Does Population Structure Predict the Rate of Speciation? A Comparative Test across Australia's Most Diverse Vertebrate Radiation

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ABSTRACT: Population divergence is the first step in allopatric speciation, as has long been recognized in both theoretical models of speciation and empirical explorations of natural systems. All else being equal, lineages with substantial population differentiation should form new species more quickly than lineages that maintain range-wide genetic cohesion through high levels of gene flow. However, there have been few direct tests of the extent to which population differentiation predicts speciation rates as measured on phylogenetic trees. Here, we explicitly test the links between organismal traits, population-level processes, and phylogenetic speciation rates across a diverse clade of Australian lizards that shows remarkable variation in speciation rate. Using genome-wide double digest restriction site–associated DNA data from 892 individuals, we generated a comparative data set on isolation by distance and population differentiation across 104 putative species-level lineages (operational taxonomic units). We find that species show substantial variation in the extent of population differentiation, and this variation is predicted by organismal traits that are thought to be proxies for dispersal and deme size. However, variation in population structure does not predict variation in speciation rate. Our results suggest that population differentiation is not the rate-limiting step in species formation and that other ecological and historical factors are primary determinants of speciation rates at macroevolutionary scales.

Keywords: population structure, isolation by distance, speciation rate, squamates, ddRAD.

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Introduction

Almost all our verbal and theoretical models for species formation describe the evolution of populations (Kirkpatrick and Ravigné 2002; Gavrilets 2004). Implicit in these models is the idea that populations differentiate, remain distinct, and persist as they evolve into separate species (Mayr 1963; Allmon 1992). Most empirical studies of speciation focus on understanding how populations remain distinct through the evolution of barriers to gene flow (i.e., reproductive isolation; Coyne and Orr 2004; Nosil 2012). Although the evolution of reproductive isolation is a key component of species formation, speciation may also be regulated by metapopulation processes that influence rates of population splitting and the persistence of populations through time (Rosenblum et al. 2012; Dynesius and Jansson 2014; Rabosky 2016*b*; Schluter 2016). Put simply, whether two populations can evolve reproductive isolation may be irrelevant for speciation if those populations never become sufficiently isolated as to allow differentiation or if they go extinct before they can differentiate.

At broader phylogenetic scales, it is clear that the speciation rate varies dramatically across the tree of life and contributes to differences in species richness across clades and among geographic regions (Sepkoski 1998; Coyne and Orr 2004; Jetz et al. 2012). Studying the underlying causes of this speciation rate variation has the potential to reveal how the tempo and mode of species formation changes across the tree of life. In particular, variation in speciation rates—as measured over macroevolutionary timescales—is likely determined by how quickly populations move through the three primary stages of species formation: population differentiation, population persistence, and the evolu-

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tion of reproductive isolation. Many studies have explored the relationship between factors that are presumed to contribute to these stages and the speciation rate. For example, variation in the strength of sexual selection might generate variation in the rate at which reproductive isolation evolves, and this conceptual linkage has motivated empirical tests of the connection between sexual selection and speciation rate (Coyne and Orr 2004; Kraaijeveld et al. 2011). Here, sexual selection is measured via surrogate variables (Kraaijeveld et al. 2011), and it is generally assumed to influence the speciation rate by increasing reproductive isolation between populations (West-Eberhard 1983). However, few studies have directly tested the relationship between any of the primary population-level controls on speciation (reproductive isolation, persistence, or differentiation) and speciation rates as measured at macroevolutionary scales (Kisel and Barraclough 2010; Rabosky and Matute 2013; Etienne et al. 2014; Harvey et al. 2017).

In this work, we focus on the link between population differentiation (population structure) and variation in speciation rates across a phylogeny. Population structure has long been appreciated as an important first step in allopatric speciation because it generates possible precursors to incipient species (Endler 1977; Levin 2000). In previous studies, researchers have measured population structure as the extent of isolation by distance (IBD) across a species' range (Wright 1943; Slatkin 1987), the number of demes or genetic clusters in a population (Hanski 1999; Pritchard et al. 2000), and the number of subspecies in a species (Haskell and Adhikari 2009). However, because of the difficulty in measuring population structure across numerous species, most studies of the relationship between population structure and diversification have relied on surrogate variables that are thought to be associated with population structure. This indirect approach assumes that dispersal-related organismal traits such as pollination mode and habitat preference are proxies for the extent to which population structure evolves, an assumption that has support from multiple taxa, including plants, birds, and fish (Loveless and Hamrick 1984; Duminil et al. 2007; Burney and Brumfield 2009; Wagner and McCune 2009). For example, in marine invertebrates, clades with long pelagic larval phases (e.g., planktotrophic; Jablonski and Lutz 1983) are assumed to have high dispersal and are thus expected to show high genetic connectivity throughout their range and less likely to form population isolates (Hansen 1983). Accordingly, putative high-dispersal clades contain fewer species than clades where pelagic larval durations (and presumably dispersal) are reduced (Shuto 1974; Jablonski 1986; but see Krug et al. 2015). Similar logic has been applied to the relationship between diversification and seed dispersal mode in angiosperms (Herrera 1989) and between diversification and wing shape in island birds (Weeks and Claramunt 2014). However, no clear generalizations have emerged from these and other studies, perhaps in part because surrogate variables are noisy measures of population structure.

Several studies have directly estimated population structure without relying on surrogate variables. For example, subspecies potentially provide information about the rate at which incipient species arise, and subspecies differentiation is weakly but significantly correlated with species diversification (Haskell and Adhikari 2009; Phillimore et al. 2010). However, subspecies are arbitrary units, and the concept is difficult to apply consistently across taxa. A more rigorous approach is to use population genetics to directly infer population structure and then test the relationship between that structure and diversification. Only a few studies have done so thus far (Kisel and Barraclough 2010; Kisel et al. 2012; Riginos et al. 2014; Gianoli et al. 2016; Harvey et al. 2017). Four of these five studies have shown that levels of population structure can predict speciation patterns. Kisel and Barraclough (2010) found that species with greater population structure (as measured using F_{ST}) can undergo speciation on smaller islands relative to species that exhibit less structure. Riginos et al. (2014) showed that coral reef fish with a benthic dispersal stage exhibited greater population structure and greater species richness compared to fish with a pelagic dispersal stage. Harvey et al. (2017) used mitochondrial phylogeographic data sets to show that bird species with greater numbers of population clusters also had higher rates of speciation. Gianoli et al. (2016) found that climbing habit in plants is linked to greater levels of population differentiation; this trait also appears to correlate with angiosperm species richness (Gianoli 2004). However, Kisel et al. (2012) found no difference in intraspecific population differentiation between pairwise comparisons of depauperate and speciose sister clades of Neotropical orchids.

