SHORT COMMUNICATION

Repeated evolution of digital adhesion in geckos: a reply to Harrington and Reeder

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Abstract

We published a phylogenetic comparative analysis that found geckos had gained and lost adhesive toepads multiple times over their long evolutionary history (Gamble *et al.*, *PLoS One*, 7, 2012, e39429). This was consistent with decades of morphological studies showing geckos had evolved adhesive toepads on multiple occasions and that the morphology of geckos with ancestrally padless digits can be distinguished from secondarily padless forms. Recently, Harrington & Reeder (*J. Evol. Biol.*, 30, 2017, 313) reanalysed data from Gamble *et al. (PLoS One*, 7, 2012, e39429) and found little support for the multiple origins hypothesis. Here, we argue that Harrington and Reeder failed to take morphological evidence into account when devising ancestral state reconstruction models and that these biologically unrealistic models led to erroneous conclusions about the evolution of adhesive toepads in geckos.

Reconstructing the evolution of phenotypic traits across the tree of life has long been a topic of interest in evolutionary biology, and numerous methods have been devised that employ phylogenies to trace a trait's evolutionary history (Swofford & Maddison, 1987; Harvey & Pagel, 1991; Schluter *et al.*, 1997; Cunningham *et al.*, 1998; Pagel, 1999; Nunn, 2011). These phylogeny-based trait reconstructions represent historical hypotheses, which can inform further investigation into that trait's development and evolution (Strathmann & Eernisse, 1994; Schluter *et al.*, 1997; Cunningham *et al.*, 1998; Lee & Shine, 1998; Griffith *et al.*, 2015). Recently developed methods, such as the binary-state speciation and extinction (BiSSE) model, allow for the co-estimation of a trait's evolutionary history along with its potential effect on diversification rates (Maddison *et al.*, 2007; FitzJohn *et al.*, 2009). BiSSE, and related models, constituted an important advance in comparative methods because they corrected a known bias that can

occur in ancestral state reconstruction methods when diversification rates are state-dependent (Maddison, 2006). Furthermore, BiSSE models offered a potential test of whether a trait can be identified as a 'key innovation': one that is associated with increased efficiency of resource usage and the promotion of ecological opportunity (Maddison *et al.*, 2007; FitzJohn *et al.*, 2009; O'Meara & Beaulieu, 2016). Unfortunately, BiSSE models, as currently implemented, are prone to high type I error rates, biased parameter estimates and incorrect ancestral state reconstructions (Davis *et al.*, 2013; Maddison & FitzJohn, 2015; Rabosky & Goldberg, 2015). Such revelations mean that the results and conclusions of some published analyses employing BiSSE may be erroneous (Maddison & FitzJohn, 2015; Rabosky & Goldberg, 2015; O'Meara & Beaulieu, 2016).

In a recent paper, Harrington & Reeder (2017), hereafter referred to as H&R, reanalysed data from two papers that had applied BiSSE to the reconstruction of ancestral states. The data sets were chosen because the results in the original papers departed from prior published hypotheses and raised the possibility that the unexpected outcomes they generated were due to BiSSE's unreliability. The first data set examined the

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evolution of adhesive toepads in geckos (Gamble *et al.*, 2012) while the second involved the evolution of viviparity across squamates (Pyron & Burbrink, 2014). H&R used several methods to diagnose and alleviate potential BiSSE-related problems with these data, which included testing *a priori* for shifts in diversification rates among sampled lineages, and exploring a wide variety of different models, including the newly developed hidden state speciation and extinction (HiSSE) models, which aim to correct some of the problems encountered when applying BiSSE (Beaulieu & O'Meara, 2016). H&R determined that their revised methodology, including use of an expanded set of models to reconstruct ancestral states, left little support for the original conclusions drawn by both papers. Thus, they considered it unlikely that geckos have repeatedly evolved adhesive toepads and that the most recent common ancestor of squamates was viviparous.

