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Novel Tests of the Key Innovation Hypothesis: Adhesive Toepads in Arboreal Lizards

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Abstract.—The evolution of key innovations—unique features that enable a lineage to interact with the environment in a novel way—may drive broad patterns of adaptive diversity. However, traditional tests of the key innovation hypothesis, those which attempt to identify the evolutionary effect of a purported key innovation by comparing patterns of diversity between lineages with and without the key trait, have been challenged on both conceptual and statistical grounds. Here, we explore alternative, untested hypotheses of the key innovation framework. In lizards, adhesive toepad structures increase grip strength on vertical and smooth surfaces such as tree trunks and leaves and have independently evolved multiple times. As such, toepads have been posited as a key innovation for the evolution of arboreality. Leveraging a habitat use data set applied to a global phylogeny of 2692 lizard species, we estimated multiple origins of toepads in three major clades and more than 100 origins of arboreality widely across the phylogeny. Our results suggest that toepads arise adaptively in arboreal lineages and are subsequently rarely lost while maintaining arboreal ecologies. Padless lineages transition away from arboreality at a higher rate than those with toepads, and high rates of invasion of arboreal niches by nonarboreal padbearing lineages provide further evidence that toepads may be a key to unlocking evolutionary access to the arboreal zone. Our results and analytical framework provide novel insights to understand and evaluate the ecological and evolutionary consequences of key innovations.[Arboreality; ecological transition; key innovation; macroevolution; phylogenetic comparative methods.]

Adaptive diversification following the invasion of new ecological zones might be facilitated by the evolution of specialized features (Simpson 1953; Mayr 1960). The appearance of such phenotypes have often been referred to as "key innovations" (Miller 1949; Simpson 1953; Mayr 1963)—new features that allow a lineage to interact with the environment in a novel way, providing ecological access to a new portion of the resource spectrum that may subsequently be evolutionarily exploited (Hunter 1998; Rabosky 2014; Stroud and Losos 2016). For example, the evolution of wings in birds provided access to aerial resources more easily accessible through flight (Mayr 1960), while the evolution of pharyngeal jaws enabled cichlid fish to diversify into a range of foraging specialists (Liem 1973; Mabuchi et al. 2007). However, while key innovations have long been of interest to evolutionary biologists, devising empirical tests of the key innovation hypothesis has been challenging and the subject of considerable debate (Cracraft 1990; Heard and Hauser 1995; de Queiroz 1998; Schluter 2000; Etienne and Haegeman 2012; Givnish 2015; Stroud and Losos 2016; Rabosky 2017).

A major issue in investigating the evolutionary consequences of potential key innovations is that many appear to be "evolutionary one-offs": features that have only evolved once (Hunter 1998; Alfaro 2014; Maddison and FitzJohn 2015; Losos 2017; Uyeda et al. 2017). As such, it is difficult—if not impossible—to investigate the evolutionary consequences of these features in an empirical comparative framework without the existence of convergent analogs (Lauder 1981; Schluter 2000; de Queiroz 2002; Losos 2010; Rabosky 2017). Similarly challenging is that studies evaluating key innovation traits must articulate a clear functional link with how the species or lineage can consequently interact with the environment in a new way or access novel ecological resources (Simpson 1953). Following that rubric, some apparent key innovations have not necessarily led to adaptive radiation (Stroud and Losos 2016). As such, common approaches that have sought to identify any trait that subsequently leads to bursts of speciation or diversification (Von Hagen and Kadereit 2003; Ree 2005; Drummond et al. 2012; Erkens et al. 2012; Silvestro et al. 2014), or to compare sister clade diversity, in which only one group evolved the proposed key trait (Mitter et al. 1988; Zeh et al. 1989; Wiens et al. 2015), have been critiqued on both conceptual and statistical grounds (Slowinski and Guyer 1993; de Queiroz 1998; Schluter 2000; Losos 2010; Drummond et al. 2012; Givnish 2015; Rabosky 2017).

Original conceptualizations of the key innovation hypothesis took a broader view of the evolutionary consequences of key innovations, encompassing a more inclusive synthesis of ecological and evolutionary patterns rather than expansions in species diversity alone (Simpson 1953; Mayr 1960; Van Valen 1971; Baum and Larson 1991; Rosenzweig and McCord 1991). A foundational concept is that the appearance of a key innovation should coincide with the invasion of a new ecological zone (Simpson 1953); however, whether the evolution of such key innovations evolve adaptively following the invasion of the new portion of the ecological resource spectrum (p. 390, Simpson 1953), or exaptively, propelling a lineage into a "*new ecologic plane*" (Miller 1949), remains unclear. Also ambiguous is whether the evolution of a key innovation leads a lineage to become

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"*more or less committed to a* [new] *way of life*" (Van Valen 1971) suggesting evolutionary transitions from the new ecological zone are either challenging or uncommon. However, tests of key innovations that explore these synthetic ecoevolutionary hypotheses are rare.

Here, we explore whether purported key innovation traits evolve adaptively after the invasion of an ecological environment in which they provide a functional advantage. Then, we test two alternative and untested hypotheses of the key innovation framework. First, we ask whether lineages that evolve a key innovation ever lose that trait while continuing to occupy the same ecological environment. And, second, we ask whether lineages that invade an ecological environment without the key innovation transition away to alternative ecologies more frequently than lineages which initially possess the key trait.

To do so, we use an often-cited key innovation that has nonetheless received relatively little critical investigation: subdigital adhesive toepad structures in arboreal lizards (Peterson 1983).

Lizards are a species-rich and globally distributed group that has diversified extensively to fill a variety of ecological niches (Pianka and Vitt 2003; Pianka et al. 2017). Adhesive toepad structures—which increase grip performance in arboreal environments, such as on vertical tree trunks and on smooth surfaces, like leaves (Irschick et al. 1996, 2006; Elstrott and Irschick 2004; Crandell et al. 2014)—have evolved in multiple lineages across a wide geographic and phylogenetic distribution (Williams and Peterson 1982; Losos 2009; Gamble et al. 2012; Hagey et al. 2017; Russell and Gamble 2019). Much debate has ensued about whether the evolution of adhesive toepads in lizards represent a key innovation underlying patterns of lizard diversity in arboreal environments (Williams and Peterson 1982; Slowinski and Guyer 1993; Irschick et al. 1996; Losos 2009; Gamble et al. 2012; Garcia-Porta and Ord 2013; Poe et al. 2018). Given lizard lineages both with and without toepads occupy arboreal niches, a comparative phylogenetic examination of the evolution of toepads and arboreality across the lizard tree of life poses an exceptional opportunity to broadly understand key innovations. Furthermore, while almost all prior investigations of key innovations have sought to associate increased diversification with the evolution of a key trait, we deviate from this framework to explore classical ecoevolutionary concepts (Miller 1949)—evaluating the correlated relationship between a key trait (toepads) and the novel environmental space in which it provides a functional advantage (arboreality).

Here, we present a conceptual and statistical framework to test multiple classical and novel aspects of the key innovation hypothesis involving the evolutionary relationship of adhesive toepad structures and arboreality in lizards. Specifically, we apply a phylogenetic approach with a time-calibrated phylogeny for 2692 lizard species to estimate i) how many times have both adhesive toepads and arboreality independently evolved in lizards, ii) if the evolution of adhesive toepads structures occurs in arboreal or nonarboreal lineages, iii) if arboreal lineages that evolve toepad structures ever transition to padless states while maintaining arboreal ecologies, and iv) if padless lineages transition away from arboreal ecologies at a higher rate than lineages with toepads.

Materials and Methods

Phylogeny and Trait Data

We used a time-calibrated squamate phylogeny (Pyron et al. 2013; Ramm et al. 2020) and isolated all lizard species (excluding snakes) for which we have ecological and morphological trait data (the final pruned tree included 2692 species representing 42 families: see Supplementary material available on Dryad at http://dx.doi.org/10.5061/dryad.4xgxd258r). To briefly characterize the taxonomic coverage of our complementary phylogeny–ecology trait data sets, we account for 39% of extant recognized lizard species diversity (Uetz et al. 2021) covering 87% of recognized genera and 100% of recognized families.

