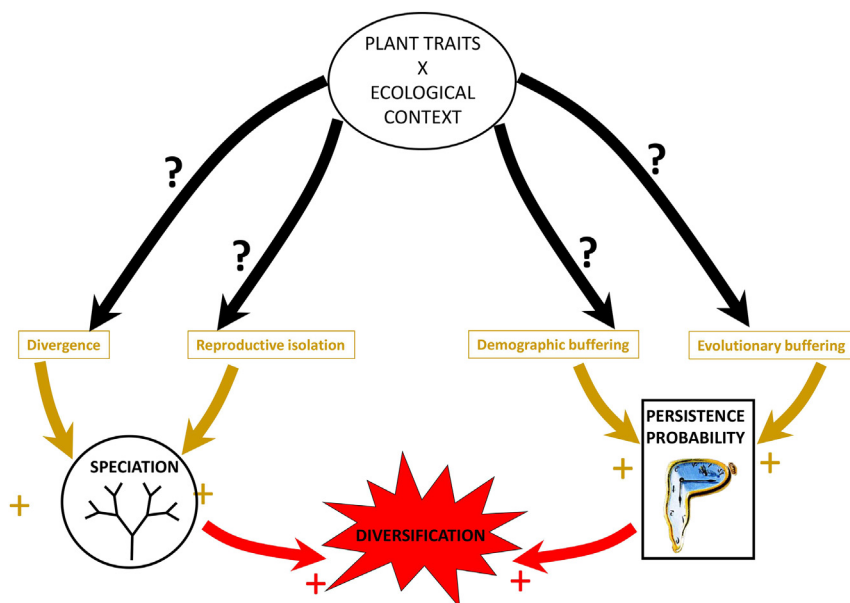


Figure 1. How plant traits and the environment affect diversification through speciation and persistence

Diversification rates are positively affected by high rates of speciation and population persistence. Speciation is influenced by the probability of population divergence and the formation of reproductive isolation barriers. Population persistence is influenced by its short-term ability to respond to ecological or demographic challenges (demographic buffering) and its long-term robustness and ability to adapt to changing environmental conditions (evolutionary buffering). This study is primarily concerned with the theoretical effects that plant traits are likely to have on these four mechanisms under different ecological contexts. See glossary (Box S1) for terminology used in this figure.



















due to context dependence. In fact, we find evidence for both of these scenarios: while Käfer et al.⁴⁸ found moderately positive effects of dioecy on diversification rates, Sabath et al.⁴⁹ found no general effect of dioecy on diversification, but rather that dioecy could have positive effects on the diversification rates of some genera and negative effects on others.

Overall, we found potential opposing effects for all examined traits. No traits were predicted to have consistent positive or negative effects on both speciation and persistence under all or almost all conditions (Tables 1 and S1), in agreement with a review of state-dependent speciation and extinction analyses across angiosperms.¹⁴ Some previous studies in angiosperms and animals have also suggested opposing effects of traits, where traits that drive high speciation rates are also expected to result in high extinction rates (so low persistence),^{50,51} leading to the assumption that speciation and extinction go hand-in-hand.^{15,50} However, we suggest that even if a trait can theoretically increase extinction and speciation rates, its ultimate effects on diversification should be determined by ecological context. For example, in certain contexts, the positive effects on speciation may outweigh its negative effects, but this may be reversed in other ecological contexts. We expect that this should give rise to variability in the associations of traits with speciation, extinction, and diversification (as demonstrated by Greenberg and Mooers⁵⁰ where extinction probability was only associated with diversification rates for amphibians but not other tetrapods).

ASYMMETRICAL AND CONTEXT-DEPENDENT EFFECTS ON DIVERSIFICATION

While the opposing effects of traits on diversification may not always cancel one another out, context is likely to dictate the circumstances under which one effect dominates the other. Ho and Agrawal⁵² predicted that these canceling effects could generate an interesting temporal pattern in the case of self-compatibility and its effect on diversification. This prediction was subsequently supported empirically in the Onagraceae by Freyman and Höhna,⁵³ who found that while self-compatibility appeared to increase speciation rates in the short term (so that it had positive effects on diversification), the speciation rate of self-compatible lineages declined in the longer term, so that net diversification rates were lower than for self-incompatible lineages.

Table 1. Summarizing different effects of plant traits on divergence, reproductive isolation, demographic buffering, and evolutionary buffering

Effects on:	SPECIATION	SPECIATION	PERSISTENCE	PERSISTENCE
Trait	<i>Divergence</i>	<i>Reproductive isolation</i>	<i>Demographic buffering</i>	<i>Evolutionary buffering</i>
Autogamy				
	<p><i>Low gene flow, low recombination, and high drift increase divergence. But low selection efficacy, low sexual and genomic conflicts decrease divergence.</i></p>	<p><i>Low gene flow, low recombination, and high drift allow accumulation of incompatibilities. But low selection efficacy, low sexual and genomic conflicts reduce accumulation of incompatibilities.</i></p>	<p><i>Uniparental reproduction allows reproductive assurance, better colonizing ability, and bigger range size.</i></p>	<p><i>Low genetic variation and high load reduce adaptive potential in changing environments.</i></p>
Self-compatibility				
	<p><i>Increased probability of selfing leads to the same predictions as autogamy.</i></p>	<p><i>Increased probability of selfing leads to the same predictions as autogamy.</i></p>	<p><i>Increased mate availability allows reproductive assurance, better colonizing ability, and bigger range size.</i></p>	<p><i>Increased probability of selfing leads to negative long-term consequences (see autogamy).</i></p>
Dioecy				
	<p><i>Obligate outcrossing, with potential exacerbated sexual conflicts, leads to the opposite predictions to autogamy.</i></p>	<p><i>Obligate outcrossing, with potential exacerbated sexual conflicts, leads to the opposite predictions to autogamy.</i></p>	<p><i>Sexual dimorphism can bias pollinator visits toward males and reduce fertilization success. Seed production by females only reduces colonizing ability (seed shadow handicap).</i></p>	<p><i>Obligate outcrossing increases adaptive potential in changing environments.</i></p>
Biotic pollination				/
	<p><i>More specialized pollination (compared to abiotic pollination) favors shifts between pollinators and increases adaptive divergence.</i></p>	<p><i>More specialized pollination (compared to abiotic pollination) favors shifts between pollinators and increases reproductive isolation.</i></p>	<p><i>More specialized pollination increases pollination efficiency but can also increase the risk of pollinator failure.</i></p>	
Zygomorphy				/














