

INVITED SPECIAL ARTICLE: COMMENTARY

# Model clades are vital for comparative biology, and ascertainment bias is not a problem in practice: a response to Beaulieu and O'Meara (2018)

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In several previous publications we have questioned the significance of large-scale phylogenetic studies, highlighting instead the value of analyzing smaller, comprehensively sampled clades (Donoghue and Edwards, 2014; Edwards et al., 2015, 2017). In the recent issue of the *American Journal of Botany* on the Plant Tree of Life (2018, vol. 105, issue 3), Beaulieu and O'Meara (2018) explored an opposing concern, namely, that the study of selected small clades could lead to incorrect conclusions about evolutionary processes. Specifically, they performed a series of simulations designed to test whether studies of smaller clades present an “ascertainment bias”. The core idea is that comparative biologists tend to study clades that show variation in a trait of interest and, correspondingly, fail to focus sufficient attention on clades that are not so variable in that trait. Consequently, this form of taxonomic sampling could result in a biased inference about the rate of change in the trait of interest across the larger clade that includes both variable and nonvariable subclades. Surprisingly, their simulations showed that this was not generally the case. In fact, averaging across many smaller, variable subclades, there turned out *not* to be a significant deviation from the rates that were estimated for a more inclusive clade based on a random sample of a similar number of species. However, they did show that sampled subclades exhibited much higher variance around the estimated transition rates for the more inclusive clade. Beaulieu and O'Meara (2018) concluded that ascertainment bias is a major problem in comparative biology and argued that large, global phylogenetic analyses are needed to overcome this bias. In

the same special issue, Folk et al. (2018) concurred with their assessment of the “risks” of relying on smaller studies.

Many details of the Beaulieu and O'Meara study are worthy of careful examination, but here we reflect solely on the central conceptual issue that they raised for comparative biologists. Our main concern is that they implicitly misrepresent the motivation behind the study of smaller variable clades. As reflected in their simulations, their premise appears to be that we study small tractable subclades (e.g., *Viburnum*) to estimate transition probabilities for a more inclusive clade (e.g., Campanulidae). But this is typically *not* the goal at all. That is, most comparative biologists are not studying a particular subclade to generate such an estimate for a more inclusive clade. And, indeed, it would be a big risk to extend the inference for a subclade to more inclusive clades, not only because the sample of species is typically much smaller, but also because, as Beaulieu and O'Meara correctly pointed out, our estimate might then be highly biased by the particular subclade in question. If we were, in fact, attempting to estimate rates for a more inclusive clade, there would be an ascertainment bias problem. *However, because those who study smaller clades typically are not intending to extend such estimates beyond the clade of interest, ascertainment bias, as described by Beaulieu and O'Meara, is simply not an issue in practice.*

One might then ask: Why bother studying a smaller variable clade if one is not interested in estimating more global transition rates (or speciation rates, or extinction rates, etc.)? Asked another way: What good are such targeted studies if not to render broader

generalizations of this type? Folk et al. (2018, p. 442) offered that “studies on smaller clades remain crucial for addressing certain specific questions”, implying that they have little relevance to more general evolutionary problems, except perhaps by providing baseline data for the assembly of large comparative data sets. In our view, these ideas miss the mark. Here it is instructive to consider the role that model organisms (species) play in biology. Detailed studies of *Arabidopsis*, maize, tomato, and snapdragon are so useful not because the results from these studies can necessarily be generalized to eudicots, monocots, or all angiosperms (though one might hope that this would be the case). Instead, they are peculiarly useful because detailed studies of these organisms, from multiple different angles, yield insights into underlying mechanisms. Such studies provide the relevant scientific community with causal hypotheses (e.g., the ABCDE model for floral organ identity) that can then be tested in other groups of organisms to try, through detailed comparisons, to establish their generality.