The ecological trait data set (Meiri 2018) assigns each lizard species to 1–3 niche classifications (e.g., a species could be only "Cryptic," or "Cryptic," and "Fossorial") from seven habitat use categories: "Arboreal," "Fossorial," "Marine," "Saxicolous," "Semiaquatic," "Terrestrial," or "Cryptic." We used these classifications to bin each species in our data set into one of three discrete ecological categories—"arboreal" (habitat use classified only as "Arboreal"), "semiarboreal" (at least one classification as "Arboreal"), or "nonarboreal" (no classifications as "Arboreal"). We also scored each species for the presence or absence of adhesive toepads using previously published data (Nicholson et al. 2006; Gamble et al. 2012). We then partitioned our global arboreality data set (Supplementary Table S1 available on Dryad) to investigate the evolution of arboreality as a binary discrete character—i) *Strict* arboreality: containing only species considered strictly arboreal (i.e., those species in our "arboreal" category), and ii) *Relaxed* arboreality: pooling both "arboreal" and "semiarboreal" categories in our data set, with the remaining species classified as "nonarboreal."

As both of our data sets include lizard species spanning a diverse range of ecologies and morphologies (e.g., limbless fossorial and quadrupedal marine), we created pruned data sets that excluded phenotypes biologically unlikely to transition to arboreality (e.g., limbless fossorial species) to avoid producing artifactually low transition rates. To do so, we filtered our "*Full*" data sets (those described above consisting of 2692 species) to a reduced data set ("*Standard*"; Supplementary Table S2 available on Dryad) composed of 2360 species by removing taxa with reduced or absent fore- or hindlegs, as well as those that are fossorial or marine specialists (sensu Meiri 2018). As with the full data set, we created "*Relaxed*" and "*Strict*" versions of

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the *Standard* data set. Thus, in total, we analyzed four data sets—"*Full*" and "*Standard*" with two versions of each ("*Strict*" and "*Relaxed*") to accommodate variable arboreality classifications.

Ancestral State Reconstruction

To determine the appropriate model of trait evolution, we independently fitted two continuous-time Markov models (*Mk* models) to our toepad trait data sets and our *Strict* and *Relaxed* arboreality data sets: an equal-rates model (ER) and an all-rates-different model (ARD) using the *fitMk* function in the R package phytools (Revell 2012; R Core Team 2014). To further investigate the evolution of habitat use, we additionally fitted an ER, ARD, and symmetric rates model (SYM) for multistate habitat use data sets (*Full* and *Standard*) including all three possible occupancy states described above (i.e., "arboreal," "semiarboreal," and "nonarboreal"). Model selection was performed using the Akaike Information Criterion and Akaike Information Criterion weights (AIC and AICw; see Supplementary Table S2 available on Dryad). Ancestral state reconstructions of toepad and habitat use evolution were then estimated with fixed root states for each data set using stochastic character mapping (Huelsenbeck et al. 2003; Bollback 2006) with 500 simulations using the *make.simmap* function in the package phytools (Revell 2012). For all models, we assigned a prior probability of 1 on the root node for the absence of toepads or nonarboreality, respectively, as suggested by paleontological evidence (Evans 2003).

Rates and Phylogenetic Signal

To test for phylogenetic signal in the evolution of arboreality and toepads across the lizard phylogeny, we calculated the D statistic using the *phylo.d* function in the package caper (Fritz and Purvis 2010; Orme 2013). If the value of D is equal to 1, then the observed trait has a phylogenetically random distribution (no phylogenetic signal), whereas if the value of D is equal to 0, the observed trait has evolved in a pattern concordant with that predicted under Brownian motion (strong phylogenetic signal). Observed values of D can also exceed the 0–1 range, suggesting either exceptionally high conservatism ($D < 0$) or overdispersion ($D > 1$) of the relevant trait. We determined significance in our estimate of D with 1000 permutations.

To investigate the evolutionary relationship between the two binary characters of toepad state and microhabitat state, we conducted Pagel's test (Pagel 1994) for evolutionary correlation between two binary characters (toepad state and arboreality state) using the *fitPagel* function with a fixed root state (nonarboreal and padless) in phytools (Revell 2012). Specifically, we tested whether rates of evolutionary transition between arboreality and toepad presence occurred independently (independent model of trait evolution) or in a correlated manner (dependent model of trait evolution).

As a simple Markov model may not capture underlying rate heterogeneity across a phylogeny, we additionally inferred hidden rate classes for the four observed state combinations by leveraging Hidden Markov Models (HMMs; see Boyko and Beaulieu [2020] for a review of the utility of HMMs in phylogenetic comparative methods), modeling both constrained (ER) and unconstrained (SYM and ARD) rate matrices. We reconstructed hidden rate categories in the R package corHMM (Beaulieu et al. 2013; Boyko and Beaulieu 2020) using the *corHMM* function, also fixing the root state to be padless or nonarboreal with a probability of 1. We designed 12 different transition rate models with the number of rate parameters (k) ranging from 1 to 26—the simplest model (least parameterized) being an ER model with a single indexed transition parameter without hidden rates categories and dual transitions disallowed ($k = 1$; a single rate matrix), and the most complex model (most parameterized) being an ARD model with two hidden rate categories and dual transitions permitted ($k = 26$; two rate matrices).

As a pervasive perspective of the key innovation hypothesis (Heard and Hauser 1995) posits that an increase in diversification over time is expected in concert with the evolution of the key trait, and that variable diversification rates can influence the evolution and distribution of traits in general (Maddison et al. 2007), we also implemented a number of speciation and extinction models (SSE) of the Multicharacter Hidden State Speciation and Extinction (MuHiSSE; Nakov et al. 2019) class. To account for/estimate levels of incomplete taxonomic sampling, we generated sampling fractions for each data set by scoring presence/absence of toepads and arboreality for an additional 2952 species that were not included in our phylogeny (5644 species total; Supplementary materials available on Dryad). We recorded the following sampling fractions which represent the proportion of species that we had ecological (arboreality), morphological (toepads) and phylogenetic information for in each state combination relative to extant species occupying those states (presented in the order of padless nonarboreal, padbearing nonarboreal, padless arboreal, and padbearing arboreal): *Full Strict* (0.48, 0.48, 0.40, 0.50), *Full Relaxed* (0.47, 0.43, 0.47, 0.52), *Standard Strict* (0.40, 0.48, 0.40, 0.50), and *Standard Relaxed* (0.37, 0.43, 0.47, 0.52). We ran a total of 16 models for each data set (*Full Strict*, *Full Relaxed*, *Standard Strict*, and *Standard Relaxed*). Note that for all SSE models, we specified a single extinction fraction parameter, which was kept constant across all states, both observed and hidden (if hidden states were present), and specified the root state to be padless nonarboreal at a probability of 1. First, we set up a MuSSE null model where turnover parameters were the same for all observed state combinations. We then followed this

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model with a "true" MuSSE model, where the turnover rate parameters corresponded to different observed state combinations.

We then ran several character-dependent (MuHiSSE) and character-independent (MuCID) multistate models. In our character-independent models, diversification rates are unlinked from observed state combinations and transition rate parameters are constrained to be identical across hidden states, whereas in the character-dependent models, rates of turnover were allowed to vary across the different states and transition rate parameters were allowed to vary across hidden states. We ran MuHiSSE and MuCID models for two through eight hidden states, for each data set, combining for a total of 16 explored models per data set with the MuSSE models. Results were visualized using the utilhisse R package (Nakov et al. 2019). We conducted marginal ancestral state reconstruction on the best-fitting models using the *MarginReconMuHiSSE* function in hisse. Tip-associated net diversification rates were computed using the *GetModelAveRates* function in hisse. We estimated rate parameter confidence intervals (uncertainty in the maximum likelihood estimates [MLE]) for the best-fitting SSE model per data set, as determined by Akaike weights, within a distance of two log-likelihood units of the MLE using the hisse function *SupportRegionMuHiSSE*, sampling 10,000 points. Computational inefficiencies in confidence interval calculations for the *Standard Relaxed* data set required reducing the random sampling variance to 0.05, whereas all other data sets were sampled at 0.1.