(Continued on next page)

Table 1. Continued

Effects on:	SPECIATION	SPECIATION	PERSISTENCE	PERSISTENCE
Trait	Divergence	Reproductive isolation	Demographic buffering	Evolutionary buffering
	More specialized pollination (compared to actinomorphy) favors shifts between pollinators and increases adaptive divergence.	More specialized pollination (compared to actinomorphy) favors shifts between pollinators and increases reproductive isolation.	More specialized pollination increases pollination efficiency but can also increase the risk of pollinator failure	
Large display size	↓ ↑	↓ ↑	↑	↓ ↑
	Better pollinator attraction increases outcrossing. But many flowers increase geitonogamous selfing. See variable divergence consequences for self-compatibility.	Better pollinator attraction increases outcrossing. But many flowers increase geitonogamous selfing. See variable reproductive isolation consequences for self-compatibility.	Better pollinator attraction increases fertilization success.	Evolutionary buffering depends on whether outcrossing or selfing is favored.
Short life span	↑	/	↓ ↑	↑
	More events per unit time (recombination, mutation, drift, ...) speed up divergence		Rapid population recovery but higher demographic stochasticity.	Faster adaptation (in unit time) to changing environments.
Small size	↓ ↑	↑	↓ ↑	↑
	Reduced dispersal ability decreases colonizing ability across divergence selection mosaic but also favors local adaptation. Alternatively, environmental heterogeneity and selection mosaics can be experienced at finer scales.	Reduced dispersal ability reduces gene flow and increases reproductive isolation.	Large local population sizes but lower competitive and dispersal abilities.	Large local population sizes increase adaptive potential in changing environments.
Biotic dispersal	↓ ↑	↓	↓ ↑	/
	More long-distance dispersal increases colonizing ability across divergent selection mosaics. However, gene flow between sites can also reduce divergence.	More long-distance dispersal favors gene flow and reduces reproductive isolation.	More long-distance dispersal increases colonizing ability. But more risk of dispersal failure leads to stochastic reproductive output.	

(Continued on next page)

Table 1. Continued

Effects on:	SPECIATION	SPECIATION	PERSISTENCE	PERSISTENCE
Trait	Divergence	Reproductive isolation	Demographic buffering	Evolutionary buffering
Small seed size				/
	<p>More long-distance dispersal increases colonizing ability, which can increase divergence across different habitats. But gene flow can also reduce divergence.</p>	<p>More long-distance dispersal increases colonizing ability, which can lead to geographically isolated populations. But, it also generates gene flow, which can reduce reproductive isolation.</p>	<p>More seeds produced and more long-distance dispersal increase colonization ability. Smaller seed size can decrease competitive ability.</p>	
Vegetative reproduction		/		
	<p>Less efficient selection under clonality reduces divergence. Uniparental reproduction allows distant colonization across divergent selection mosaic, which increases divergence.</p>		<p>Uniparental reproduction allows reproductive assurance, better colonizing ability, and bigger range size.</p>	<p>Less efficient selection under clonality reduces adaptive potential in changing environments.</p>
Seed dormancy		/		
	<p>Generation overlap increases generation time, so decreases divergence.</p>		<p>Long-lived seed bank buffers against stochastic conditions.</p>	<p>Generation overlap maintains genetic variation, and high adaptive potential but slower adaptation per unit time.</p>
Polyploidy				
	<p>Gene redundancy allows evolution of new functions and facilitates divergence.</p>	<p>Divergent resolution of gene redundancy leads to post-zygotic incompatibilities.</p>	<p>Instability in meiosis and minority cytotype disadvantage leads to mating difficulties</p>	<p>Gene redundancy buffers deleterious mutations and allows evolution of new functions, which increases adaptive potential in changing environments.</p>

“Up” arrows indicate expectations of increased diversification, and “down” arrows indicate expectations of reduced diversification associated with a trait. Both “up” and “down” arrows indicate that a trait has variable effects on diversification. Most traits have mixtures of “up” and “down” arrows, suggesting inconsistent trait effects on all four factors key to speciation and population persistence. “/” symbols indicate fields where we were not able to think of direct trait effects. See [Table S1](#) for more detailed reasoning and references.