There is no doubt that BiSSE and related models have serious methodological flaws (Davis *et al.*, 2013; Maddison & FitzJohn, 2015; Rabosky & Goldberg, 2015). However, not every study that has used BiSSE is necessarily unsound, and each should be reanalysed and judged independently. In particular, analyses that failed to find an association between trait evolution and net diversification rates may be less suspect than analyses that find positive associations between these parameters because one of BiSSE's failures is increased type I errors (Rabosky & Goldberg, 2015). With this in mind, we respond to H&R's comments regarding the reanalysis of the gecko toepad data (Gamble *et al.*, 2012). In particular, we focus on two of the main points from the H&R paper. First, we argue that the results and conclusions of Gamble *et al.* (2012) were neither unexpected nor a BiSSE artefact. Second, we posit that ambiguity in H&R's gecko toepad ancestral state reconstructions resulted from their use of irreversible models, which are biologically unrealistic given our knowledge of gecko morphology. When just the biologically plausible reversible models are examined, the HiSSE results are concordant with those advanced by Gamble *et al.* (2012). Finally, we offer a more general comment about the use of ancestral state reconstructions to study the repeated evolution of phenotypes.

Before we address our concerns with H&R, we feel it is worth reiterating our previously published conclusions (Gamble *et al.*, 2012). Using a large, multigene gecko phylogeny, Gamble *et al.* (2012) found that the most recent common ancestor to geckos lacked adhesive toepads, that adhesive toepads have been gained and lost multiple times with approximately equal frequency and that this has occurred within several lineages of the Gekkota. Importantly, the BiSSE analyses revealed no association between toepad gains or losses and diversification rates. These results were placed into a broader context by re-examining some of the morphological data. In particular, Gamble *et al.* (2012)

highlighted morphological transformations in an unambiguous gain of adhesive toepads in the genus *Hemidactylus* and several losses of adhesion in the southern African *Pachydactylus/Chondrodactylus* group.

H&R claim that the Gamble *et al.*'s (2012) results were unexpected and that this departure from the 'traditional' view was due to problems associated with BiSSE. First, Gamble *et al.*'s (2012) results were not unique to their BiSSE analyses but were broadly concordant with additional results using parsimony and maximum likelihood. Moreover, Gamble *et al.*'s (2012) failure to find an association between toepad gains or losses and diversification rates places it outside the zone of type I errors that make BiSSE problematic. Concordance across multiple comparative phylogenetic methods, on its own, provides insufficient grounds for accepting or rejecting one hypothesis relative to another, particularly when all available methods have some failings (Schluter *et al.*, 1997; Zhang & Nei, 1997; Maddison, 2006; Goldberg & Igić, 2008; Rabosky & Goldberg, 2015; Wright *et al.*, 2015). However, concordance can indicate signal in the data that is robust to the varying assumptions of the different methods used. Furthermore, concordance can help evaluate support for one hypothesis or another when combined with additional means of hypothesis testing, such as comparative morphology or developmental data (see below), (Cunningham *et al.*, 1998; Lee & Shine, 1998; Griffith *et al.*, 2015; Rabosky & Goldberg, 2015). H&R emphasize the conflict between the results of Gamble *et al.* (2012) and an earlier work (Underwood, 1954) (Fig. 1). However, in the time between Underwood's (1954) and Gamble *et al.* (2012) contributions, other authors had addressed the evolution of adhesive toepads in geckos and had arrived at conclusions that differed from those of Underwood (Haacke, 1976; Russell, 1976, 1979, 2002; Joger, 1985; Kluge & Nussbaum, 1995; Russell *et al.*, 1997; Bauer *et al.*, 2005). To that end, it is worth laying out the details of Underwood's (1954) hypothesis and exploring differences between his and Gamble *et al.*'s (2012) conclusions, and the phylogenetic and anatomical framework within which each was set. Underwood (1954), like others (Haacke, 1976; Russell, 1979), noted that members of the family Eublepharidae exhibit no morphological evidence of ever having had adhesive toepads. Underwood's (1954) estimates of gekkotan phylogeny at the time placed eublepharids as the sister clade to the remaining geckos (Fig. 1). Using this phylogenetic arrangement, Underwood hypothesized that adhesive toepads evolved just once in the most recent common ancestor to the non-eublepharid geckos and that all padless non-eublepharid geckos exhibited a secondary loss of toepads. However, recent phylogenies contradict the idea that eublepharids are the sister clade to all other geckos and instead place them nested within the Gekkota as the sister lineage to a clade composed of the
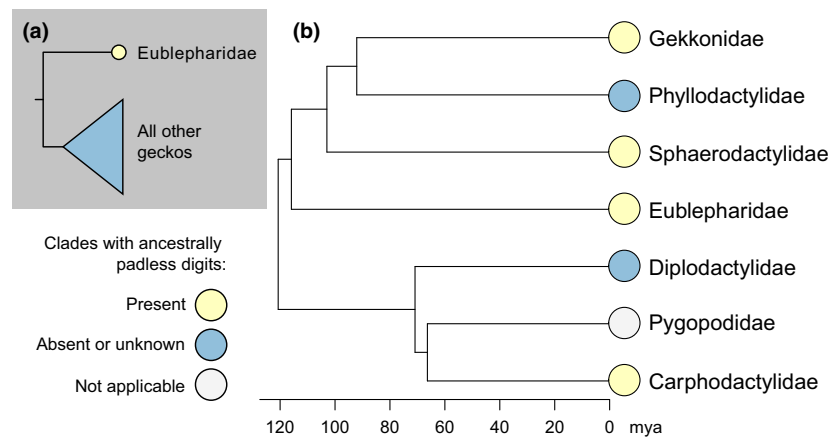
Fig. 1 Morphological evidence countering the hypothesis of a single origin of adhesive toepads in geckos. (a) Phylogenetic hypothesis proposed by Underwood (1954) that prompted him to suggest a single origin of toepads in the non-eublepharid geckos. (b) Current estimates of phylogenetic relationships among gecko families (Gamble *et al.*, 2012), with timescale from Gamble *et al.* (2015b). Four of the seven families have some species with ancestrally padless digits, as identified through detailed morphological examination. These include all species in the Carphodactylidae and Eublepharidae and one or more species in the following genera: *Agamura, Crossobamon, Narudasia, Ptenopus, Stenodactylus* and *Tropiocolotes* (Gekkonidae); *Gonatodes* and *Quedenfeldtia* (Sphaerodactylidae) (Underwood, 1954; Haacke, 1976; Russell, 1976, 1979; Russell *et al.*, 2015). Note that the Pygopodidae are limbless and limb-reduced gekkotans.