Sampling and Parameter Estimation Sensitivity

To explore potential issues of taxonomic sampling and sensitivity biases in our data sets, we asked two questions: i) do any individual species exert substantial influence on rate parameter estimates and ii) how robust are our rate parameter estimates to species exclusions? To answer these questions, we leveraged several features of the sensiPhy R package (Paterno et al. 2018). To examine and identify individual species that may be imposing high influence on rate parameter estimates, we used the *influ_discrete* function to sequentially remove a single species at a time (for each data set independently), estimated rate parameters using both ER and ARD models, and compared these rates to a "full" model with all species included for the respective data set. We consider species imposing a 10% or greater influence on rate parameter estimates (Paterno et al. 2018) as "influential." Using the *samp_discrete* function, we evaluated uncertainty in rate parameters q_{12}(gain of toepads or arboreality) and q_{21} (loss of toepads or arboreality) by randomly removing 10%, 20%, and 30% of all species iteratively 25 times and subsequently calculated rate estimates under ER and ARD models using the *fitDiscrete* function in Geiger (Harmon et al. 2008). We ran these aforementioned sensitivity analyses on all four data sets. Additionally, we exhaustively sampled the Reptile Database (Uetz et al. 2021), the most comprehensive catalog of reptile taxonomic diversity, to review which taxonomic lizard groups are not included in our data set.

Results

Evolution of Arboreality and Toepads in Lizards

In all standard *Mk* models fit for ancestral state reconstructions, an all-rates-differing (ARD) model of evolution was favored by AIC model selection (Supplementary Table S3 available on Dryad). In both the *Full* and *Standard* data sets, ancestral state reconstructions of toepad (Supplementary material available on Dryad) evolution estimated an average of four independent evolutionary origins (95% highest posterior density [HPD]: 3–5 [*Full*], 3–5 [*Standard*]), with reversions to padless states 22 times (95% HPD: 19–26 [*Full*], 20–26 [*Standard*]). An ER model of rate evolution yielded an average of 10 independent origins of toepads (95% HPD: 9–12 [*Full*], 8–11 [*Standard*]), and 12 losses (95% HPD: 10–13 [*Full*], 11–14 [*Standard*]). Our ancestral state reconstructions of habitat use with the *Full Strict* arboreality data set estimated 115 independent evolutionary transitions from nonarboreality to arboreality (95% HPD: 102–131) and 229 reversions from arboreality to nonarboreality (95% HPD: 198–261; Fig. 1), whereas the *Full Relaxed* data set estimated a comparatively far higher average estimate of 267 origins of arboreality (95% HPD: 243–287; Fig. 1) and 236 mean losses (95% HPD: 209–264; Fig. 1). Similarly, the *Standard Strict* data set yielded an estimated 128 average origins of arboreality (95% HPD: 109–144) and 208 average reversions from arboreality to nonarboreality (95% HPD: 171–243), whereas the *Standard Relaxed* data set yielded 277 average transitions from nonarboreality to arboreality (95% HPD: 255–300) and 235 average reversions to nonarboreality (95% HPD: 206–261).

The *Full* reconstruction using a multistate arboreality data set (Supplementary Figs. S3 and S4 available on Dryad)—one which included the intermediate ecology of "semiarboreal"—estimated 252 transitions from nonarboreality to semiarboreality (95% HPD: 223–280), and 174 transitions from semiarboreality to arboreality (95% HPD: 154–193), as well as 183 transitions from arboreality to semiarboreality (95% HPD: 157–211) and 363 transitions from semiarboreality to nonarboreality (95% HPD: 326–397). This model also estimated zero direct transitions from nonarboreality to arboreality, and on average a single transition from arboreality to nonarboreality (95% HPD: 0–3). The *Standard* multistate arboreality data set reconstruction yielded an average of 286 transitions from nonarboreality to semiarboreality (95% HPD: 261–318), 180 transitions from semiarboreality to arboreality (95% HPD: 161–197), 164 transitions from arboreality to semiarboreality (95% HPD: 141–189), and 330 transitions from semiarboreality to nonarboreality (95% HPD: 296–367). This model also estimated zero direct transitions from nonarboreality directly to arboreality, and a mean

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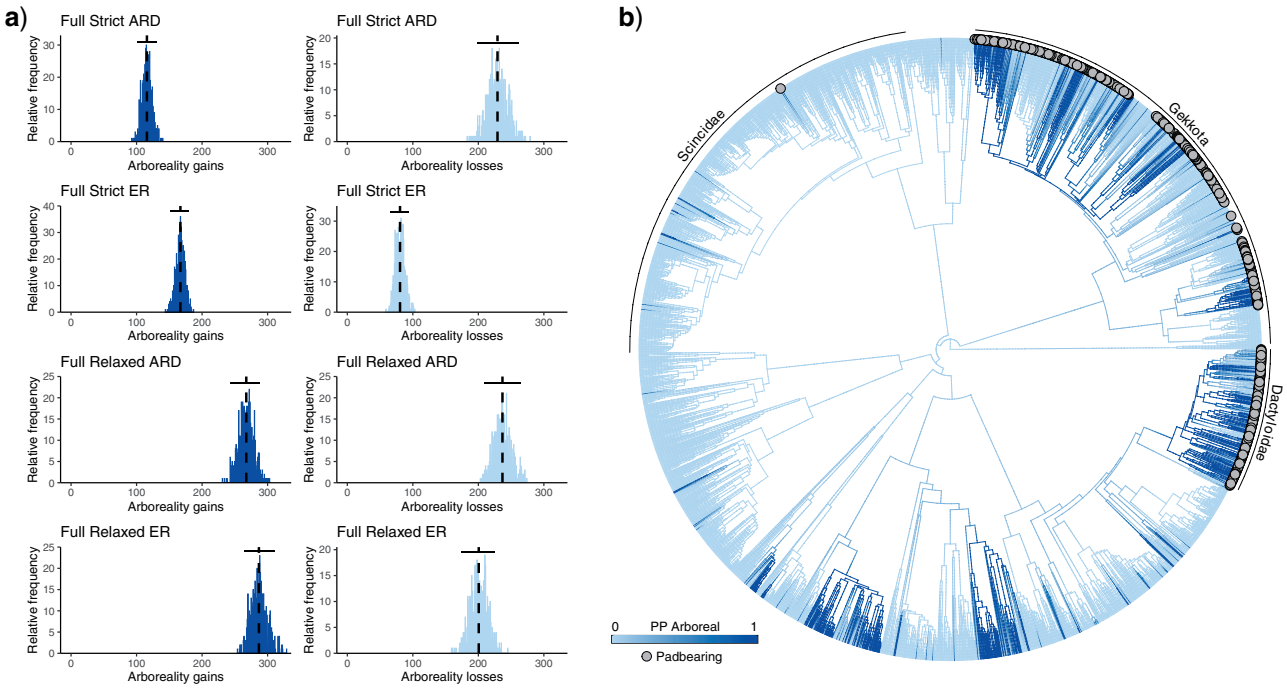

FIGURE 1. The evolution of arboreality in lizards. a) Frequency distribution of gains and losses of arboreality among 500 stochastic maps in the *Full* data set across *Strict* and *Relaxed* variants and ER and ARD models—vertical dashed line indicates mean value, and horizontal black bar indicates 95% highest posterior density interval (95% HPD). b) Phylogeny of 2692 lizards mapping the evolution and origins of arboreal specialists with the *Full Strict* data set. Posterior density for arboreality plotted from 500 stochastic character mappings. Light versus dark color scheme indicates low versus high posterior probability (PP) of arboreality. Gray circles at tips denote the presence of toepads. Major clades with padbearing taxa are labeled.

of 3 transitions (95% HPD: 1–7) from arboreality to nonarboreality.