It is possible that traits with consistent directional effects on speciation or persistence will have clearer effects on diversification than traits that have inconsistent or opposing effects on speciation or persistence alone. Zygomorphy, biotic pollination, short lifespan, and polyploidy would appear to be four such traits (see [Table 1](#)): When speciation or persistence is considered alone, these traits do not appear to have conflicting outcomes on

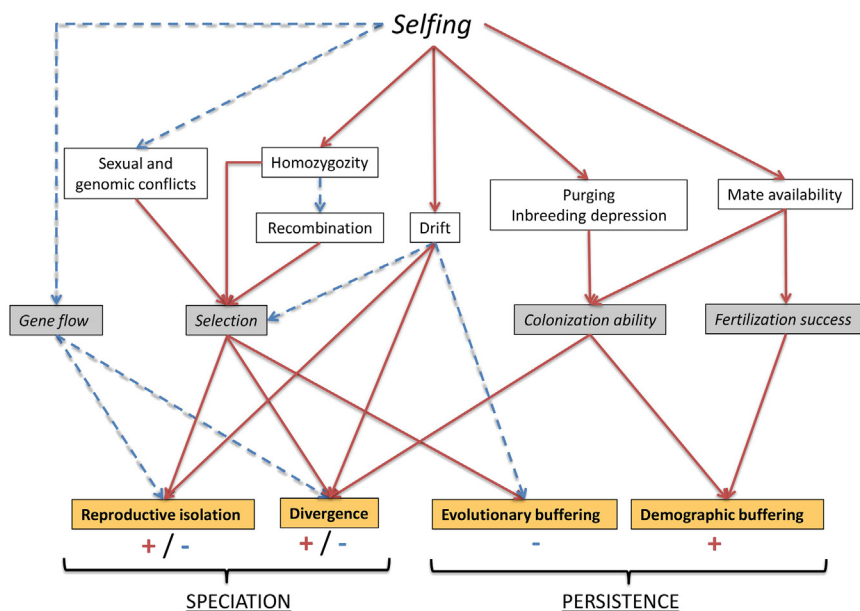


Figure 2. Complex and opposing effects of selfing on speciation and persistence

Selfing may have positive or negative effects on the four processes (orange) linked to speciation and persistence (positive associations in red and negative associations in blue). For example, selfing decreases reproductive isolation and divergence through its negative effects on sexual and genomic conflicts. However, selfing also reduces gene flow which is likely to increase both reproductive isolation and divergence. Selfing may also reduce selection through drift and low effective recombination. Selfing also has discordant effects on persistence: Its effects on evolutionary buffering are consistently negative but its effects on demographic buffering are consistently positive. These conflicts make the ultimate effect of selfing on diversification unpredictable and possibly influenced by environmental context.

diversification. Instead, the conflicts only become evident when trait effects on speciation and persistence are considered simultaneously. If we consider zygomorphy, it is clear that it should always promote speciation because of greater pollinator specialization^{54,55} or because it allows more precise manipulation of pollinator position and behavior in the flower.^{54,56} But at the same time, pollinator specialization (a frequent consequence of zygomorphy) may increase the risk of extinction through pollinator failure⁵⁷ (Table 1). Importantly, the positive effect of zygomorphy on speciation through its effects on divergence and reproductive isolation^{54,56} may be stronger in stable environments (where pollinator failure is unlikely) than its negative effect on persistence. Consequently, we might expect a greater proliferation of zygomorphic species in the environmentally stable wet tropics. In contrast, less-stable conditions may result in persistence being a more important diversification generator than speciation, perhaps leading to a greater prevalence of pollinator generalism and actinomorphy in higher latitudes. This example suggests that the effects of traits like zygomorphy may be highly predictable in certain ecological conditions but may be much less predictable if environmental context is not considered. Failure to take environmental context into account (e.g., by analyzing large datasets within which the implications of specialization vary strongly among clades and environmental conditions) risks obscuring important associations. Despite this risk, some taxonomically diverse analyses (e.g.⁵⁸) have found overall effects on diversification for some traits, notably for zygomorphy, but also for biotic pollination and annuality. Tantalizingly, these are all traits with consistent effects on either speciation or persistence (Table 1), although it is also possible that, with further study, traits which appear to have consistent effects on diversification may turn out to have inconsistent effects.

TRAIT CORRELATIONS AND THEIR OPPOSING EFFECTS

Failure to find straightforward effects of traits on diversification may also be influenced by their associations with other traits⁸ that, themselves, may either promote or reduce diversification in different contexts. In some cases, a trait may only have a positive effect on diversification when it is associated with another specific trait. For example, vertebrate dispersal is thought to increase diversification in woody plants, but not herbaceous plants.^{8,59} Many traits are also highly inter-correlated due to life history trade-offs, or because of allometric associations (such as between plant size and lifespan⁶⁰). Consequently, associated traits may

have indirect, opposing effects on diversification. For instance, self-fertilization is associated with an annual life history, so that a greater speciation rate in selfing plants might be ultimately attributable to short generation times,^{61,62} or because small size could allow plants to diversify into more niches, especially under extreme conditions.⁶¹ At the same time, however, small size could also be associated with lower competitive and persistence abilities in mature communities,⁶³ leading to lower diversification.

Trait combinations may also be adaptive and conceivably evolve in individuals because one trait may offset the negative effects of another trait. For example, specialized pollination systems may have positive effects on gene flow,² but they may also increase the risk of pollination failure. Consequently, the evolution of specialization may be facilitated when individuals possess traits with the ability to buffer the effects of stochastic pollinator failure (see⁶⁴), such as delayed selfing (e.g.⁶⁵) or cleistogamy (e.g.⁶⁶), long lifespans or long-lived seed banks. Thus, complementary plant traits may evolve as syndrome associations.