Here, we test whether population differentiation is a rate-limiting control on speciation rates as measured at macroevolutionary scales in a group of lizards that are characterized by considerable among-clade variation in speciation rate (Rabosky et al. 2014*a*). This clade—Australia's sphenomorphine skinks—is Australia's largest radiation of vertebrates and contains 266 species in 17 genera. This clade is an ideal system for exploring this question for three primary reasons. First, sphenomorphines exhibit noted variation in species richness; two genera—*Ctenotus* and *Lerista*, with approximately 100 species each—are far more species rich than the remaining 15 genera of sphenomorphines. Previous studies have suggested that this pattern is partially driven by an increase in speciation rate at the base of the clade that includes *Ctenotus* and *Lerista* (Rabosky et al. 2007, 2014*a*). Second, burrowing morphologies (limb reduction, body elongation) have evolved repeatedly within the sphenomorphines (Brandley et al. 2008; Skinner et al. 2008; Skinner 2010), with at least 15 independent instances of complete limb loss. Because fossorial animals are expected to show reduced dispersal relative

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to species that are active on the surface (Gans and Fusari 1994), we expect that limb reduction should be associated with variation in levels of population structure across this group. Finally, the vast majority of these species are restricted to the arid and semiarid regions of Australia, which allows us to partially control for the role of biogeographic history in structuring populations (Avise 2000).

To test the relationship between population structure and speciation, we generated standardized estimates of population differentiation (e.g., slope of IBD relationships; β_{IBD}) for 104 putative species-level lineages (operational taxonomic units [OTUs]) of sphenomorphine skinks, using genome-wide sequence data from 892 individual lizards across 10 genera (fig. 1). We then evaluated which organismal traits—if any—explain variance in β_{IBD} across the clade. In doing so, we quantitatively delimited putative species-level taxa for analysis using genomic data, thus ensuring that the basic unit of analysis in macroevolutionary analysis—the species—has been characterized uniformly across the clade. We then used the same data to infer population structure. This introduces potential circularity and bias; to determine whether this bias affects our results, we repeated all analyses across both the existing and the revised taxonomies. Finally, we estimated phylogenetic rates of speciation across this group of lizards and tested whether they are correlated with β_{IBD}, as would be expected if the formation of population structure is a rate-limiting control on the speciation process.

Material and Methods

Sampling and Genetic Data Collection/Analysis

As of June 2017 (Uetz et al. 2018), the existing taxonomy for the sphenomorphine clade consists of 266 nominal species, all but four of which are endemic to Australia (Cogger 2014; Uetz et al. 2018). We sampled broadly across species in the clade and across the ranges of these species based on available tissue samples at several major Australian and US natural history museums. In total, we sampled 892 individuals across 98 nominal species (average: 9.1; range: 3–92). We sampled 34%, 47%, and 28% of the species in *Ctenotus*, *Lerista*, and all other genera, respectively. Table S1 (tables S1–S3 are available online) lists the individuals sampled and their associated details. Four hundred eighty-three of the individuals included in this study were previously published (Singhal et al. 2017).

To determine patterns of genetic differentiation and population structure, we collected and analyzed double digest restriction site–associated DNA (ddRAD) sequencing data for each sampled individual (Peterson et al. 2012). The approach for data collection and analysis followed previously published approaches (Singhal et al. 2017); we briefly summarize it here. For each individual, we cut genomic DNA using the enzymes EcoRI and MspI and ligated barcoded adaptors. We then generated equimolar pools of 24 individuals and size selected these pools from 290 to 390 bp. We combined four of these 24-individual pools to sequence 96 individuals to a lane on an Illumina HiSeq 2500 sequencer at three facilities: the UC Berkeley Vincent Coates Genome Sequencing Laboratory (Berkeley), the Center for Applied Genomics at the Hospital for Sick Children (Toronto), and the Genomics Service Laboratory at HudsonAlpha (Montgomery, AL). Following sequencing, we demultiplexed the data using the process_radtags module in Stacks (Catchen et al. 2011) and assembled each individual using Rainbow v2.0.4 (Chong et al. 2012). For each of the 10 genera, we then used VSEARCH v2.0.2 to identify homologous loci across individuals in that genus (Rognes et al. 2016), allowing for an 80% similarity cutoff.

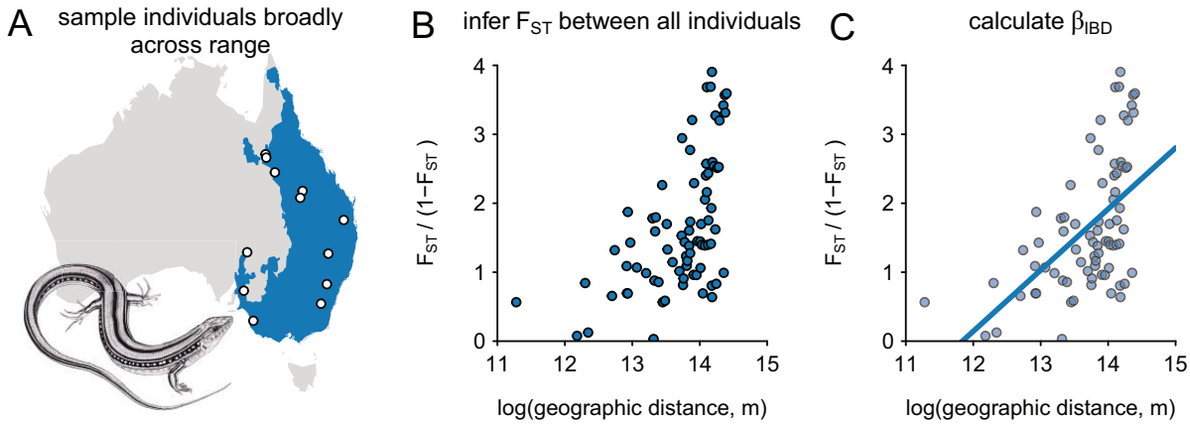

Figure 1: Approach used to calculate our metric for population structure, β_{IBD}, shown for an exemplar taxon, *Ctenotus robustus*. *A*, For each species, we sampled individuals broadly across their range. *B*, We estimated pairwise F_{ST} between all individuals from genome-wide double digest restriction site–associated DNA data. *C*, We used the Rousset et al. (1997) approach to estimate β_{IBD} or the slope of F_{ST} against pairwise geographic distances. The value of β_{IBD} is expected to vary as a function of a species' population size and dispersal length. IBD = isolation by distance.