Gekkonidae, Sphaerodactylidae and Phyllodactylidae (Han *et al.*, 2004; Townsend *et al.*, 2004; Gamble *et al.*, 2008a,b, 2011; Wiens *et al.*, 2012; Zheng & Wiens, 2016; Fig. 1). This alone renders a single origin of adhesive toepads in geckos far less likely. When one considers that eublepharids are not the only ancestrally padless geckos (Haacke, 1976; Russell, 1976, 1979; Russell *et al.*, 2015), a single-origin hypothesis becomes even more untenable.

Morphological data, when examined in the light of the current gekkotan phylogeny, make it clear that geckos have evolved adhesive toepads on multiple occasions (Fig. 1). Several lineages have a pedal morphology that is fully consistent with the ancestrally padless condition, which is expressed widely among lizard families in general. Also, it has been known for decades that the morphology of geckos with ancestrally padless digits can be distinguished from secondarily padless forms (Haacke, 1976; Russell, 1976, 1979, 2002; Russell *et al.*, 2015). Reversion to a secondarily padless condition carries with it evidence of the prior presence of this structural complex and is fully consistent with Dollo's law (Gould, 1970; Haacke, 1976; Russell, 1976, 1979, 2002; Russell *et al.*, 2015). The complete secondary absence of the adhesive toepad apparatus, as in *Chondrodactylus angulifer* (Gekkonidae) and *Lucasium damaeum* (Diplodactylidae), does not result in reversion to the ancestral phalangeal morphology or proportionality. Thus, it is highly improbable that fully expressed toepads could be forsaken and the ancestral state of all digital features (skeletal, muscular, tendinous, circulatory) be fully reconstituted. Functionally adhesive toepads are complex, integrated, modular structures that

are gained, and lost, in a stepwise fashion (Russell, 1976, 1979; Russell *et al.*, 2015; Higham *et al.*, 2016). The fully expressed adhesive apparatus includes the transformation of epidermal spinules into elaborate setae that enable adhesion through frictional and van der Waals interactions (Russell, 1975, 2002; Autumn *et al.*, 2000, 2002, 2006), expansion of the subdigital surface into scansors (Russell, 1975, 1976, 1979) and modification of muscles and tendons to control the scansors (Russell, 1975, 1976, 1979). Additional, lineage-specific, adaptations include the evolution of specialized phalangeal morphology, blood sinuses and adipose pads to enhance scansor contact with the substrate (Russell, 1981; Russell & Bauer, 1988; Bauer & Russell, 1990), reconfiguration of the form of the autopodium bringing about a symmetrical disposition of the digits (Russell *et al.*, 1997), the elevation of the penultimate and ungual phalanges to segregate these from the underlying scansors (Russell, 1976; Gamble *et al.*, 2012), scansors (with fundamentally different control mechanisms than those exhibited by basally derived toepads) limited to the distal tips of the digit to facilitate movement across dusty substrates (Russell & Delaugerre, 2017) and various modifications of digit I (Russell & Bauer, 1990). Indeed, because the ancestral condition in digit I is to possess only two phalanges, it is subject to constraints with regard to how an adhesive apparatus can be accommodated and operated (Russell & Bauer, 2008). Digit I, therefore, by virtue of the various patterns of configuration that it shows, provides support for the multiple origins of toepads in the Gekkota. In lineages that have acquired adhesive toepads, digit I is variously: 1) omitted from the