Tests of Phylogenetic Signal

Our analysis of phylogenetic signal via D statistic calculations (Table 1) with the *Full* and *Standard Strict* arboreality data sets suggest phylogenetic signal in arboreality is present ($D=0.15$; $D=0.11$) and not statistically departing from expectations under a model of Brownian motion ($D=0, P=0.207$; $D=0, P=0.127$). The *Full* and *Standard Relaxed* arboreality data sets yielded D values ($D=0.21$; $D=0.26$) discordant both with a pattern of evolution following a random phylogenetic distribution ($D=1$, $P<0.001$) and a model of Brownian motion ($D=0$, $P=0.024$; $D=0$, $P=0.004$), suggesting weak phylogenetic signal. Toepads displayed a highly conserved pattern of evolution across the lizard phylogeny, both in the *Full* ($D=-0.51$; $D=0$, $P=1.00$) and the *Standard* data sets ($D=-0.49$; $D=0$, $P=1.00$), thus suggesting species more closely related are likely to share this trait.

Evolutionary Rates and the Correlated Evolution of Toepads and Arboreality

Across all data sets, Pagel's test (1994) for correlated evolution among toepads and arboreality favored an ARD model of evolution where these two characters have coevolved, with the exception of the *Standard Relaxed* data set, which favored an ARD model where the rate of change in arboreality state depends on toepad state, but not the converse (AICw = 0.96; Fig. 2; Supplementary Table S4 available on Dryad). In models from all data sets, lineages without toepads transitioned from arboreality to nonarboreality at rates between 1.6 (*Standard Relaxed*) to 7.7 times greater (*Full Strict*) than from nonarboreality to arboreality, respectively. In padbearing lineages, a substantially weaker pattern was observed; transition rates from arboreality to nonarboreality ranged from 1.8 times greater (*Full Strict*, *Standard Strict*, 0.009 vs. 0.017; Fig. 2) to 3 times less (*Standard Relaxed*, 0.007 vs. 0.020; Fig. 2) than the rate of nonarboreality to arboreality.

Transitions to arboreality were more frequent in padbearing lineages, ranging from transition rates 2 (*Standard Relaxed*, 0.021 vs. 0.011; Fig. 2) to 4 times greater (*Full Strict*, 0.010 vs. 0.002; Fig. 2) than in padless lineages. Padless lineages transitioned from arboreality to nonarboreality at a rate 1.1 (*Strict* data sets) to 2.5 (*Relaxed* data sets) times greater than padbearing lineages. With all data sets, we estimated a small but detectable transition rate from padless to padbearing arboreal lineages ([*Standard Relaxed*] 0.0001–0.0003 [*Standard Strict*, *Full Strict*]). However, toepad gain in nonarboreal lineages was estimated to be substantially rarer, with transition rates as low as 5 times less than the corresponding transition rate in arboreal lineages (*Full Relaxed*: 0.00005

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TABLE 1. Tests of phylogenetic signal in the evolution of arboreality and toepads using Fritz and Purvis' D statistic

Data set	Estimated *D*	*P* value *D* different from random phylogenetic distribution (no signal)	*P* value *D* different from Brownian motion (strong signal)
Standard Strict arboreality	0.15	<0.001***	0.127
Standard Relaxed arboreality	0.26	<0.001***	0.004**
Full Strict arboreality	0.11	<0.001***	0.207
Full Relaxed arboreality	0.21	<0.001***	0.024*
Standard toepads	−0.49	<0.001***	1.000
Full toepads	−0.51	<0.001***	1.000

P values denote if the D statistic significantly differs from 0 (Brownian motion) or 1 (randomly distributed). *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

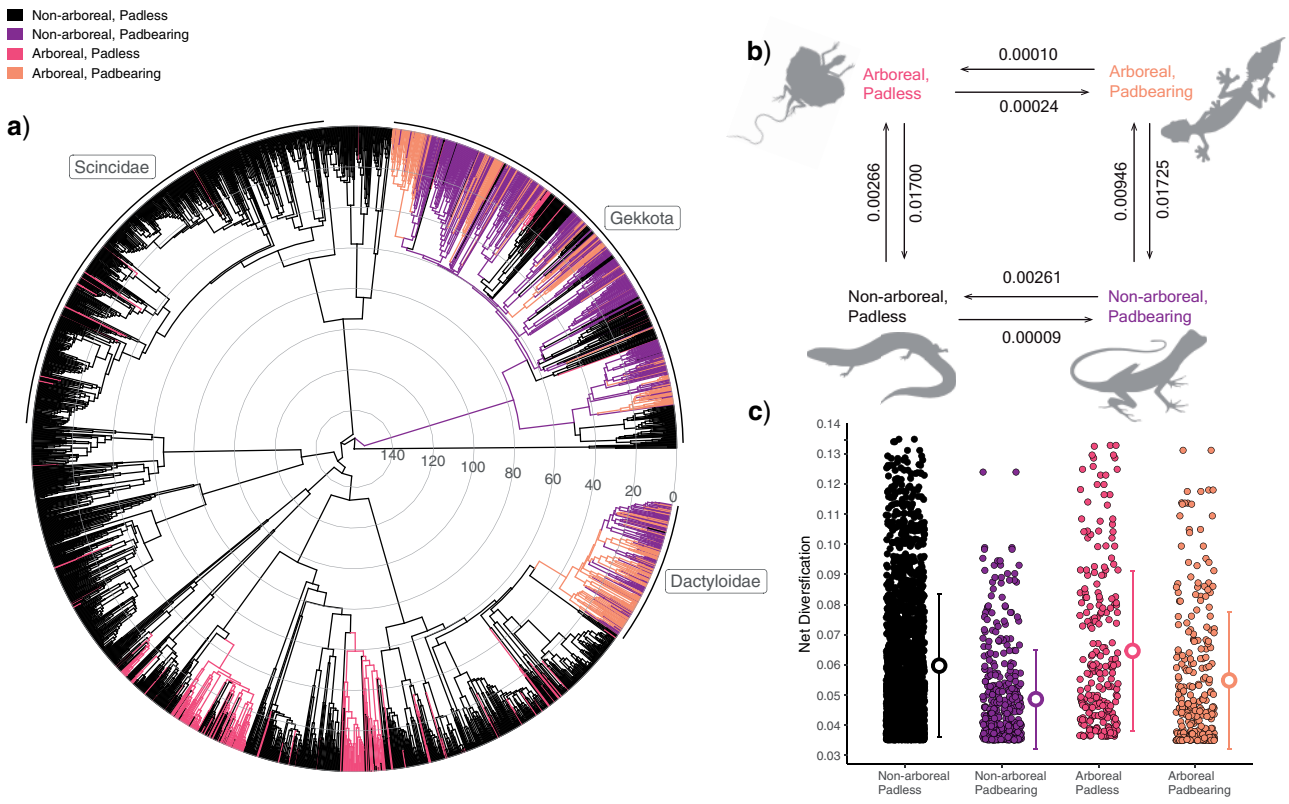

FIGURE 2. The codiversification of arboreality and adhesive toepads in lizards, as modeled in MuCID-7 *Full Strict*. a) Ancestral state reconstruction from the MuCID-7 model, the best-fit diversification model for the *Full Strict* data set. Major clades with padbearing taxa are labeled, and internal numbers denote time (Ma). b) Graphical depiction of transition rate matrix as estimated from the MuCID-7 model. c) Tip-associated net diversification rates from the MuCID-7 model.

nonarboreal gain of toepads vs. 0.00024 arboreal gain of toepads). The loss of toepads in nonarboreality occurred at a rate between 14 (*Full Relaxed*: 0.0039) and 52 times higher (*Standard Strict*: 0.0026) than the loss of toepads in arboreality (*Full Relaxed*: 0.00028; *Standard Strict*: 0.00005), with the exception of the *Standard Relaxed* model in which these rates were equivalent (0.00162) as a result of the best-fit model being arboreality state dependent.