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Previous studies (Rabosky et al. 2014*c*, 2017*a*; Singhal et al. 2017) have revealed that sphenomorphine nominal species, like other squamate species (Oliver et al. 2009; Potter et al. 2016), often contain multiple, deeply divergent, and morphologically cryptic lineages. To ensure that we analyzed equivalent taxonomic units across the clade, we applied a coalescent-based method to delimit OTUs. Although coalescent-based methods can overdelimit taxonomic units (Sukumaran and Knowles 2017), we believe that this provisional taxonomy reflects "good" biological species, subject to further taxonomic revision (see "Results" and "Discussion"). For each of the 10 genera, we generated concatenated alignments of ddRAD loci that were ≥60% complete across individuals. Then, using the GTRGAMMA model, we inferred a topology and branch lengths using the randomized accelerated maximum likelihood (RAxML) v8.2.8 rapid hill-climbing algorithm (Stamatakis 2014). To infer a dated tree on a relative timescale, we ran treePL for a range of λ values (0.001, 0.01, 0.1, 1), picking the final value of λ that reduced the coefficient of variation (Smith and O'Meara 2012). We then used the single-threshold method in the generalized mixed Yule-coalescent (GMYC) approach to infer putative OTUs (Fujisawa and Barraclough 2013). GMYC is a coalescent-based approach that delineates OTUs by identifying the nodes in phylogenies where branching processes transition from coalescent to Yule patterns. This can result in both nominal species being split into multiple OTUs and multiple species being collapsed into a single OTU.

We then generated a pseudoreference genome (PRG) for each OTU using VSEARCH with a ≥95% similarity cutoff. To identify variants, we mapped reads from each individual to the PRG using Burrows-Wheeler aligner (BWA) v0.7.12 (Li 2013), called variant and invariant sites using SAMtools v1.3.1 (Li et al. 2009), and then filtered sites to remove sites with <10× coverage, ≥3× the median coverage for the individual, and <20 quality scores.

Estimation of Rates of Population Differentiation

For each OTU, we inferred the rate at which populations differentiated across space. Using the filtered variant data sets, we calculated pairwise F_{ST} estimates and D_{xy} between all individuals in a given OTU (Nei and Li 1979; Reich et al. 2009). Where possible, we used previously published data to infer mtDNA D_{xy} as well (Rabosky et al. 2014*a*, 2014*c*). We calculated both geographic and environmental distance between points. For environmental distance, we extracted climatic and environmental data for each individual using 19 Bioclim variables and two supplementary variables using the R package raster (Hijmans and van Etten 2014; Fick and Hijmans 2017). The supplementary variables were an index of vegetation density and a measure of soil mineral content; this latter variable tracks broadscale distributions of mineral-containing substrates across the continent. We used these data to calculate Euclidean distances in environmental space between individuals.

To infer how F_{ST} changes across geographic space, we used the IBD approach introduced by Rousset (1997). Because these habitats are nonlinear, we regressed pairwise $F_{ST}/(1 - F_{ST})$ estimates against the natural log of geographic distance. The resultant slope (here defined as β_{IBD}) is expected to vary as a function of both population size and dispersal. We assessed significance of the slope using a Mantel test (Oksanen et al. 2007). For all other measures of genetic distance and all other measures of geographic and environmental distance, we simply found the slope of genetic distance against geographic or environmental distance, again assessing significance via Mantel tests.

Phylogenetic Framework and Diversification Rate Analysis. We focused on "recent" speciation rates in our analyses rather than net diversification (speciation minus extinction) for two reasons. First, as has been recognized since birth-death models were applied to species-level phylogenies, speciation rates are more robustly estimated than extinction rates (Nee et al. 1994; Davis et al. 2013). Indeed, the present-day slope of lineage accumulation curves derived from phylogenetic trees is expected to converge on the instantaneous speciation rate (Nee et al. 1994), although we expect this estimate to be somewhat biased by protracted speciation (Rosindell et al. 2010; Etienne and Rosindell 2012) or lag times to species recognition in species-level phylogenies (Weir and Schluter 2007). Second, extinction estimates are particularly sensitive to model misspecification (Rabosky 2016*a*).

To estimate rates of speciation across the sphenomorphines, we first inferred a phylogeny for the sphenomorphine clade and five outgroups. Rabosky et al. (2014*a*) published the most complete phylogeny for the sphenomorphines, which was based on four mitochondrial genes and two nuclear genes for 216 of the 266 nominal species. Because our range-wide sampling identified several new OTUs, we updated this phylogenetic analysis to include previously published data from the mitochondrial locus cytochrome *b* for newly delimited OTUs (Rabosky et al. 2014*c*). We additionally downloaded sequence data from GenBank to include 10 previously unrepresented nominal species (table S2). For the phylogenetic analysis, we implemented a relaxed uncorrelated lognormal clock in Bayesian evolutionary analysis by sampling trees (BEAST) 2 v2.4.5 (Bouckaert et al. 2014). We analyzed the data under a model with eight partitions: each of the three coding positions plus the noncoding portion of the two coding mitochondrial loci and the other four loci individually. We assigned a GTR + I + G model of molecular evolution to each partition. The clock and tree models were linked across all loci. We calibrated the tree as done by Rabosky et al. (2014) by placing lognormal priors on three key nodes

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of the phylogeny that had been dated in a previous, more extensive analysis (Skinner et al. 2011). We ran the phylogenetic analysis for 300e6 generations with a 40% burn-in and evaluated convergence using Tracer v1.6.0. We summarized the maximum clade credibility tree using TreeAnnotator v2.4.5.

We inferred speciation rates for each tip in the phylogeny using two complementary approaches. First, we calculated the inverse equal-splits measure of speciation rate (diversification rate [DR] statistic; λ_{DR}), which is effectively a weighted inverse of branch lengths leading to a tip in a phylogeny (Jetz et al. 2012; Belmaker and Jetz 2015). The equal-splits approach can capture variation in the rate of speciation at the tips of the tree, although it is sensitive to incomplete sampling and cannot account for variation in extinction rate (Belmaker and Jetz 2015; Rabosky and Goldberg 2017). We sampled 85% of nominal sphenomorphine taxa for our study, and taxon sampling is not appreciably different among three major taxonomic subgroups of the data (*Ctenotus*: 93% sampling; *Lerista*: 83% sampling; other sphenomorphine taxa: 80% sampling). Through simulation, we demonstrate that the variance of the λ_{DR} remains relatively constant with respect to taxon sampling (fig. S1; figs. S1–S13 are available online), indicating that extreme imbalances in sampling are necessary to generate spurious patterns of among-clade rate variation. In addition, we use rarefaction to demonstrate that variation in sampling among the major groups of sphenomorphines has minimal impact on λ_{DR} across the phylogeny (fig. S2). Thus, incomplete taxon sampling is unlikely to be problematic for the relative (λ_{DR}) rate estimates across clades given that broadly comparable fractions of taxa were sampled in each case.