Associations and correlations between traits may contribute to the typically weak associations between traits and diversification,¹⁴ but they also reduce the interpretability of results from phylogenetic comparative studies.⁶⁷ Poor understanding of how, why, and when traits are correlated,⁶⁸ exacerbates the cause-and-effect traps of phylogenetic comparative studies. While this may not render such studies useless, it does call for very cautious interpretations of associations, *a priori* hypothesis building based on theoretical links between traits and diversification, as well as experimental studies to support the findings. In this regard, Nurk et al.⁶⁹ provide a useful analytical framework termed the “evolutionary arena” where they envisage diversification through the interactions between the biotic environment, the abiotic environment, and clade-specific traits.

IMPLICATIONS FOR MACROEVOLUTION ANALYSIS

It is clear that the evolutionary mechanisms linking traits and diversification rates are complex. Recognition of this complexity is important for designing comparative approaches, choosing appropriate clades for analysis, formulating realistic hypotheses, and for the interpretation of correlative diversification studies.⁷⁰ Below, we summarize four important points.

- Opposing effects of traits on diversification appear to be widespread and this is expected to result in widespread context dependence. This underpins the need to explicitly include environmental and ecological context into our analyses⁷¹ instead of asking more simple questions such as which traits increase or decrease diversification in general. More specifically, this would entail statistical tests of the interactive effects between traits and the environment on diversification.
- Trait effects on diversification also need to be considered in the context of associated traits and their indirect effects on diversification, especially since such trait correlations are likely to reduce the certainty of cause-and-effect inferences. Consequently, research should be strongly directed by theory and *a priori* hypotheses, as proposed in [Table 1](#).
- Most traits appear to affect diversification through multiple mechanisms; however, theoretical studies usually consider the effects of traits on diversification through just one or two mechanisms (e.g. the effects of selfing on the efficacy of selection). Thus, the results of single theoretical studies are limited in their ability to make general predictions of how traits affect diversification.
- Associations between traits and diversification rates offer a glimpse into evolution’s mechanics through a very particular and narrow lens (i.e. key innovations). But because of context dependence and trait correlations, trait possession may not be strongly associated with the dramatic variation in species richness observed among clades¹⁴ as is so often assumed (e.g.⁵⁸). For instance, the traits most important in the speciation process are often likely to be the ones that change most, not the ones that are retained in diverse lineages. Greater understanding of how traits affect diversification may be gleaned by using complementary approaches that combine field ecology and phylogeny to mechanistically link traits with ecology, lineage splitting, reproductive isolation, and lineage termination.⁷² For example, by mapping trait change and ecological conditions onto phylogenies, one can study trait lability^{71,73} and how different ecological conditions are associated with trait divergence.⁷⁴ Associative studies should be backed up by experimental studies. For example, Cossard et al.⁷⁵ studied evolutionary transitions from dioecy to hermaphroditism by manipulating sex ratios in experimental plots, while field studies by Minnaar et al.⁷⁶ were able to document how trait

divergence affected pollen movement patterns and the formation of reproductive barriers. These mechanistic approaches to studying traits and their effects on diversification should be strongly embedded in the theory of how diversification occurs. For example, the process of ecological speciation is mimicked through studies that examine firstly how exposure to different ecological niches drives phenotypic diversification and secondly how that divergence in turn results in speciation by reinforcing reproductive isolation and ultimately speciation,^{77,78} as predicted by the process of ecological speciation.⁷⁹

While we are not advocating that researchers stop using state-dependent speciation and extinction models, we do think that they should be used with caution to uncover directional effects of traits on diversification in context-dependent ways³⁴ (also see⁸⁰ for ecological studies). For example, multistate methods can be used to explicitly parameterize context dependence or correlation between traits. Alternatively, the use of hidden-state models may also be a useful way to assess the effects of unmeasured variables (e.g. correlated traits or ecology) on diversification.⁸¹ Such unmeasured variables were found to be an underlying cause^{82,83} behind the finding that birth-death models often support trait-dependent diversification, irrespective of whether traits actually affect speciation or extinction. Consequently, Beaulieu et al.⁸² developed hidden-state models to address this issue and these have been further developed⁷⁰ and implemented.³⁵

Our findings also support the calls for explicit hypothesis testing in comparative studies, and the tables presented here provide a starting point for developing theoretical frameworks to underpin such hypotheses. Höhna et al.⁸⁴ (also see⁷⁰) provide additional methods on how hypothesis testing could be made more explicit, through the development of graphical modeling frameworks. These can be used to depict relationships between traits, selective agents, and diversification as a first step to generate more explicit *a priori* hypotheses. Similar ideas were utilized by Givnish et al.,⁸⁵ who used well-developed *a priori* hypotheses to distinguish direct from indirect effects of traits on diversification.

The findings of this paper may also provide insights into the appropriate scales for conducting comparative analyses, which has become a polarizing debate, pitting the utility of small, focused analyses against the utility of broad-scale analyses spanning the Tree of Life (e.g.^{86–88}). Although there are many arguments both for and against large- versus small-scale analyses (see^{86–88}), our paper suggests that the mechanics of traits and their effects on diversification are extremely complex. Consequently, associations with diversification rates may be hard to interpret because of the complexity of linked factors affecting diversification, and this is likely to be amplified with large analyses where context dependence is harder to take into account. For example, in large analyses, conflicting effects of traits may often cancel one another out across diverse samples, potentially hiding important context-dependent trends. Furthermore, large analyses lose their ability to dissect out the mechanics leading to their results (also see^{89,90}). The result is that large analyses lose their ability to provide credible answers to the so called “big questions” that they purportedly ask.⁸⁸ Instead, we maintain that smaller analyses potentially offer a greater degree of analytical control and the greater depth of understanding required for more robust interpretations of results. Choosing clades with limited diversity in ecological or life history traits may reduce the complexity of interactions and interwoven confounding factors. We maintain that the usefulness of individual comparative studies may not be their ability to answer broad questions (arguments above) but to investigate and compare the confluence of factors that spark diversification in different clades and in different environmental conditions.³⁹ In addition, controlled, small-scale analyses may be replicated across independent clades to answer broader questions about the effects of traits on diversification.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.106362>.