Multidimensional studies of particular, comprehensively sampled, model clades can and should play an analogous role with respect to evolutionary phenomena. That is, they can be (and have actually been) crucibles for the generation of test-worthy hypotheses about underlying evolutionary causes. Model clades are not in any sense “typical”, nor are they generally selected, as Folk et al. (2018) implied, because they are known to capture “generalizable ecological and evolutionary processes” (p. 440) or because they “cross-section broader diversity patterns” (p. 441). As an example, we have pursued a variety of evolutionary questions in *Viburnum* (e.g., Chatelet et al., 2013; Edwards et al., 2014, 2017; Scoffoni et al., 2016), but *Viburnum* was not chosen for these studies because we considered it “typical” of Dipsacales, or campanulids, or asterids, or of angiosperms as a whole, or because we think that we can necessarily generalize in any way to these more inclusive clades. Instead, we simply decided to study it from many different perspectives and to sample it as completely as possible. Our hope is that *Viburnum* will reveal unique insights into the mechanisms underlying evolutionary patterns simply by virtue of paying very close and prolonged attention to it. This long-term attention is what makes model species so important, after all. It is not that *Arabidopsis thaliana* is a “typical” angiosperm that makes it so important, or that we can necessarily generalize from it (despite a prevailing attitude that we can). Indeed, some of the attributes that render *Arabidopsis* an excellent experimental subject (short generation time and tiny genome) are distinctly *not* typical. And, *Arabidopsis* is, at best, only slightly informative when it comes to the study of certain phenomena of tremendous interest, such as the development of secondary xylem or tubular corollas. What makes it valuable is just that we now know so much about it. An entire community of scientists has “piled on” to this system for generations and continues to add value by applying new approaches and investigating new dimensions of *Arabidopsis* biology. The well-known result is that dozens of fundamental discoveries have been made in *Arabidopsis*—discoveries of underlying mechanisms that are actively being tested for their generality.

How do these reflections on model systems specifically relate to the arguments advanced by Beaulieu and O’Meara (2018)? Our view is that, although mega-phylogenetic studies of rates of character evolution across campanulids may be of some interest, such studies are unlikely to yield much insight into the mechanisms responsible for whatever broad patterns are observed at that scale. A global estimate of transition rates between tropical and temperate biomes might demonstrate an overall asymmetry, but it will not,

by itself, provide a direct understanding of what generated that asymmetry. And, in so far as these large-scale studies generally steer clear of identifying any particular evolutionary events, they are nearly powerless to identify specific factors that might underlie such transitions (Donoghue and Edwards, 2014; Edwards et al., 2015). Progress on this front requires far more detailed analyses of the circumstances surrounding multiple individual transitions, and these analyses are precisely what model clades can offer. Ideally, such studies will bring together many more sources of relevant evidence than would be possible in mega-phylogenetic analyses. Furthermore, studies in model clades may allow the integration of phylogenetically informed field studies and/or experimental tests. Our work in *Viburnum* on tropical–temperate biome transitions and the evolution of the deciduous habit provides a concrete example (Edwards et al., 2017). In this case, we were able to integrate extensive field observations on leafing phenology to identify coordinated leaf senescence, in particular, as a parallel response to prolonged freezing during the winter, and a possible mechanism underlying the repeated emergence of a deciduous leafing habit in the temperate zone. Just as studies of model species have yielded testable genetic and developmental hypotheses, studies of model clades are capable of yielding brand new hypotheses on the mechanisms that underlie macro-evolutionary dynamics. It cannot be *assumed* that mechanisms identified in model clades apply more generally, but it can be *tested*!

It is important to note that the comments above do not specifically concern the scope of a study or the size of the clade under consideration. Nor do they concern the presumed homogeneity or heterogeneity of underlying processes. For the study of some particularly conserved traits, it will, of course, be necessary to consider larger trees, and, depending on the trait, the degree of heterogeneity may become problematical at different scales (Graham et al., 2018). Instead, we are emphasizing the great power in actually identifying specific instances of evolutionary change in whatever traits are under consideration, with the aim of discovering the local factors that may have driven such changes.

In making their argument, Beaulieu and O’Meara (2018) imply that we should be spending more time studying clades that do not show variation in the trait of interest (even if these are less tractable). As they expressed it: “the problem comes from looking only at variable, tractable cases” (p. 11)—studying “the hotrods of biology” as opposed to the “minivans” (p. 12). Of course, we agree that nonvariable clades are valuable, especially as they can sometimes provide clear-cut tests of hypotheses derived from studies of variable clades. However, the very natural tendency of evolutionary biologists to first concentrate on clades that show obvious (even extreme) variation in a phenomenon of interest seems completely right-headed to us. For example, if we are interested in the phenomenon of adaptive radiation, it makes perfect sense to start with clades such as the Hawaiian silverswords. Detailed studies of silverswords are most certainly not expected to yield reliable diversification rate estimates for the more inclusive Madianeae, or for the Asteraceae, or the Campanulidae. But we have every reason to expect them to yield concrete hypotheses on the causes of radiation, which can then be tested more broadly.