expression of the adhesive mechanism (thus retaining the ancestral pattern of phalanx configuration, in contrast to the modifications shown by the other digits); 2) greatly reduced in size (to the point of being vestigial in some lineages); or 3) exhibits extreme morphological modifications (such as hyperphalangy or the loss of the claw and massive elongation of the ungual phalanx) in association with the accommodation and operation of the adhesive mechanism (Russell, 2002).

All of the structural diversity observed across the Gekkota, and the presence of adhesive phenotypes at all stages of transformation (Russell *et al.*, 2015), points to multiple origins of this complex trait. Furthermore, as mentioned, many gecko species are secondarily padless but retain clear anatomical indicators of their pad-bearing ancestry (Haacke, 1976; Russell, 1976, 1979; Lamb & Bauer, 2006). The reduction of 'adhesive toepads' to a single trait, without consideration of the unique morphological intricacies (Russell, 1976, 1979) that characterize the adhesive apparatus of various gekkotan lineages, greatly oversimplifies the situation and overlooks biological evidence essential to the interpretation of the origin of novel morphologies that exhibit functional equivalency (Russell, 1979; Koehl, 1996). This does not mean that comparative analyses that code complex traits, such as adhesive digits in geckos (Gamble *et al.*, 2012), as a single, binary trait are without merit. Such studies can provide a 'bird's-eye view' of a complex trait's evolution and can inform future research, including the evolution of eyes (Oakley & Cunningham, 2002); sex chromosomes (Gamble *et al.*, 2015a); vocal learning in birds (Jarvis *et al.*, 2000); or whole-organism ecomorphologies (Losos *et al.*, 1998). However, it is important that such studies do not become so reductionist that they fail to acknowledge their own assumptions and limitations.

H&R also claim that their results are consistent with '... recent discoveries of stem gekkotan fossils possessing toepads, suggesting that toepads arose very early in the diversification of Gekkota'. The existence of Cretaceous fossil geckos with adhesive toepads is not evidence that the most recent common ancestor of geckos possessed toepads just that toepads arose early in gekkotan evolution. Furthermore, the fragmented nature and uncertain phylogenetic placement of most gekkotan fossils limits their usefulness in understanding toepad evolution. The phylogeny of Cretaceous gekkotans, in particular, remains fluid. As is often the case for fossils, these taxa, with one exception noted by Daza *et al.* (2016) – which is itself a hatchling – are limited to isolated partial skulls (e.g. Mongolian material), or partial limbs/digits, (e.g. Myanmar amber fossils) (Daza *et al.*, 2014). Thus, their precise position in any phylogenetic hypothesis is tentative at best. This is demonstrated by phylogenetic discordance evident in a series of papers as a result of the inclusion of just one additional specimen (Daza *et al.*, 2012, 2016; Conrad & Daza, 2015).