ACKNOWLEDGMENTS

This research is product of the “DiveRS” group funded by the synthesis center CESAB of the French Foundation for Biodiversity Research (FRB; www.fondationbiodiversite.fr). C.B. received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Fellowship Programme (grant agreement No. 839643).

AUTHOR CONTRIBUTIONS

Manuscript idea was conceived by B.A. Writing was done by B.A., J.P., S.G., and J.K. Figures and Tables were done by B.A., S.G., and J.K. All authors contributed equally to editing and compilation of theoretical predictions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Davies, T.J., Barraclough, T.G., Chase, M.W., Soltis, P.S., Soltis, D.E., Savolainen, V., Crane, P.R., Davies, T.J., Barraclough, T.G., Chase, M.W., et al. (2004). Darwin's abominable mystery : insights from a supertree of the angiosperms. *Proc. Natl. Acad. Sci. USA* 101, 1904–1909. <https://doi.org/10.1073/pnas.030812710>.
- Magallón, S., and Sanderson, M.J. (2001). Absolute diversification rates in angiosperm clades. *Evolution* 55, 1762–1780. <https://doi.org/10.1111/j.0014-3820.2001.tb00826.x>.
- Rabosky, D.L. (2010). Primary controls on species richness in higher taxa. *Syst. Biol.* 59, 634–645. <https://doi.org/10.1093/sysbio/syq060>.
- Rabosky, D.L., Slater, G.J., and Alfaro, M.E. (2012). Clade age and species richness are decoupled across the eukaryotic tree of life. *PLoS Biol.* 10, 1001381. <https://doi.org/10.1371/journal.pbio.1001381>.
- Sauquet, H., and Magallón, S. (2018). Key questions and challenges in angiosperm macroevolution. *New Phytol.* 219, 1170–1187. <https://doi.org/10.1111/nph.15104>.
- Raup, D.M., Gould, S.J., Schopf, T.J.M., and Simberloff, D.S. (1973). Stochastic models of phylogeny and the evolution of diversity. *J. Geol.* 81, 525–542. 30060095.
- Gould, S.J., Raup, D.M., Sepkoski, J.J., Schopf, T.J.M., and Simberloff, D.S. (1977). The shape of Evolution: a comparison of real and random clades. *Paleobiology* 3, 23–40. <https://doi.org/10.1017/S009483730000508X>.
- De Queiroz, A. (2002). Contingent predictability in evolution: key traits and diversification. *Syst. Biol.* 51, 917–929. <https://doi.org/10.1080/10635150290102627>.
- Guyer, C., and Slowinski, J.B. (1991). Comparisons of observed phylogenetic topologies with null expectations among three monophyletic lineages. *Evolution* 45, 340–350. <https://doi.org/10.1111/j.1558-5646.1991.tb04408.x>.
- Guyer, C., and Slowinski, J.B. (1993). Adaptive radiation and the topology of large phylogenies. *Evolution* 47, 253–263. <https://doi.org/10.1111/j.1558-5646.1993.tb01214.x>.
- Heard, S.B. (1992). Patterns in tree balance among cladistic, phenetic, and randomly generated phylogenetic trees. *Evolution* 46, 1818–1826. <https://doi.org/10.1111/j.1558-5646.1992.tb01171.x>.
- Mooers, A.Ø. (1995). Tree balance and tree completeness. *Evolution* 49, 379–384. <https://doi.org/10.1111/j.1558-5646.1995.tb02251.x>.
- Purvis, A., and Agapow, P.-M. (2002). Phylogeny imbalance: taxonomic level matters. *Syst. Biol.* 51, 844–854. <https://doi.org/10.1080/10635150290155809>.
- Helmstetter, A.J., Zenil-Ferguson, R., Sauquet, H., Otto, S.P., Méndez, M., Vallejo-Marin, M., Schönenberger, J., Burgarella, C., Anderson, B.J., et al. (2023). Trait-dependent diversification in angiosperms: patterns, models and data. *Ecol. Lett.* <https://doi.org/10.1111/ele.14170>.
- Stanley, S.M. (1975). A theory of evolution above the species level. *Proc. Natl. Acad. Sci. USA* 72, 646–650. <https://doi.org/10.1073/pnas.72.2.646>.
- Coyne, J.A., and Orr, H.A. (2004). *Speciation* (Sinauer Associates, Inc).
- Maddison, W.P. (2006). Confounding asymmetries in evolutionary diversification and character change. *Evolution* 60, 1743–1746. <https://doi.org/10.1111/j.0014-3820.2006.tb00517.x>.
- Ricklefs, R.E. (2007). History and diversity: explorations at the intersection of ecology and evolution. *Am. Nat.* 170, S56–S70. <https://doi.org/10.1086/519402>.
- Vamosi, J.C., and Vamosi, S.M. (2010). Key innovations within a geographical context in flowering plants: towards resolving Darwin's abominable mystery. *Ecol. Lett.* 13, 1270–1279. <https://doi.org/10.1111/j.1461-0248.2010.01521.x>.
- Vamosi, J.C., and Vamosi, S.M. (2011). Factors influencing diversification in angiosperms: at the crossroads of intrinsic and extrinsic traits. *Am. J. Bot.* 98, 460–471. <https://doi.org/10.3732/ajb.1000311>.
- Sargent, R.D. (2004). Floral symmetry affects speciation rates in angiosperms. *Proc. Biol. Sci.* 271, 603–608. <https://doi.org/10.1098/rspb.2003.2644>.
- Hodges, S.A., and Arnold, M.L. (1995). Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. R. Soc. B Biol. Sci.* 262, 343–348. <https://doi.org/10.1098/rspb.1995.0215>.
- Fernández-Mazuecos, M., Blanco-Pastor, J.L., Juan, A., Carnicero, P., Forrest, A., Alarcón, M., Vargas, P., and Glover, B.J. (2019). Macroevolutionary dynamics of nectar spurs, a key evolutionary innovation. *New Phytol.* 222, 1123–1138. <https://doi.org/10.1111/nph.15654>.
- Govindaraju, D.R. (1988). Relationship between dispersal ability and levels of gene flow in plants. *Oikos* 52, 31–35. <https://doi.org/10.2307/3565978>.
- Wessinger, C.A. (2021). From pollen dispersal to plant diversification: genetic consequences of pollination mode. *New Phytol.* 229, 3125–3132. <https://doi.org/10.1111/nph.17073>.
- Igic, B., Lande, R., and Kohn, J. (2008). Loss of self-incompatibility and its evolutionary consequences. *Int. J. Plant Sci.* 169, 93–104. <https://doi.org/10.1086/523362>.
- Stebbins, G.L. (1957). Self fertilization and population variability in the higher plants. *Am. Nat.* 91, 337–354. <https://doi.org/10.1086/281999>.
- Holsinger, K.E. (2000). Reproductive systems and evolution in vascular plants. *Proc. Natl. Acad. Sci. USA* 97, 7037–7042. <https://doi.org/10.1073/pnas.97.13.7037>.
- Takebayashi, N., and Morrell, P.L. (2001). Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *Am. J. Bot.* 88, 1143–1150. <https://doi.org/10.2307/3558325>.
- Wright, S.I., Kalisz, S., Slotte, T., and Wright, S.I. (2013). Evolutionary consequences of self-fertilization in plants. *Proc. Biol. Sci.* 280, 20130133. <https://doi.org/10.1098/rspb.2013.0133>.
- Burgarella, C., and Glémin, S. (2017). Population genetics and genome evolution of selfing species. *eLS*, 1–8. <https://doi.org/10.1002/9780470015902.a0026804>.
- Loveless, M.D., and Hamrick, J.L. (1984). Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.* 15, 65–95. <https://doi.org/10.1146/annurev.es.15.110184.000433>.
- Hu, X.S. (2015). Mating system as a barrier to gene flow. *Evolution* 69, 1158–1177. <https://doi.org/10.1111/evo.12660>.
- Goldberg, E.E., Kohn, J.R., Lande, R., Robertson, K.A., Smith, S.A., and Igić, B. (2010). Species selection maintains self-incompatibility. *Science* 330, 493–495. <https://doi.org/10.1126/science.1194513>.
- Zenil-Ferguson, R., Burleigh, J.G., Freyman, W.A., Igić, B., Mayrose, I., and Goldberg, E.E.