Second, we used Bayesian analysis of macroevolutionary mixtures (BAMM) v2.5.0, a Bayesian approach for inferring speciation and extinction rate variation across a phylogeny (Rabosky et al. 2017*b*). Our BAMM analyses assumed time-varying rates of speciation within macroevolutionary rate regimes; previous analyses recovered strong evidence for temporal declines in the rate of speciation during the sphenomorphine radiation (Rabosky et al. 2014*a*). We used a prior mean of 1.0 for the expected number of rate shifts and proportion of sampled taxa as 85%. We ran BAMM for 100e6 generations with a 30% burn-in. We assessed convergence by comparing the marginal posterior distributions on the number of shifts across multiple runs and by ensuring that the effective sample size for log likelihoods and shifts exceeded 150. For each species, we used BAMMtools v2.1.6 to estimate the present-day speciation rate as the mean of the corresponding marginal posterior distribution of tip rates for the species (λ_{BAMM}; Rabosky et al. 2014*b*). As an additional test for overall diversification rate variation, we computed a tree-wide balance statistic (I_c; Colless 1982; Heard 1992). We then estimated the significance of the I_c statistic by comparing it to a null distribution generated by simulating phylogenies of the same size as the focal tree under a constant-rate birth-death process.

Analysis of Predictive Factors Influencing Rates of Differentiation. We used a multipredictor model-averaging approach (Burnham and Anderson 2003) to simultaneously test three main hypotheses for the relationship between specific organismal traits and population differentiation as measured through β_{IBD}. Population genetic theory proposes that at equilibrium, population structure should vary as an inverse function of population deme size (N_e) and migration (m; Wright 1943). Accordingly, we included three variables in our candidate set that might be expected to correlate with population size: within-population genetic diversity, geographic range size, and body size. For migration, we added proxies for predicted dispersal patterns: degree of limb loss and digit loss. Additionally, local adaptation to spatially varying environmental conditions can influence population differentiation (Endler 1977; Bradburd et al. 2013). To capture environmental predictors of population differentiation, we included the range of climatic space spanned by an OTU's geographic range as a proxy for environmental heterogeneity. Finally, we included two nuisance variables in our model-testing approach: the latitudinal midpoint of each OTU's geographic range and the number of individuals sampled for that OTU. Below, we briefly summarize how we collated data on geographic and morphological predictor variables; detailed explanations are available in Singhal et al. (2017).

Accurate geographic ranges are not available for most squamates. Instead, we generated our own range maps as the intersection of the alpha convex hull spanning all biodiversity database occurrences and the ecological niche model for the species (Phillips and Dudík 2008; Pateiro-López and Rodrıguez-Casal 2010). We then used these geographic ranges to calculate range size and climatic space spanned. With respect to morphology, we summarized data from Cogger (2014) and Rabosky et al. (2014*a*) on body size (snout-vent length) and five measurements of limb loss (forelimb length, hindlimb length, toe length, number of hand digits, number of foot digits). We first took the mean of measurements across individuals for a species. For each limb measure, we regressed species means against body size using a phylogenetic linear model and scaled and centered the resulting residuals. We ordinated these values using a phylogenetic principal components analysis (PCA; Revell 2012), retaining the first two axes. The first axis explained 75.2% of the variation and corresponded to the degree of limb reduction, and the second axis explained 18.0% of the variation and corresponded to the degree of digit reduction.

We generated a full set of additive linear models across all nine factors and fitted them to the data using phylogenetic linear models (Ho et al. 2016). We calculated rel-

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ative importance for a variable by summing the relative Akaike information criterion weights of the models in which the variable appears. To estimate the significance and coefficient estimates for variables, we constructed a full phylogenetic linear model that contained all variables with a relative importance greater than 0.6 (Wagner et al. 2012).

Analysis of Relationship between Differentiation and Diversification. To determine whether β_{IBD} and the speciation rate were correlated, we conducted three analyses. First, we tested the correlation between β_{IBD} and λ_{DR} using phylogenetic generalized least squares (PGLS; Grafen 1989; Freckleton et al. 2008; Pinheiro et al. 2014). Second, we used a simulation-based approach (ES-Sim) to test whether the observed correlation between trait values and speciation rates is significantly different from a null model in which traits evolve under Brownian motion. This approach has been shown to perform better than PGLS for assessing the association between λ_{DR} and continuous traits under some simulation scenarios (Harvey and Rabosky 2017). Third, due to the low number of rate shifts inferred using BAMM, we lacked power to test for trait-dependent speciation using structured rate permutations on phylogenies (STRAPP; Rabosky and Huang 2015). Rather, we visually compared patterns of β_{IBD} between subclades inferred to have different rates of speciation.

Sensitivity and Power Analysis. We conducted a series of analyses to determine the robustness of our results to known sources of error and sampling biases. Perhaps our largest source of bias is the use of the same genomic data to both delimit taxa and infer population structure. Accordingly, we repeated all analyses, including estimating β_{IBD} and inferring diversification rates, for the existing taxonomy.

Additionally, we conducted a series of five analyses (*a*–*e*), which modified the pairwise F_{ST} estimates included in estimating β_{IBD}. After applying these filters, we reestimated β_{IBD}. These analyses were (*a*) bootstrapping loci in each F_{ST} estimate to account for error in F_{ST} estimation; (*b*) bootstrapping all pairwise F_{ST} estimates to account for sampling biases within OTUs; (*c*) removing pairwise F_{ST} comparisons <20 km, as plots of pairwise F_{ST} estimates often showed an apparent breakpoint, with comparisons at local geographic scales appearing disjointed from those at broader scales; (*d*) removing pairwise F_{ST} comparisons where $F_{ST} > 0.7$, as $F_{ST} > 0.7$ is higher than might be expected for within-species comparisons and might reflect unnamed cryptic lineages or technical artifacts; and (*e*) restricting analyses to only pairwise F_{ST} estimated from the same biome (here, the desert). Our results suggested that biome affects rates of population differentiation (fig. S8), so we control for this by analyzing individuals only from the same biome. We then redid our analyses after subsampling the data set to include or drop OTUs across different classifiers. Our approaches and rationale were (*f*) removing species with less than five individuals, as rates based on fewer individuals might be more likely subject to sampling artifact; further, sampling, while correlated with taxon range size, was uneven across taxa; (*g*) removing species with nonsignificant rates of differentiation; (*h*) removing recently diverged species, which ensures that our results are robust to taxonomic uncertainty in this clade; and (*i*) repeating analyses with a random selection of 80% of the taxa, ensuring that our results are robust to our sampling across the clade, including our slightly imbalanced sampling across genera. Additionally, we accounted for phylogenetic uncertainty by (*j*) repeating our analyses across 100 samples from the posterior distribution of phylogenies sampled using BEAST. We also considered other measures of differentiation rates: (1) the rate at which D_{xy} accumulates across geographic space and (2) the rate at which F_{ST} accumulates across environmental space. Across those analyses where we evaluated alternate tree topologies, we estimated speciation rates as λ_{DR} and then used PGLS to test their correlation with β_{IBD}.

Because we found no effect of population structure on speciation rate, we designed a power analysis to infer our ability to infer a correlation had one existed. To do so, we simulated our trait (here, β_{IBD}) with a known correlation to λ_{DR} under a Brownian model of trait evolution. We estimated power as the percentage of 100 replicates in which our simulated trait was correlated with λ_{DR}, using both PGLS and ES-Sim to assess the significance of the relationship.