Of the 16 SSE models explored for each data set, a character-independent (MuCID) model with several hidden states was thoroughly favored (*Standard Relaxed* MuCID8: AICw = 0.96; *Standard Strict* MuCID5: AICw = 0.83; *Full Relaxed* MuCID8: AICw = 1; *Full Strict* MuCID7: AICw = 0.52; Fig. 3; Supplementary Table S5 available on Dryad) over character-dependent models and MuSSE models. The best-fit diversification models—null models in which diversification occurs independent of the traits—yielded rate parameter estimates (Table 2; Fig. 2) largely concordant with those estimated from the Pagel (1994) inference (Fig. 2), with confidence interval calculations (Table 2; Fig. 4) indicating generally steep likelihood surfaces surrounding rate parameter maximum likelihood estimates (narrow intervals). Padless lineages transitioned from arboreality to nonarboreality at a rate 2 (*Standard Relaxed*) to 6.4 times (*Full Strict*) higher than the rate in the opposite direction. On the contrary, padbearing lineages transitioned from arboreality to nonarboreality as low as nearly half the rate of the opposite direction (*Full Relaxed*: 0.00835 vs. 0.01789;

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TABLE 2. Maximum likelihood estimates for rate parameters in the best-fitting SSE model (multistate character-independent models [MuCID]) of each data set, and two log-likelihood confidence interval (in brackets)

Data set	q00 → 10	q10 → 00	q00 → 01	q01 → 00	q01 → 11	q11 → 01	q10 → 11	q11 → 10
Standard Strict MuCID5	0.00297 [0.00270–0.00326]	0.01861 [0.01590–0.02166]	0.00008 [0.00007–0.00016]	0.00296 [0.00276–0.00352]	0.00936 [0.00803–0.01015]	0.01734 [0.01539–0.01918]	0.00029 [0.00029–0.00080]	0.00004 [0.00004–0.00064]
Standard Relaxed MuCID8	0.00962 [0.00854–0.01064]	0.01886 [0.01653–0.02186]	0.00007 [0.00007–0.00019]	0.00431 [0.00357–0.00460]	0.01787 [0.01715–0.01929]	0.00841 [0.00761–0.00985]	0.0002 [0.00019–0.00040]	0.00029 [0.00026–0.00066]
Full Strict MuCID7	0.00266 [0.00225–0.00296]	0.017 [0.01500–0.02155]	0.00009 [0.00012–0.00028]	0.00261 [0.00182–0.00306]	0.00946 [0.00762–0.01187]	0.01725 [0.01211–0.02084]	0.00024 [0.00042–0.00100]	0.0001 [0.00053–0.00094]
Full Relaxed MuCID8	0.00826 [0.00693–0.00926]	0.01903 [0.01566–0.02209]	0.00006 [0.00006–0.00025]	0.00377 [0.00316–0.00453]	0.01789 [0.01462–0.02128]	0.00835 [0.00644–0.01171]	0.00022 [0.00018–0.00066]	0.0003 [0.00028–0.00071]

00: nonarboreal padless; 01: nonarboreal padbearing; 10: arboreal padless, 11: arboreal padbearing. The number next to each multistate character-independent (MuCID) model in the "Data set" column indicates the number of hidden states.

Standard Relaxed: 0.00841 vs. 0.01787), and as high as 1.8 times greater (*Full Strict*: 0.01725 vs. 0.00946; *Standard Strict*: 0.01734 vs. 0.00936). Padless lineages transitioned from arboreality to nonarboreality at a rate 1.1 to 2.3 times greater than padbearing lineages, with the exception of *Full Strict* data set (nearly equal rates of transition).

Transitions to arboreality in padbearing lineages across these SSE models occurred at a rate between 1.8 and 3.5 times greater than in padless lineages (*Standard Relaxed*, 0.01787 vs. 0.00962; *Full Strict*, 0.00946 vs. 0.00266). Similar to the rates estimated by the Pagel method, we estimated a small transition rate from padless to padbearing arboreal lizards ([*Standard Relaxed*] 0.0002–0.00029 [*Standard Strict*]), a high enough magnitude to outpace the rate of toepad evolution in nonarboreal lizards by a factor of 2.8 (*Full Strict*: 0.000242 vs. 0.000086) to 3.8 times (*Full Relaxed*: 0.00022 vs. 0.000057). Furthermore, the loss of toepads in nonarboreality occurred at a factor of 12 (*Full Relaxed*: 0.00377) to 75 (*Standard Strict*: 0.003) times higher than the loss of toepads while maintaining an arboreal ecology (*Full Relaxed*: 0.0003; *Standard Strict*: 0.00004).

Net diversification rates (Fig. 3) were highest in the padless arboreal state (with the exception of the *Standard Relaxed* data set, in which padless nonarboreal taxa took top honors), and consistently lowest in the padbearing nonarboreal state. To briefly summarize SSE-derived ancestral state reconstructions (see Fig. 3 for *Strict Full* reconstruction; Supplementary material available on Dryad for others), marginal ancestral state reconstructions across all data sets for the best-fitting models yielded agreement in a padbearing, arboreal, most recent common ancestor (MRCA) for anoles (Dactyloidae), a padbearing, nonarboreal, MRCA of geckos (Gekkota), and a padless, nonarboreal, MRCA for skinks (Scincidae).

Across all data sets, corHMM analyses (Fig. 5; Supplementary Table S6 available on Dryad) yielded highest AICw support to SYM/SYM models (two rate matrices under symmetric rate conditions), although the *Strict* data set models favored allowance of diagonal transitions (14 rate parameters) while the *Relaxed* versions favored disallowance of such transitions (10 rate parameters). The dominance of these aforementioned models over single-rate class models indicates the presence of rate heterogeneity across the phylogeny. Within rate classes, transitions to arboreality from nonarboreality were always higher in padbearing lineages, with the exceptions of the rate class 1 in the *Standard Strict* data set (Fig. 5f) and rate class 2 in the *Standard Relaxed* data set (Fig. 5e). In both *Relaxed* data sets (Fig. 5b,e), toepads evolve in arboreality (q10 → 11) at a rate 1.8 to 3.7 times higher than in nonarboreality. Dual transitions (e.g., q00 → 11, the "simultaneous" evolution of toepads and arboreality) appear to be quite rare, with all dual transition rates as zero in the *Strict* data sets with the exception of q00 → 11 in rate class 2 (*Standard Strict*: 0.0001; *Full Strict*: 1.21×10^{-6}).

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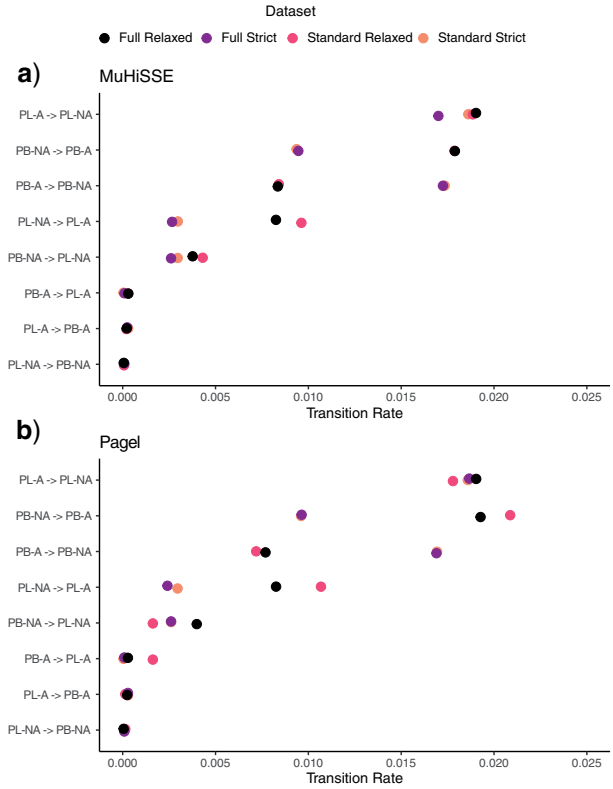

FIGURE 3. Transition rate estimates across best-fitting a) MuCID and b) Pagel models for each data set. PL = padless; PB = padbearing; A = arboreal; NA = nonarboreal.