- (2019). Interaction among ploidy, breeding system and lineage diversification. *New Phytol.* 224, 1252–1265. <https://doi.org/10.1111/nph.16184>.
36. Barrett, S.C., and Harder, L.D. (1996). Ecology and evolution of plant mating. *Trends Ecol. Evol.* 11, 73–79. [https://doi.org/10.1016/0169-5347\(96\)81046-9](https://doi.org/10.1016/0169-5347(96)81046-9).
 37. Baker, H.G. (1955). Self-compatibility and establishment after "Long-Distance" dispersal. *Evolution* 9, 347–349. <https://doi.org/10.2307/2405656>.
 38. Bouchenak-Khelladi, Y., Onstein, R.E., Xing, Y., Schwery, O., and Linder, H.P. (2015). On the complexity of triggering evolutionary radiations. *New Phytol.* 207, 313–326. <https://doi.org/10.1111/nph.13331>.
 39. Donoghue, M.J., and Sanderson, M.J. (2015). Confluence, synnovation, and depauperons in plant diversification. *New Phytol.* 207, 260–274. <https://doi.org/10.1111/nph.13367>.
 40. Vamosi, J.C., Magallón, S., Mayrose, I., Otto, S.P., and Sauquet, H. (2018). Macroevolutionary patterns of flowering plant speciation and extinction. *Annu. Rev. Plant Biol.* 69, 685–706. <https://doi.org/10.1146/annurev-arplant-042817-040348>.
 41. Jablonski, D. (2008). Species selection: theory and data. *Annu. Rev. Ecol. Syst.* 39, 501–524. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173510>.
 42. Parker, G.A., and Partridge, L. (1998). Sexual conflict and speciation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 353, 261–274. <https://doi.org/10.1098/rstb.1998.0208>.
 43. Renner, S.S., and Ricklefs, R.E. (1995). Dioecy and its correlates in the flowering plants. *Am. J. Bot.* 82, 596–606. <https://doi.org/10.2307/2445418>.
 44. Heilbut, J.C., Ilves, K.L., and Otto, S.P. (2001). The consequences of dioecy for seed dispersal: modeling the seed-shadow handicap. *Evolution* 55, 880–888. 055 [0880:tcodfs]2.0.co. <https://doi.org/10.1554/0014-3820>.
 45. Wolf, D.E., and Takebayashi, N. (2004). Pollen limitation and the evolution of androdioecy from dioecy. *Am. Nat.* 163, 122–137. <https://doi.org/10.1086/380493>.
 46. Vamosi, J.C., and Otto, S.P. (2002). When looks can kill: the evolution of sexually dimorphic oral display and the extinction of dioecious plants. *Proc. Biol. Sci.* 269, 1187–1194. <https://doi.org/10.1098/rspb.2002.2004>.
 47. Glémin, S., and Ronfort, J. (2013). Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. *Evolution* 67, 225–240. <https://doi.org/10.1111/j.1558-5646.2012.01778.x>.
 48. Käfer, J., de Boer, H.J., Mousset, S., Kool, A., Dufay, M., and Marais, G.A.B. (2014). Dioecy is associated with higher diversification rates in flowering plants. *J. Evol. Biol.* 27, 1478–1490. <https://doi.org/10.1111/jeb.12385>.
 49. Sabath, N., Goldberg, E.E., Glick, L., Einhorn, M., Ashman, T.L., Ming, R., Otto, S.P., Vamosi, J.C., and Mayrose, I. (2016). Dioecy does not consistently accelerate or slow lineage diversification across multiple genera of angiosperms. *New Phytol.* 209, 1290–1300. <https://doi.org/10.1111/nph.13696>.
 50. Greenberg, D.A., and Mooers, A.Ø. (2017). Linking speciation to extinction: diversification raises contemporary extinction risk in amphibians. *Evol. Lett.* 1, 40–48. <https://doi.org/10.1002/evl3.4>.
 51. Davies, T.J., Smith, G.F., Bellstedt, D.U., Boatwright, J.S., Bytebier, B., Cowling, R.M., Forest, F., Harmon, L.J., Muasya, A.M., Schrire, B.D., et al. (2011). Extinction risk and diversification are linked in a plant biodiversity hotspot. *PLoS Biol.* 9, e1000620. <https://doi.org/10.1371/journal.pbio.1000620>.
 52. Ho, E.K.H., and Agrawal, A.F. (2017). Aging asexual lineages and the evolutionary maintenance of sex. *Evolution* 71, 1865–1875. <https://doi.org/10.1111/evo.13260>.
 53. Freyman, W.A., and Höhna, S. (2019). Stochastic character mapping of state-dependent diversification reveals the tempo of evolutionary decline in self-compatible onagraceae lineages. *Syst. Biol.* 68, 505–519. <https://doi.org/10.1093/sysbio/syy078>.