Phylogenetic and genetic data, including the BEAST XML file used in the phylogeny, are deposited to the Dryad Digital Repository (https://datadryad.org/resource/doi:10.5061/dryad.j6823nt; Singhal et al. 2018) and in BioProjects PRJNA382545 and PRJNA476569. Scripts used in the analysis are at https://github.com/singhal/Spheno_Gene_Flow.

Results

Genomic and Phylogenetic Analysis

Our ddRAD approach successfully generated an average of 2.5 Mb of sequence for each of 885 individuals; seven individuals with ≤10 kb of sequence were dropped from further analysis. These data revealed multiple, deeply divergent OTUs within nominal species. As reported previously, we identified putatively cryptic OTUs in 39% of *Ctenotus* nominal species (Singhal et al. 2017). Here, we find the same pattern is true across other genera. Based on our coalescent-based species delimitation, 9% of the nominal species in *Lerista* and 30% of the species in the other eight genera were split into multiple cryptic OTUs. Across all genera, 6% of OTUs collapsed multiple nominal species.

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Our updated phylogenetic analysis for the sphenomorphines included many of these newly identified OTUs (fig. 2). The resulting tree revealed that several nominal species are polyphyletic (fig. S3). For example, *Ctenotus strauchii* was split into three OTUs, whose most recent common ancestor also includes *Ctenotus zebrilla* as a descendant. The inferred tree is otherwise very similar to previous trees with respect to topology, support for key nodes, and branch lengths (Rabosky et al. 2014*a*; fig. S3).

Patterns of Population Differentiation and Its Determinants. On average, we estimated F_{ST} across 59,000 variable sites and D_{xy} across 1.25 Mb of sequence. Our three estimates of differentiation (nuclear F_{ST}, nuclear D_{xy}, and mtDNA D_{xy}) are highly

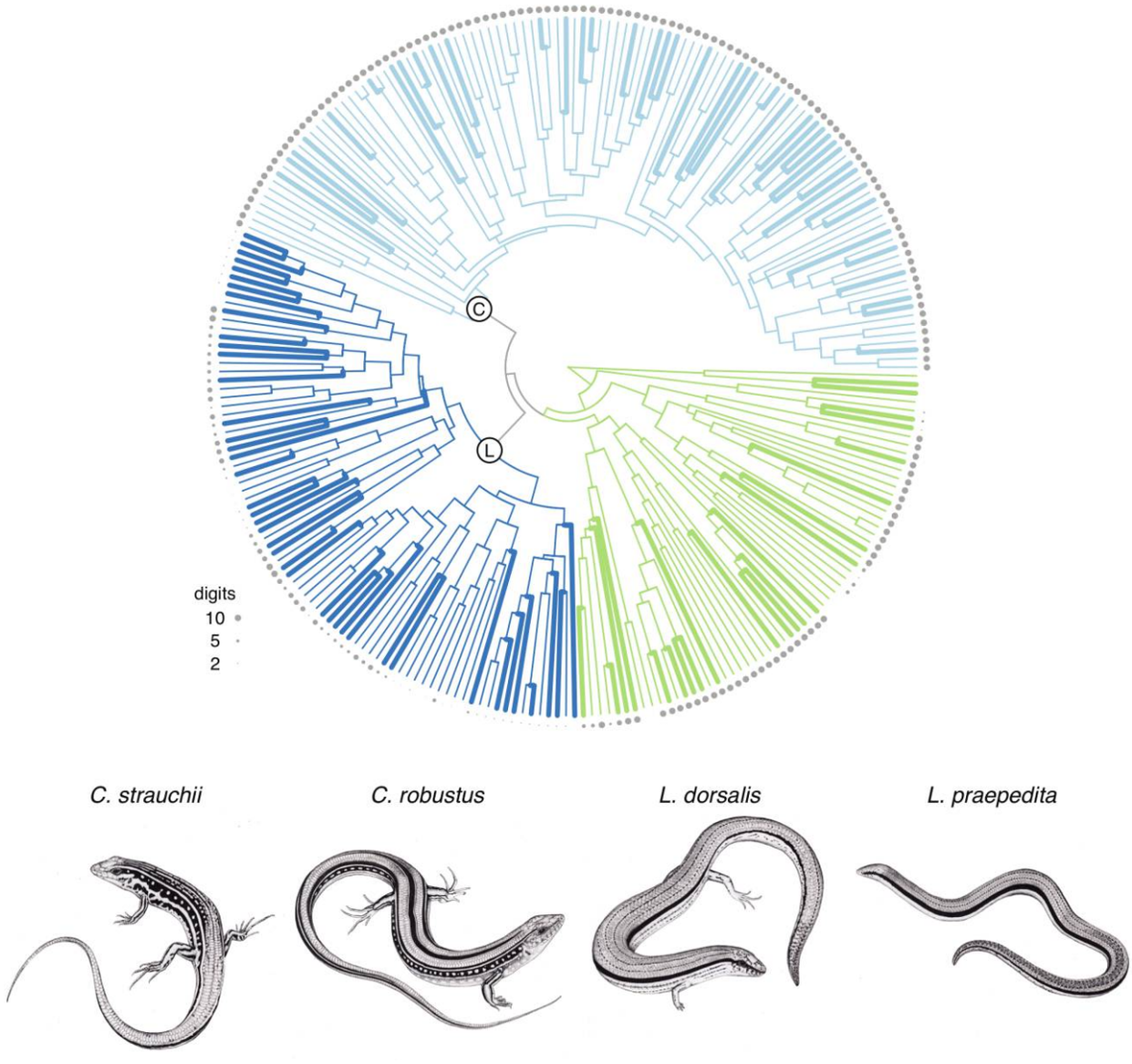

Figure 2: Phylogeny of Australian sphenomorphine scincid lizards, representing 248 operational taxonomic units and 85% of described nominal species-level diversity. The two most speciose genera *Ctenotus* and *Lerista* are depicted in light blue and dark blue and are labeled with a C and an L, respectively; all other genera are shown in green. Branches for which we inferred patterns of population structure are in boldface, and tip labels indicate the relative extent of limb reduction (here measured as the total number of hand and toe digits). Drawings show four exemplar species arranged from left to right in order of extent of limb reduction (courtesy of M. R. Grundler); size of images does not reflect relative body size of species. Annotated phylogeny showing posterior support for nodes, tip labels, and error in branch lengths is depicted in fig. S3, available online. Limb reduction has evolved over 15 independent times in the sphenomorphines but never in *Ctenotus*; this variation in a key dispersal trait is predicted to influence patterns of population structure.