Sensitivity Analyses and Diagnosis of Potential Sampling Bias

In both the *Full* and *Standard* data sets, including both the *Strict* and *Relaxed* variants, leave-one-out analyses detected no species exerting a 10% or greater change in the rate parameters values for the evolution or loss of arboreality, regardless of the model of rate evolution (ER or ARD), with the singular exception of the *Strict Standard* ARD model, in which four gecko species (*Cnemaspis podihuna*, *Ebenavia inunguis*, *Lygodactylus pictus*, and *Lygodactylus tuberosus*) exhibited between a 10.4% and 11.6% influence on arboreality gain, and five species between 11.3% and 14.4% (the aforementioned four and *Paroedura masobei*) on the loss of arboreality.

In the *Full* and *Standard* data sets, an ARD model of rate evolution yielded high estimates of taxon-specific phylogeny-wide influence in the evolution of toepads for five species—*Alsophylax pipiens* (*Full*: 11.3%; *Standard*: 11.8%; Gekkonidae), *Microgecko helenae* (*Full*: 13.2%; *Standard*: 14.0%; Gekkonidae), *Prasinohaema virens* (*Full*: 27%; *Standard*: 30%; Scincidae), *Euleptes europaea* (*Full*: 93%; *Standard*: 94%; Sphaerodactylidae), and *Chatogekko amazonicus* (*Full*: 108%; *Standard*: 116%; Sphaerodactylidae). An ER model of rate evolution did not recover this same result, yielding no influential species beyond 10%, with the most influential species being *Anolis onca* (Dactyloidae) and *Lucasium damaeum* (Diplodactylidae), both of which imposed a 4.6% change in toepad rate parameters in both directions.

In evaluating the effect of sampling uncertainty (Fig. 6) in estimating rate parameters for the gain or loss of arboreality, iterative random removal of 10%, 20%, and 30% of sampled species in both the *Full* and *Standard* data sets (Supplementary Tables S7 and S8 available on Dryad) yielded a mean percent change in rate parameter estimates greater than 10% in only the *Full Strict* ARD and *Standard Strict* ARD models at the 30% taxon removal treatment level (11.0–12.8%). Random proportional taxon removal had a far greater consequence when inferring toepad evolution rate parameters, although disproportionally in the direction of toepad gain, where the maximum mean percent change in the gain of toepads was nearly 48% (*Standard* ARD at 30% removal treatment), whereas the maximum mean percent change in the loss of toepads was a 23% mean change (*Full* ARD at 30% removal treatment).

The average proportion of species sampled per family in the *Full* data set was 52%. Of the large lizard families, we have low sampling of Gekkonidae and Scincidae. Specifically, we sample 25% of Gekkonids (341 of ca. 1400 species) and 36% of skinks (Scincidae; 618 of 1715 species). However, there are only two families with >20 species in which we sample $<25\%$ of species: Amphisbaenidae (20/182 species; a nearly all limbless

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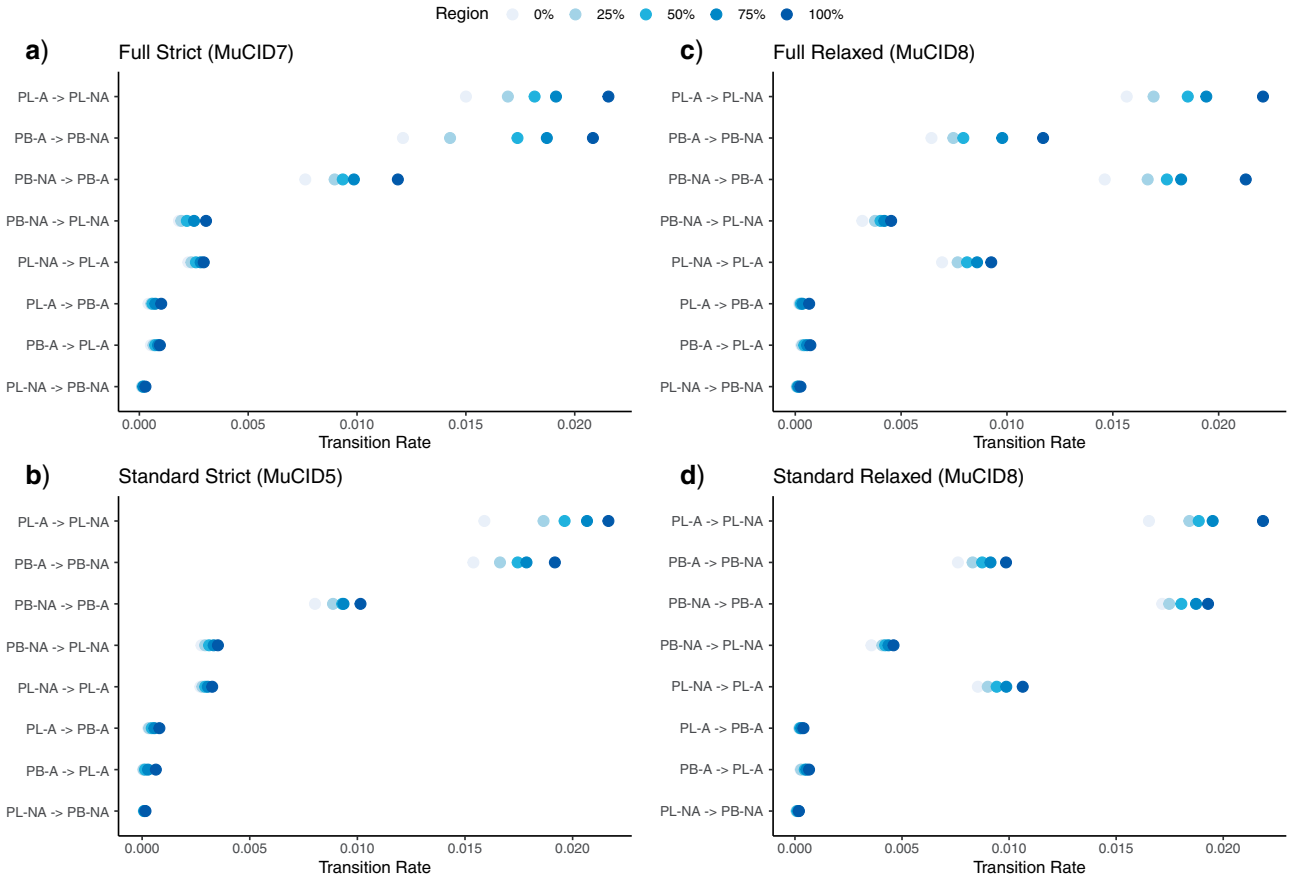

FIGURE 4. Uncertainty (confidence intervals) in the maximum likelihood estimates for transition rates estimated in SSE analyses for the best-fitting model of each data set, as calculated by adaptive sampling of the likelihood surface. Points are sampled within a two log-likelihood unit distance from the maximum likelihood estimate. Character states are abbreviated as follows PL = padless; PB = padbearing; A = arboreal; NA = nonarboreal.

group) and Leiocephalidae (6/29 species). Such data limits are difficult to avoid in an era experiencing an explosion of biodiversity discovery (Hortal et al. 2015); more than 1300 new lizard species have been described since 2010 (Uetz et al. 2021), comprising approximately 20% of extant diversity, yet for many species basic ecological data such as habitat use remain scarce (Stroud and Thompson 2019).

DISCUSSION

Independent evolutionary transitions to arboreality have occurred extensively throughout the diversification of lizards (Fig. 1). Using a conservative data set of strictly arboreal specialists (*Full Strict*), we estimate that the independent colonization of arboreal niches has occurred repeatedly (mean estimate 115 transitions [95% HPD: 102–131]) and broadly across the lizard phylogeny in 22 of 42 sampled families. Using a more inclusive data set (*Full Relaxed*), one that also includes an intermediate category of semiarboreal species as "arboreal," we estimate an average of 267 independent transitions to arboreality (95% HPD: 243–287).