 54. Grant, V. (1981). *Plant Speciation* (Columbia University Press).
 55. Yoder, J.B., Gomez, G., and Carlson, C.J. (2020). Zygomorphic flowers have fewer potential pollinator species. *Biol. Lett.* 16, 20200307. <https://doi.org/10.1098/rsbl.2020.0307>.
 56. Neal, P.R., Dafni, A., and Giurfa, M. (1998). Floral symmetry and its role in plant-pollinator systems: terminology, distribution, and hypotheses. *Annu. Rev. Ecol. Syst.* 29, 345–373. <https://doi.org/10.1146/annurev.ecolsys.29.1.345>.
 57. Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., and Ashman, T.-L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Syst.* 36, 467–497. <https://doi.org/10.1146/annurev.ecolsys.36.102403.115320>.
 58. Hernández-Hernández, T., and Wiens, J.J. (2020). Why are there so many flowering plants? A multiscale analysis of plant diversification. *Am. Nat.* 195, 948–963. <https://doi.org/10.1086/708273>.
 59. Tiffney, B.H., and Mazer, S.J. (1995). Angiosperm growth habit, dispersal and diversification reconsidered. *Evol. Ecol.* 9, 93–117. <https://doi.org/10.1007/BF01237700>.
 60. Pianka, E.R. (1970). On r- and K-Selection. *Am. Nat.* 104, 592–597.
 61. Boucher, F.C., Verboom, G.A., Musker, S., and Ellis, A.G. (2017). Plant size: a key determinant of diversification? *New Phytol.* 216, 24–31. <https://doi.org/10.1111/nph.14697>.
 62. Lanfear, R., Ho, S.Y.W., Jonathan Davies, T., Moles, A.T., Aarssen, L., Swenson, N.G., Warman, L., Zanne, A.E., and Allen, A.P. (2013). Taller plants have lower rates of molecular evolution. *Nat. Commun.* 4, 1879–1887. <https://doi.org/10.1038/ncomms2836>.
 63. Falster, D.S., and Westoby, M. (2003). Plant height and evolutionary games. *Trends Ecol. Evol.* 18, 337–343. [https://doi.org/10.1016/S0169-5347\(03\)00061-2](https://doi.org/10.1016/S0169-5347(03)00061-2).
 64. Bond, W.J. (1994). Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. London B* 344, 83–90. <https://doi.org/10.1098/rstb.1994.0055>.
 65. Fenster, C.B., and Martín-Rodríguez, S. (2007). Reproductive assurance and the evolution of pollination specialization. *Int. J. Plant Sci.* 168, 215–228. <https://doi.org/10.1086/509647>.
 66. Joly, S., and Schoen, D.J. (2021). Repeated evolution of a reproductive polyphenism in plants is strongly associated with bilateral flower symmetry. *Curr. Biol.* 31, 1515–1520.e3. <https://doi.org/10.1016/j.cub.2021.01.009>.
 67. Maddison, W.P., and Fitzjohn, R.G. (2015). The unsolved challenge to phylogenetic correlation tests for categorical characters. *Syst. Biol.* 64, 127–136. <https://doi.org/10.1093/sysbio/syu070>.
 68. Rabosky, D.L., and Goldberg, E.E. (2015). Model inadequacy and mistaken inferences of trait-dependent speciation. *Syst. Biol.* 64, 340–355. <https://doi.org/10.1093/sysbio/syu131>.
 69. Nürk, N.M., Linder, H.P., Onstein, R.E., Larcombe, M.J., Hughes, C.E., Piñeiro Fernández, L., Schlüter, P.M., Valente, L., Beierkuhnlein, C., Cutts, V., et al. (2020). Diversification in evolutionary arenas—assessment and synthesis. *Ecol. Evol.* 10, 6163–6182. <https://doi.org/10.1002/ece3.6313>.
 70. Uyeda, J.C., Zenil-Ferguson, R., and Pennell, M.W. (2018). Rethinking phylogenetic comparative methods. *Syst. Biol.* 67, 1091–1109. <https://doi.org/10.1093/sysbio/syy031>.
 71. Onstein, R.E., Carter, R.J., Xing, Y., and Linder, H.P. (2014). Diversification rate shifts in the Cape Floristic Region: the right traits in the right place at the right time. *Perspect. Plant Ecol. Evol. Syst.* 16, 331–340. <https://doi.org/10.1016/j.ppees.2014.08.002>.
 72. van der Niet, T. (2021). Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytol.* 229, 1201–1205. <https://doi.org/10.1111/nph.16813>.
 73. Onstein, R.E. (2020). Darwin's second 'abominable mystery': trait flexibility as the innovation leading to angiosperm diversity. *New Phytol.* 228, 1741–1747. <https://doi.org/10.1111/nph.16294>.
 74. Anderson, B., Ros, P., Wiese, T.J., and Ellis, A.G. (2014). Intraspecific divergence and convergence of floral tube length in