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correlated across pairwise comparisons (fig. S4). The slopes of differentiation calculated from these pairwise estimates, however, are correlated between differentiation estimated only using nuclear and mitochondrial D_{xy} (fig. S5). A simple model of IBD explained an average of 50% of the variation in how F_{ST} changed over geographic space, although the extent explained varied considerably across taxa (fig. S6). Models of isolation by environment—that is, where differentiation among populations was modeled as a function of environmental distance—were less powerful, explaining only an average of 34% of the variation.

The rate at which populations differentiate across space (β_{IBD}) ranged from −0.09 to 4.38 across the sampled OTUs (fig. 3). The parameter β_{IBD} exhibits moderate phylogenetic signal ($\lambda = 0.34$; $P = .007$) across the sampled tips (fig. 3). We tested three primary hypotheses that predict how and why β_{IBD} might vary across this clade: variance in deme size, variance in migration, and variance in environmental heterogeneity. Our model-fitting approach supported all three hypotheses and identified four factors that significantly predicted population structure: within-population genetic diversity (a proxy for deme size), degree of limb reduction (a proxy for dispersal), range of climatic space spanned (a proxy for environmental heterogeneity), and latitudinal midpoint (figs. 4, S7, S8). As expected, β_{IBD} decreases as within-population diversity increases; conversely, β_{IBD} increases with limb reduction (fig. 4). Further, these two factors are expected to interact, because lizards with more reduced limbs tend to have smaller range sizes and less genetic diversity (fig. S9; Lee et al. 2013). Our sensitivity analyses found that these results are robust to the effects of technical and sampling limitations (table S3). The correlations between climatic space and latitudinal midpoint and differentiation are driven by OTUs restricted to the tropical grasslands and temperate forest biomes in eastern Australia (fig. S8), however. We refrain from discussing this result further until additional data confirms that this pattern is not a biome-specific effect.

Analysis of Relationship between Differentiation and Diversification

We first tested whether there is evidence for variation in speciation rate across the sphenomorphine phylogeny. Overall patterns of tree balance revealed significant heterogeneity

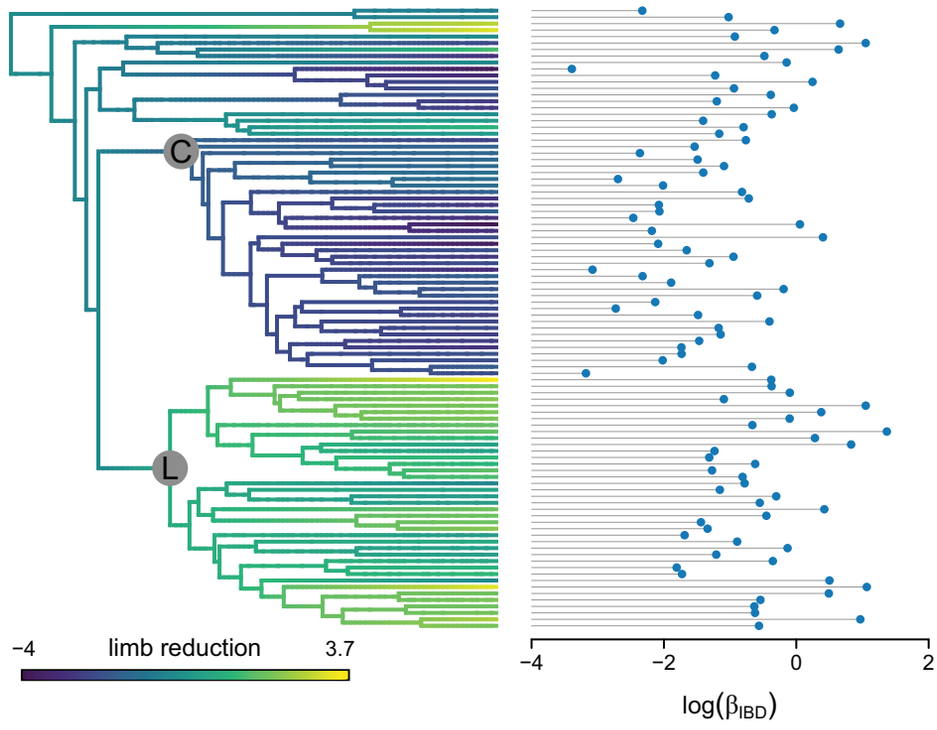

Figure 3: Relationship between a dispersal-related trait (degree of limb reduction) and population structure (β_{IBD}) across the 104 operational taxonomic units (OTUs) included in this study. *A*, Ancestral state reconstruction for morphology principal component 1 (PC1), inferred using the contMap function in phytools (Revell 2012). Morphology PC1 reflects the extent of limb reduction, and positive values reflect decreases in both limb length and the number of terminal digits. *Ctenotus* and *Lerista* clades are labeled with a C and an L, respectively. *B*, Estimated β_{IBD} for each OTU. Both extent of limb reduction and β_{IBD} vary substantially, and these two traits also covary substantially (fig. 4*A*). IBD = isolation by distance.

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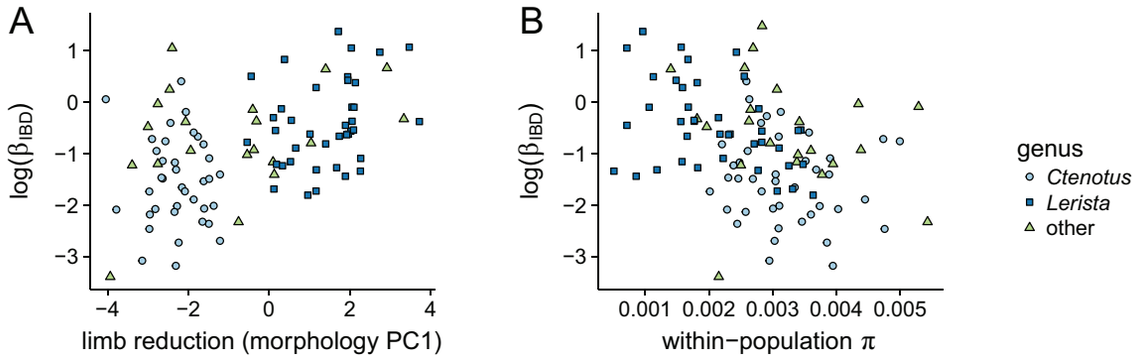

Figure 4: Relationship between extent of population structure (β_{IBD}) and two of its significant predictors, morphology principal component 1 (PC1; *A*) and within-population genetic diversity (*B*). Greater values of PC1 indicate greater limb reduction. Points are coded by genera; "other" reflects a nonmonophyletic group (fig. 2). As expected, as dispersal (limb reduction as a proxy) decreases, β_{IBD} increases, and as deme size (within-population genetic diversity as a proxy) increases, β_{IBD} decreases. IBD = isolation by distance.

in the diversification rate ($I_c = 0.061$, effect size = 3.66, $P = .003$; fig. S10). BAMM analyses recovered strong support for a model with a single rate shift (M_1; fig. S11). The posterior probability of a one-shift model was $P = .86$, versus $P = 0$ for a model with zero shifts (M_0), providing strong support in favor of a model with rate heterogeneity. As has been previously reported (Rabosky et al. 2014*a*), this rate variation inferred with BAMM appears to reflect an increase in the speciation rate at the base of the *Ctenotus* and *Lerista* clade (figs. 5, S11). Further, λ_{DR} exhibits greater heterogeneity than λ_{BAMM} (figs. 5, 6). The DR statistic can overestimate rate variation and will reveal apparent heterogeneity in tip rates even for constant-rate trees, because their values will also reflect stochastic variation in branch lengths and node densities. However, BAMM has demonstrably low power to infer rate shifts that lead to small-rate regimes (Rabosky et al. 2017*b*). Hence, the true magnitude of rate variation presumably lies somewhere in between the variation revealed by BAMM and that revealed by the other metrics.