In agreement with previous studies (Gamble et al. 2012; Hagey et al. 2017; Russell and Gamble 2019), we infer multiple origins of adhesive toepads in lizards; once each in Dactyloidae (*Anolis* spp.) and Scincidae (*P. virens*) and multiple origins in Gekkota (geckos) (Supplementary Fig. S3 available on Dryad). Our analyses also highlight that adhesive toepads are highly phylogenetically conserved in lizards (Table 1).

Evolutionary Evidence for a Key Innovation

To investigate the role of adhesive toepads as a key innovation in the evolution of arboreality in lizards, we tested three features of the key innovation framework i) if the evolution of a key trait occurs adaptively or exaptively, in other words either before or after the invasion of an ecological environment in which it provides a functional benefit, ii) if lineages which possess the key trait ever lose that trait while remaining in that ecological environment, and iii) if lineages without the key trait which also enter the ecological environment transition away to alternative ecological space at a higher rate.

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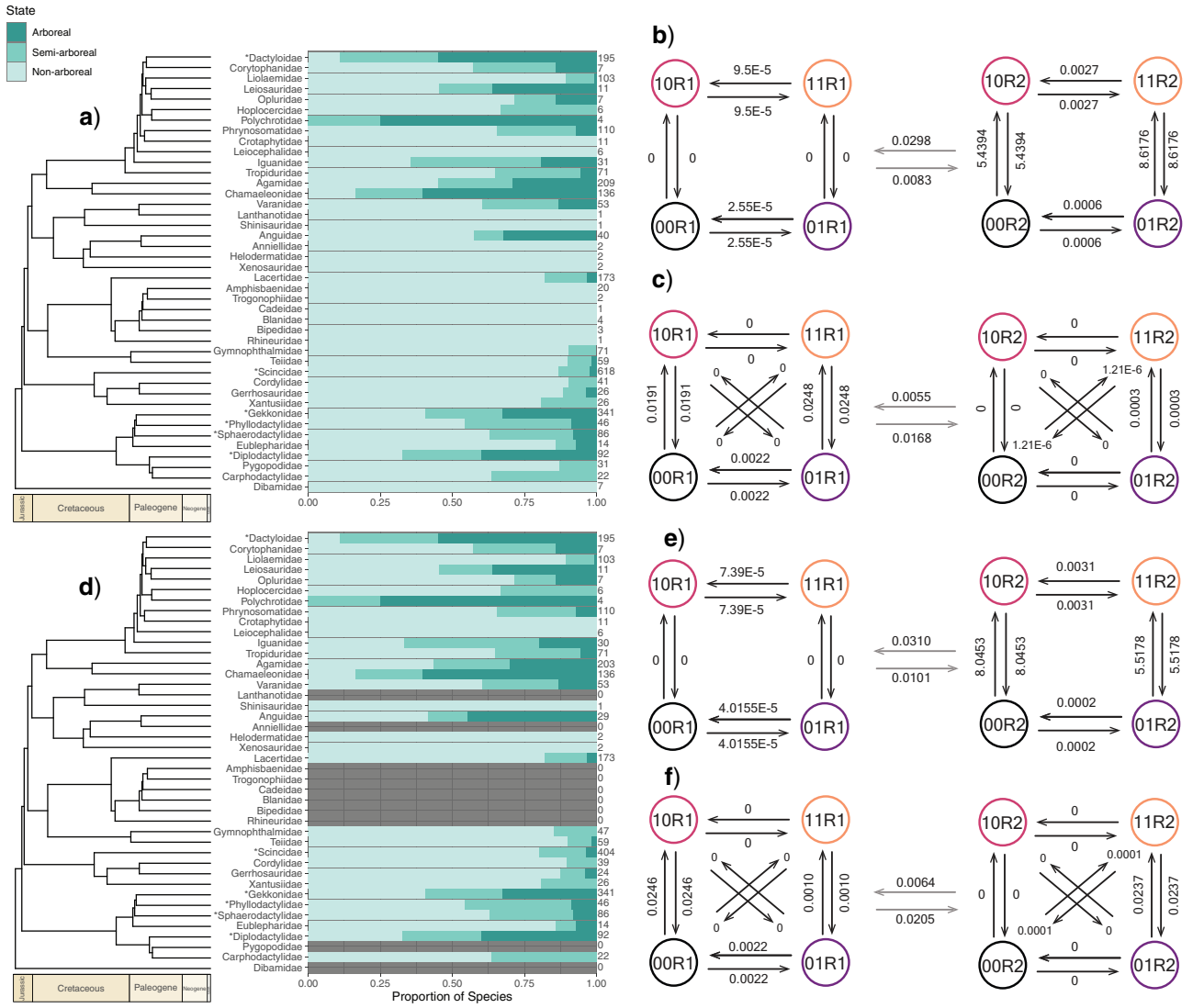

FIGURE 5. Hidden rate estimates for the coevolution of arboreality and toepads. Family-level lizard phylogeny (sensu Pyron et al. [2013]) for the *Full* (a–c) and *Standard* (d–f) data sets and the proportion of species within each lizard family occupying either arboreal, semiarboreal, or nonarboreal microhabitats (relative to sampled taxa). Families with asterisks contain species with toepads. Values adjacent to bars (right) are the total number of species sampled for each family. Rate matrices visualizations as estimated in corHMM for b) *Full Relaxed*, c) *Full Strict*, e) *Standard Relaxed*, and f) *Standard Strict*. Arrows between rate matrices denote the parameter process, the rate of transition between rate categories. "R1" indicates rate category 1, and "R2" indicates rate category 2. States follow as: 00: nonarboreal padless; 01: nonarboreal padbearing; 10: arboreal padless, 11: arboreal padbearing.

The form–function relationship between adhesive toepads and arboreal habitat use in lizards is well established (Irschick et al. 1996, 2006; Elstrott and Irschick 2004; Crandell et al. 2014), providing clear functional evidence for the role that toepads may play as a key innovation to arboreal environments. Digital toepads greatly increase clinging ability (Irschick et al. 1996), as well as locomotor performance on vertical or inclined surfaces (Russell 1975; Williams and Peterson 1982; Irschick et al. 1996; Vanhooydonck et al. 2005; Donihue et al. 2018, 2020), due to microscopic setae promoting adhesion via van der Waals forces and complex frictional interactions (Autumn et al. 2000, 2006; Rizzo et al. 2006). Given strong evidence for the correlated and repeated evolution of adhesive toepad structures and arboreality across the lizard phylogeny (Fig. 2; Supplementary Table S3 available on Dryad), and a clear form–function relationship between toepads and structural habitat features found in arboreal environments, we first asked a classic question of the key innovation hypothesis (Simpson 1953): are such traits adaptive, evolving *in situ* after the colonization of arboreal niches, or exaptive, evolving prior to transitions to the arboreal environment? Our rate parameter estimates uncovered that the evolution of toepads while in an arboreal state has occurred at a rate far greater (as high as a factor of 5) than lineages in nonarboreal states (Supplementary Table S4 available on Dryad). As such, our analyses

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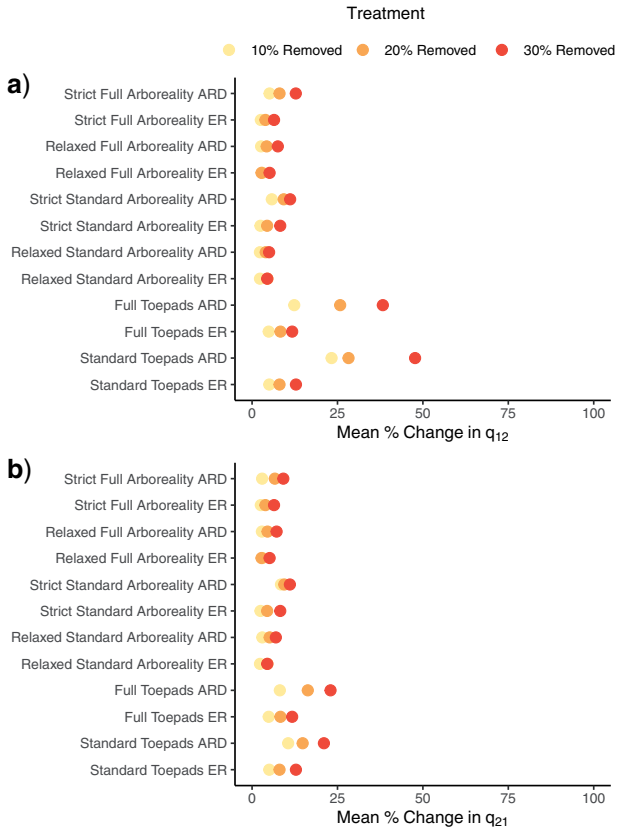

FIGURE 6. The influence of taxon sampling on rate parameter estimates, as determined by the iterative random removal of a proportion of species amounting to either 10%, 20%, or 30% of total sampling. Y-axis ticks indicate the applied categorization condition (*Strict* or *Relaxed*), the data set (*Full* or *Standard*), the character (arboreality or toepads), and model of trait evolution (ER or ARD).

suggest that toepads have primarily evolved in arboreal lineages (Figs. 2–5).