None of the Cretaceous fossils preserves characters that would unambiguously place them in the crown Gekkota; however, molecular dating suggests that many crown gekkotan lineages were present at that time (Gamble *et al.*, 2008a, 2015b; Zheng & Wiens, 2016). The one apparent stem-group gekkotan is a hatchling, and some of the character states that place it in that position may be a reflection of its developmental stage (e.g. large basicranial fenestra, unfused frontal bones) and even if it is correctly placed – it cannot be determined whether it had toepads or not (Daza *et al.*, 2016). Evidence of toepads from the same time frame comes from specimens known only from hands or feet which lack any characters that are diagnostic at a hierarchically more inclusive level than genus (Arnold & Poinar, 2008; Daza *et al.*, 2016). Thus, although we know that toepads were present by 99 million years ago, we cannot determine the phylogenetic placement within Gekkota of the taxa that bore them. As for the digital morphologies, these are represented by (1) *Cretaceogekko* (Arnold & Poinar, 2008), the manus briefly described by Daza *et al.* (2016) and (2) the large foot from the same paper. The former pattern is not unlike the toepad configurations seen in many modern geckos with 'basal' pads (i.e. physically proximal on the digits), whereas the latter is very distinctive and most similar to *Paragehyra*, an extant Madagascan genus. So, to some extent, it could be stated that these digital morphologies are very similar to those of crown-group gekkotans. It is not, however, possible to determine whether one pattern arose from another or whether either of these patterns could be considered plesiomorphic for Gekkota. Although we accept the possibility that padded stem gekkotans may have existed, this cannot currently be demonstrated. Given our argument that toepad 'gains' really are independent elaborations of a system based on the spinulate Oberhäutchen, a feature present ancestrally in crown gekkotans, and presumably also in stem gekkotans, we would not find it surprising if character state changes in adhesive toepads occurred among Cretaceous lineages as well. In other words, the presence of toepads in a stem gekkotan lineage would not preclude lack of toepads as being the plesiomorphic condition for crown gekkotans. This could only be resolved with a robust phylogeny that includes Cretaceous geckos, which is lacking, and by examining larger sample sizes of Cretaceous fossils that might reveal whether both padded and padless conditions were present at that time (currently $n = 3$ for specimens with preserved digits). Recently, Simões *et al.* (In Press) argued that two Jurassic lizards are stem gekkotans and that they show evidence of adhesive toepads. Although their interpretations are interesting and may well be valid, it is worth noting the following: (1) there is disagreement on the placement of *Eichstaettisaurus* and *Ardeosaurus* as stem gekkotans (Evans & Barbadillo, 1999; Evans & Wang, 2005; Conrad, 2008; Reeder

et al., 2015); (2) other authors have found similarities of digit structure between *Eichstaettisaurus* and primitively padless geckos (Russell *et al.*, 1997); and (3) toepads, whether present or absent in these Jurassic forms, do not refute the hypothesis that independent gains or re-elaborations of the fully expressed adhesive apparatus (based on the plesiomorphic spinulate Oberhäutchen) can occur.

Newly developed HiSSE models may be one way of remedying some of the problems inherent in BiSSE analyses (Beaulieu & O'Meara, 2016). H&R examined 16 variations of HiSSE models to reconstruct the evolution of gecko adhesive toepads. These included five irreversible models that restrict character transitions to only change from a padded to a padless state. Thus, an irreversible model forces the most recent common ancestor of geckos to have possessed adhesive toepads, with all padless geckos being accounted for by a loss of toepads (Harrington & Reeder, 2017), similar to Underwood's (1954) hypothesis (but note that Underwood excluded the Eublepharidae). H&R found the model that best fit their data was one of their irreversible models based on the corrected Akaike information criterion (AICc) scores. However, two reversible models had AICc scores only slightly worse than their best fit