We then asked whether β_{IBD} could predict this variation in speciation rate. We recovered no correlation across our multiple approaches to address this question, as is perhaps best illustrated by a visual comparison of the distributions of β_{IBD} and speciation rate across major clades (fig. 6). More quantitative approaches confirmed this intuition. Both PGLS ($P = .47$) and ES-Sim ($\rho = -0.22$, $P = .30$) failed to recover a correlation between β_{IBD} and speciation rates (fig. 7). Further, although tip speciation rates estimated with BAMM (λ_{BAMM}) were faster under the existing taxonomy than under the revised taxonomy, relative differences in rates among major clades were approximately the same for both taxonomies (fig. S11). All subsequent analyses were qualitatively and quantitatively similar whether the existing or revised taxonomy was used (fig. S11; table 1). This null result was robust to the effects of other technical and sampling limitations as well (table S3).

Because we recovered a null result, we conducted a power analysis to estimate our ability to recover a true correlation between β_{IBD} and the speciation rate if it existed. The false positive rate was 0.04, similar to the false discovery rate of 0.05 at $\alpha = 0.05$. Across the true correlations, for both methods we had weak power until $\rho \geq 0.6$, at which point ≥70% of our simulated data sets recovered a significant correlation (fig. S12). However, because this power analysis did not account for error in estimating β_{IBD} or speciation rates, our power calculations are likely overestimates.

Discussion

We found that species exhibit considerable variation in rates of population genetic differentiation across space and that this variation is predictable from species traits. However, although population differentiation is one of the key early steps in speciation, we found no evidence for a correlation between the extent of population differentiation and species formation. Below, we consider a few possible explanations for this primary result.

Population differentiation and speciation rate are challenging quantities to estimate, and error in estimating either of these properties could potentially obscure a true pattern. However, such technical artifacts are unlikely to explain our results for two reasons. First, our estimates of population structure exhibited phylogenetic signal, and the extent of population structure was predictable by biologically meaningful variables and in the direction of our a priori hypotheses (figs. 4, S7). These results mirror those from other studies, which have found correlations between

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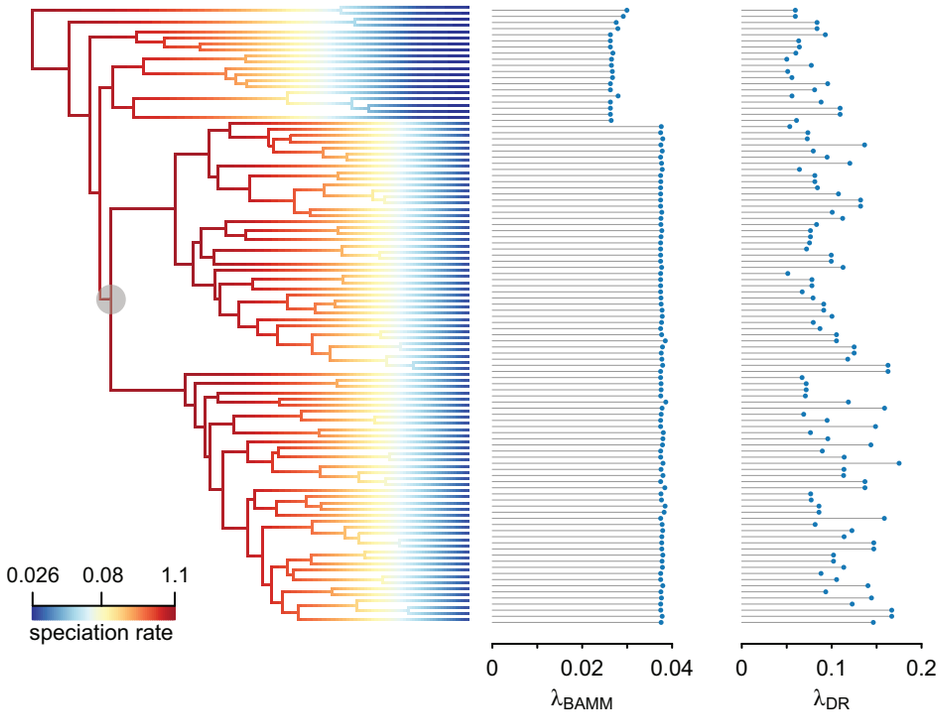

Figure 5: Variation in speciation rates across the sphenomorphine clade as estimated by two metrics for speciation rates at the tip: Bayesian analysis of macroevolutionary mixtures (BAMM) tip rates (λ_{BAMM}) and the equal-splits metric (λ_{DR}). The phylogeny depicts the BAMM-inferred speciation rates through time mapped onto a pruned phylogeny of the 104 taxa included in this study. The gray circle marks the location of the inferred rate shift in speciation rates at the base of the *Ctenotus* and *Lerista* clades. Across both metrics, speciation rate varies across the tree. DR = diversification rate.

genetic differentiation and dispersal-related traits such as habitat preference in birds, breeding mode in plants, and reproductive mode in frogs (Loveless and Hamrick 1984; Hamrick and Godt 1996; Burney and Brumfield 2009; Paz et al. 2015). Significant error in our estimates should have prevented us from recovering these predicted relationships. Second, our sensitivity analyses filtered our data set to help address many of the known possible quality issues—for example, sampling biases and error in phylogenetic estimation. Yet, throughout this series of tests, the lack of relationship between speciation rate and population differentiation remained robust. That said, our analyses have modest power (fig. S12) to detect a true positive association between β_{IBD} and speciation rate, given the amount of phylogenetically independent variation in speciation rate across the skink phylogeny. It is noteworthy that we detect a substantial difference in β_{IBD} between the genera *Ctenotus* and *Lerista*, which differ markedly in ecology. *Lerista*, as a limb-reduced fossorial clade, has greater rates of population differentiation with respect to geographic distance. However, the profound differences in dispersal ecology between *Lerista* and *Ctenotus*, with demonstrable effects on β_{IBD}, have not led to substantial differences in the rate of speciation between these genera. Indeed, as measured by λ_{DR} (fig. 6*C*), *Lerista* appears to have slower rates of speciation than *Ctenotus*, which directly contradicts the hypothesis that population differentiation is the rate-limiting step for phylogenetic speciation rates.