- specialized pollination interactions. *Proc. Biol. Sci.* 281, 20141420. <https://doi.org/10.1098/rspb.2014.1420>.
75. Cossard, G.G., Gerchen, J.F., Li, X., Cuenot, Y., and Pannell, J.R. (2021). The rapid dissolution of dioecy by experimental evolution. *Curr. Biol.* 31, 1277–1283.e5. <https://doi.org/10.1016/j.cub.2020.12.028>.
 76. Minnaar, C., de Jager, M.L., and Anderson, B. (2019). Intraspecific divergence in floral-tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytol.* 224, 1160–1170. <https://doi.org/10.1111/nph.15971>.
 77. Van der Niet, T., and Johnson, S.D. (2012). Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends Ecol. Evol.* 27, 353–361. <https://doi.org/10.1016/j.tree.2012.02.002>.
 78. Lagomarsino, L.P., Forrester, E.J., Muchhala, N., and Davis, C.C. (2017). Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade. *Evolution* 71, 1970–1985. <https://doi.org/10.1111/evo.13297>.
 79. Nosil, P. (2012). *Ecological Speciation* (Oxford University Press).
 80. Catford, J.A., Wilson, J.R.U., Pyšek, P., Hulme, P.E., and Duncan, R.P. (2022). Addressing context dependence in ecology. *Trends Ecol. Evol.* 37, 158–170. <https://doi.org/10.1016/j.tree.2021.09.007>.
 81. Beaulieu, J.M., and O'Meara, B.C. (2016). Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Syst. Biol.* 65, 583–601.
 82. Beaulieu, J.M., O'Meara, B.C., and Donoghue, M.J. (2013). Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Syst. Biol.* 62, 725–737. <https://doi.org/10.1093/sysbio/syw022>.
 83. Beaulieu, J.M., and O'Meara, B.C. (2014). Hidden Markov models for studying the evolution of binary morphological characters. In *Modern phylogenetic comparative methods and their application in evolutionary biology*, L.Z. Garamszegi, ed. (Springer), pp. 395–408.
 84. Höhna, S., Heath, T.A., Boussau, B., Landis, M.J., Ronquist, F., and Huelsenbeck, J.P. (2014). Probabilistic graphical model representation in phylogenetics. *Syst. Biol.* 63, 753–771. <https://doi.org/10.1093/sysbio/syu039>.
 85. Givnish, T.J., Barfuss, M.H.J., Van Ee, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A.C., et al. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Mol. Phylogenet. Evol.* 71, 55–78. <https://doi.org/10.1016/j.ympev.2013.10.010>.
 86. Beaulieu, J.M., and O'Meara, B.C. (2018). Can we build it? Yes we can, but should we use it? Assessing the quality and value of a very large phylogeny of campanulid angiosperms. *Am. J. Bot.* 105, 417–432. <https://doi.org/10.1002/ajb2.1020>.
 87. Beaulieu, J.M., and O'Meara, B.C. (2019). Model clades are vital for comparative biology, and ascertainment bias is not a problem in practice: a response to Beaulieu and O'Meara (2018). *Am. J. Bot.* 106, 613–617. <https://doi.org/10.1002/ajb2.1278>.
 88. Folk, R.A., Sun, M., Soltis, P.S., Smith, S.A., Soltis, D.E., and Guralnick, R.P. (2018). Challenges of comprehensive taxon sampling in comparative biology: wrestling with rosids. *Am. J. Bot.* 105, 433–445. <https://doi.org/10.1002/ajb2.1059>.
 89. Donoghue, M.J., and Edwards, E.J. (2014). Biome shifts and niche evolution in plants. *Annu. Rev. Ecol. Evol. Syst.* 45, 547–572. <https://doi.org/10.1146/annurev-ecolsys-120213-091905>.
 90. Edwards, E.J., de Vos, J.M., and Donoghue, M.J. (2015). Doubtful pathways to cold tolerance in plants. *Nature* 521, E5–E6. <https://doi.org/10.1038/nature14393>.