irreversible model. To account for this model uncertainty, H&R illustrated their ancestral state reconstruction averaged across all models, weighted according to AICc score. Because the irreversible models force the most recent common ancestor of geckos to have possessed adhesive toepads, some proportion of their results must exhibit a single origin of adhesive toepads. The resulting uncertainty in their ancestral state reconstructions led H&R to state, '…we find no instances of decisive support for multiple gains of toepads, in contrast with the results of Gamble *et al.* (2012)'. However, because geckos with digit morphologies entirely consistent with ancestral padlessness occur across the gecko phylogeny (Fig. 1), the employment of irreversible models appears biologically improbable. We reanalysed the Gamble *et al.*'s (2012) data using H&R's models (Fig. 2). We were able to recapitulate their results and show uncertainty at the root state when ancestral state reconstruction is averaged across all models, weighted according to AICc scores (Fig. 2a). However, examining the reversible and irreversible models separately confirms that the irreversible models are solely responsible for that root uncertainty (Fig. 2b). Indeed, an analysis of just the reversible models recovers a padless gekkotan ancestor with multiple gains and losses of adhesive
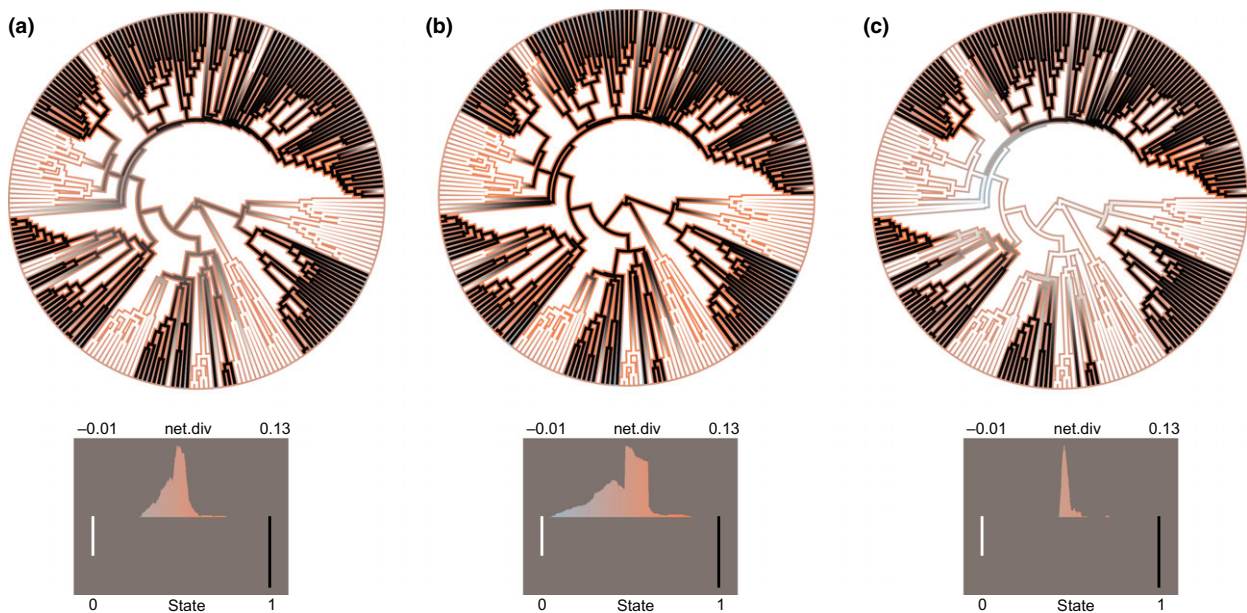

Fig. 2 Ancestral state reconstruction of adhesive toepad evolution in geckos using model-averaged HiSSE. Black branches indicate toepads present (state 1), white branches indicate toepads absent (state 0), and grey branches indicate uncertainty across sampled models. The colour of the branch outline indicates net diversification rates. Histograms below the trees designate the frequency and value of net diversification rates as well as state frequencies (0 or 1) for extant taxa. (a) HiSSE results model-averaged across all tested models. Combined, these models suggest considerable uncertainty about the presence of adhesive toepads in the most recent common ancestor of geckos. This is equivalent to Figure 2 of Harrington & Reeder (2017). (b) HiSSE results model-averaged across only irreversible models. Irreversible models support the presence of adhesive toepads in the most recent common ancestor of geckos. (c) HiSSE results model-averaged across all models except the irreversible models. Reversible models support the absence of adhesive toepads in the most recent common ancestor of geckos. This is consistent with the conclusions of Gamble *et al.* (2012).

toepads (Fig. 2c), consistent with the Gamble *et al.*'s (2012) results using BiSSE, maximum likelihood and parsimony. Thus, H&R's lack of support for multiple gains of gecko toepads results from an artefact of their application of models that are *a priori* unrealistic based on our current knowledge of morphology. It is the ubiquitous presence of epidermal spinules (Ruibal & Ernst, 1965; Maderson, 1970) and their modification into setae (Peattie, 2008; Russell *et al.*, 2015) that serves as the key innovation (Russell, 1979) leading, on multiple occasions, to the subsequent elaboration of an adhesive apparatus (Russell *et al.*, 2015). These outcomes ultimately become clustered under the blanket term 'toepads', but it is not the latter that is the presumed key innovation.

The ability to reconstruct a trait's evolution onto a phylogeny is an important tool for developing and testing hypotheses about the evolution of repeated patterns observed across the tree of life (Harvey & Pagel, 1991). Although the power and general applicability of modern model-based comparative methods are grounded in their quantitative nature, methodological rigour and repeatability, this does not argue for their application in a vacuum. Multiple lines of biological evidence can play a role in model-based phylogenetic comparative methods, both by corroborating or falsifying the homology of the traits under consideration and by informing the models under which these traits may evolve (Koehl, 1996). Careful examination of morphology, along with developmental data, can distinguish ancestral from derived forms and subsequently be incorporated into tests of specific hypotheses emergent from phylogenetic comparative analyses (Bock, 1959; Strathmann & Eernisse, 1994; Blackburn, 2015; Griffith *et al.*, 2015).

Acknowledgments

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