Second, we asked if arboreal lineages that evolve toepad structures ever transition to padless states while maintaining arboreal ecologies. In other words, if, once evolved, a key innovation is ever lost while maintaining occupation of the same ecological niche space in which it evolved. While we recorded evidence of low transition rates from padbearing to padless states in arboreality, these transitions occurred at a factor as high as 75 times lower than the loss of toepads in nonarboreality.

Lastly, we asked whether padless lineages transition away from arboreal ecologies at a higher rate than lineages with toepads. While the presence of specialized adhesive toepad structures is not necessary for lineages to become arboreal, our analyses estimated that padless lineages transitioned from arboreality to nonarboreality more frequently than lineages which possessed toepads. As our previous results suggest that toepad structures mainly evolved in arboreal lineages, the presence of toepads in most nonarboreal lineages likely represent transitions to terrestriality from arboreality. As such, toepads in nonarboreal lineages may represent exaptations to arboreality; preadapting or evolutionarily predisposing lineages for re-invasion of the arboreal environment, which our analyses estimate frequently occurred (Fig. 2). Together, our results suggest that adhesive toepad structures, which originally evolved adaptively following the invasion of an arboreal environment, represent key innovations to arboreality in lizards.

While there exists strong support for the correlated nature of the evolution of toepads and arboreality, we also detected multiple transitions of padbearing lineages from arboreality to nonarboreality. Specifically, 393 of 646 padbearing species in our study are not classified as strictly arboreal specialists. Instead, many are considered "semiarboreal" (191 of 393 species); in other words, some portion of their ecology encompasses arboreal habitats. For example, only one of 436 extant species in Dactyloidae (i.e., *Anolis* spp.) lacks toepads—the terrestrial Venezuelan sand dune specialist *A. onca* (Nicholson et al. 2006)—despite many species having semiarboreal, rather than exclusively arboreal, ecologies (67 of 195 sampled *Anolis* spp. are "semiarboreal"). It is possible that semiarboreal species may still experience selection pressures that favor toepad possession, while not being as ecologically restricted to arboreal environments as exclusive arboreal specialists. It is also possible that structural features of some nonarboreal habitats, such as saxicolous (rock-dwelling) environments (Zhuang et al. 2019), present species with similar selection landscapes

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to arboreality, maintaining the high functional advantage of increased grip performance. It is clear that a better understanding of the function of toepads in lizards in nature will be crucial to understand their mechanistic role in underlying evolutionary patterns of ecological diversity (Irschick et al. 1996; Elstrott and Irschick 2004; Losos 2009; Gamble et al. 2012; Stroud and Losos 2020).

Although transition rate estimates for the evolution of toepads—and, dependent upon the data set and condition, arboreality—appears to be partially sensitive to a handful of species under an ARD model of rate evolution, we find biological and statistical resilience in our results for a number of reasons. For example, it may be expected for some species to yield inherent influence over parameter estimates when possessing a trait unique relative to other clade members. We observed rate parameter estimates for the evolution of toepads to be sensitive to inclusion/exclusion of *P. virens*, a species that represents the only case of adhesive toepad evolution in Scincidae (>1700 species) and the only example in lizards outside of Gekkota (geckos) and Dactyloidae (anoles). We also detected a strong influence of *C. amazonicus* and *E. europaea*, but these results are less biologically interpretable; both species are monotypic in their genera and have complex histories of phylogenetic uncertainty (Gamble et al. 2008, 2011). However, the ARD and ER models agree on a general hypothesis of multiple origins of toepads within both lizards broadly (Gekkota, Dactyloidae, and Scincidae) and Gekkonidae specifically, supporting other studies with similar results (Gamble et al. 2012; Hagey et al. 2017; Russell and Gamble 2019). Furthermore, we found strong concordance between ARD and ER models in our rate parameter estimates using the Pagel (1994) method, with the mean variance across all data sets for a given transition between ER and ARD models estimated to be exceptionally small (1.6×10^{-5}; e.g., *Full Strict* ARD coevolving q00 → 01 vs. *Full Strict* ER coevolving q00 → 01). Likewise, the mean variance for a rate parameter estimate within the same data set for a best-fitting SSE model and best-fitting Pagel model (e.g., *Full Strict* SSE q00 → 01 vs. *Full Strict* Pagel q00 → 01) was 4.27×10^{-7}.

Here, in identifying the correlated relationship between the evolution of adhesive toepads and the evolution of arboreality in lizards, several questions specific to this model system remain: did similar selective pressures lead to the independent adaptive evolution of toepads? Do toepads serve different functions in different lineages? Why have adhesive structures not evolved in padless lineages? What role has behavior played in the evolution of toepads? It is likely that the answers to these questions may only come to light through a broader synthesis of comparative morphology, experimental tests of the form–function relationship, and detailed natural history observations of habitat use and associated behaviors in the wild. To fully understand the origins of lizard diversity, and the evolutionary impact of toepads as a key innovation, approaches will need to be designed to bridge the divide linking ecological and microevolutionary processes with macroevolutionary patterns (Stroud and Losos 2020).

Determining the "Evolutionary Success" of a Key Innovation

Throughout much of the contemporary history of evolutionary biology, tests of the key innovation hypothesis have centered on exploring whether the presence of key traits can explain comparative differences in diversity (Von Hagen and Kadereit 2003; Ree 2005; Alfaro et al. 2009; Erkens et al. 2012; Silvestro et al. 2014; Fernández-Mazuecos et al. 2019). The reliance on such lines of evidence as measures of the "evolutionary success" of a key innovation grew in tandem with the wider availability of densely sampled, time-calibrated molecular phylogenies (Hunter 1998; Alfaro 2014). However, such metrics represent a restrictive measure of "evolutionary success." Fundamentally, the key innovation hypothesis predicts that lineages in possession of key novelties should be more successful in the ecological environment in which the trait(s) provides a novel, functional benefit than closely related lineages which have not evolved the key trait. Here, we develop the theory of key innovations to include novel measures of ecological and evolutionary success. Specifically, using a hypothesized model system of adhesive toepad structures as key innovations in the evolution of arboreal specialization in lizards, we test whether a key innovation is ever lost while lineages remain in the ecological niche space in which the trait adaptively evolved, and explore whether lineages in possession of the putative key trait transition out of the ecological niche space to alternate ecologies at a lower rate than lineages without the key trait. With increasing availability of ecological trait databases spanning vast geographic and taxonomic distributions, and in partnership with densely sampled, time-calibrated molecular phylogenies, it will be possible to apply these novel tests to many putative cases of evolutionary key innovations.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4xgxd258r. Code available on GitHub: https://github.com/AryehMiller/Lizard-Key-Innovations.

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using resources at the Center for High Performance computing (CHPC) within the Mallinckrodt Institute of Radiology, Washington University School of Medicine in St. Louis (WUSM). We thank Malcolm Tobias at the WUSM CHPC for troubleshooting assistance.

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