Beaulieu and O’Meara (2018) also convey the sense that estimates of transition rates at more inclusive levels such as campanulids are somehow more important (or useful) than are such estimates at less inclusive levels. But, why is this the case? One might just as well defend the opposite proposition. That is, estimates

using mega-phylogenies at the level of campanulids may be subject to enormous error, owing to the high likelihood of heterogeneity across clades of such size. Beaulieu and O'Meara are well aware of the heterogeneity problem, having themselves devised methods to address it (e.g., Beaulieu et al., 2013; Beaulieu and O'Meara, 2016). But, a much more fundamental issue is this: What actual work can we do with a rate estimate for a clade such as the campanulids, or for any other clade? What does a transition rate, of say, 42%, mean? What does it tell us about evolution? If one imagines that the goal of such a study is to generalize to an even more inclusive clade (as implied by Beaulieu and O'Meara, [2018], and by Folk et al., [2018]), *then ascertainment bias does become a genuine problem*. Similarly, numbers for campanulids should not be extended to less inclusive clades that have not been studied individually. So, the potential uses for such estimates remain to be clearly identified, and we admit that we continue to puzzle over the biological significance of such parameter estimates. One possibility is that they are informing us about an underlying generative process that has somehow guided the evolution of a clade from the outset. Although this interpretation seems a bit mysterious, if it were true, then a number like 42% could provide genuinely interesting insights into character evolution and the ways in which organismal structure and development may constrain or enable certain evolutionary outcomes (Maynard Smith et al., 1985). Even then, however, rates estimated from phylogenies have obviously been influenced by an untold number of other factors (e.g., exposure to particular environmental conditions), so a particular estimate is necessarily an amalgamation of all of these things. Our point is that the interpretation of what appears to be a simple number is a difficult task and, at the very least, requires additional knowledge of the organisms in question. It is precisely for this reason that studies of well-sampled model clades will take on special importance as comparative biology continues to mature and become more deeply integrative.

From the discussion here, one might imagine that we favor the study of smaller clades because we think they are more likely to be homogeneous with respect to underlying processes and, therefore, to yield more reliable rate estimates. This is not the case. We did not choose to study *Viburnum*, for example, because we determined beforehand that it represented a sweet spot in phylogeny with respect to the homogeneity of processes. In fact, we decided to pursue studies of leaf evolution in this group because we already had some background knowledge of potentially related factors such as branching architecture and because we stood a fighting chance of obtaining a comprehensive sample of the living species. This thinking aligns perfectly with the reasons that people gravitate to model species; that is, it definitely helps to have some background knowledge of a system, and there are existing resources to take advantage of. While we are confident that insights into mechanisms can be gained from the study of smaller model clades, we do not think that such clades are particularly special, either in the sense of being necessarily able to generalize from them (the ascertainment bias problem) or in their unique suitability for evolutionary and ecological studies.

One last point. The most common response that we have heard to our arguments and those of Beaulieu and O'Meara is that, in the end, we are all correct. The idea is that large- and small-scale studies are complementary in the sense that they are doing similar things and offering similar insights, only at different scales. Smaller studies provide insights into recent evolutionary events, while larger ones go deeper in time and tackle the more general evolutionary phenomena. And,

of course, studies at both scales have their risks. This seems like a satisfying resolution, but it is manifestly not the position that we are promoting here. On the one hand, we are arguing that the main risk that has been identified with small studies—ascertainment bias—is not a significant problem for comparative biology. But, more importantly, we hold that small and large studies are not just different in scale, but in kind, especially because small studies, for the reasons outlined above, offer the possibility of identifying evolutionary mechanisms in ways that mega-analyses do not. And, in any case, it is incorrect to suppose that mechanisms discovered in relatively small comprehensively sampled clades are applicable only at that scale. In fact, they can inform the most general issues in evolution, including events in deep time. For their part, megaphylogenies are useful in identifying general patterns, and as we emphasized elsewhere (Donoghue and Edwards, 2014), they light up areas in a large tree that may be especially promising for in-depth studies. In this we agree with Beaulieu and O'Meara (2018, p. 15): “large scale comparative studies... allow for exploration of patterns and for identifying locations of important changes that may or may not correspond to any formally named group and that may require closer examination.”

In summary, we think that Beaulieu and O'Meara (2018) have not fully reflected the intentions of comparative biologists who carry out comprehensive analyses of model clades, and this is at the heart of their concern about ascertainment bias. When such targeted studies are understood not as a means to estimate transition probabilities (and other such rates obtained in phylogenetic studies) at other hierarchical levels, but instead as a means to generate testable mechanistic hypotheses on evolutionary processes, the “problem” of ascertainment bias simply disappears. In our view, model clades are *not* models in the sense that they are somehow representative of a more inclusive lineage, and the term “exemplar clade” (Folk et al., 2018) only serves to increase this confusion. Instead, model clades are models in the alternative sense that they are arenas within which detailed studies, undertaken from multiple angles, may provide us with a deeper understanding of underlying mechanisms.

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