An outstanding challenge is the potential bias introduced by species delimitation on estimation of both population structure and speciation rates. For our primary analyses, we used a provisional taxonomy derived from coalescent-based species delimitation. More than 70% of OTUs recovered correspond to nominal species; this coalescent-based approach largely validates the existing taxonomy. However, for many species (fig. S13), our data suggest that the existing taxonomy does not accurately reflect species boundaries in the sphenomorphine group, consistent with recent taxonomic work in this clade (Rabosky et al. 2014*c*, 2017*a*). These OTUs appear to be evolving independently (fig. S13), although validating their robustness as "good" biological species will require extensive additional taxonomic work (Fišer et al. 2018). Until then, using quantitative species delimitation increases the comparability of units for which speciation rates are estimated and reduces error in rate estimates attributable to lumping multiple distinct species together as a single tip in a reconstructed phylogeny. Further, relying on existing taxonomies

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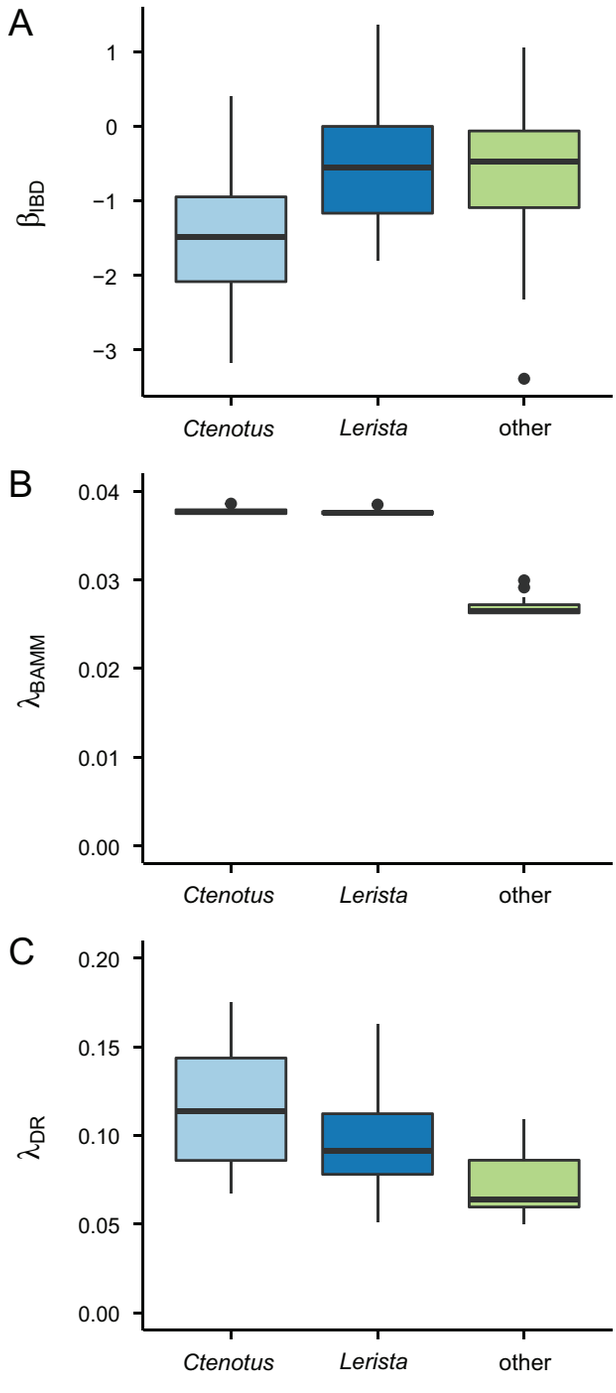

Figure 6: Distributions of population structure (β_{IBD}; *A*) and speciation rates (*B*, *C*) for *Ctenotus*, *Lerista*, and all other operational taxonomic units. Although *Ctenotus* and *Lerista* exhibit higher speciation rates than other sphenomorphine skinks, we see no corresponding increase in the extent of population structure. BAMM = Bayesian analysis of macroevolutionary mixtures; DR = diversification rate; IBD = isolation by distance.

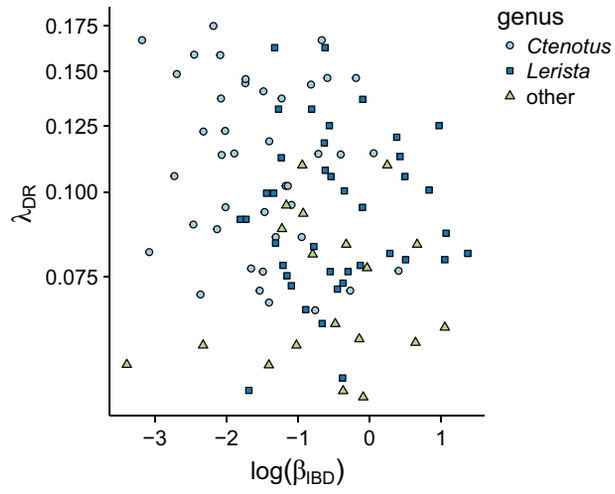

Figure 7: Relationship between population structure (β_{IBD}) and speciation rate at the tips as measured by the equal-splits (λ_{DR}). Points are coded by genera; "other" includes all non-*Ctenotus*/non-*Lerista* operational taxonomic units (fig. 2). Speciation rate and β_{IBD} are not correlated (phylogenetic generalized least squares; $P = .47$, $r = 0.004$), suggesting that the tempo of speciation is unrelated to the extent of population structure in this clade. DR = diversification rate; IBD = isolation by distance.

can also introduce error or even bias if the species under study have diverged on axes that are difficult to study, including physiology and pheromones (de León and Poulin 2016).

However, using population genetic data to both delimit units and estimate IBD potentially introduces bias into our analyses. Splitting nominal species that exhibit high levels of structure might reduce estimates of β_{IBD} for those taxa; conversely, this splitting would increase speciation rate estimates. Together, these two effects could weaken the association between β_{IBD} and speciation rates. We assessed the effects of this bias by performing several additional analyses. First, we tested the relationship between β_{IBD} and λ_{DR} using nominal species. This analysis minimized potential circularity, because few nominal species in the sphenomorphine clade have been described using genetic data. We also repeated our analyses after removing young OTUs, thus minimizing biases due to taxonomic oversplitting. Our results remain unchanged (tables 1, S3; fig. S11). As such, our analyses are likely robust to how errors and biases in species delimitation affect our estimates of β_{IBD} and speciation rate.

In addition to species delimitation, other biological factors can affect our estimates of population structure (Whitlock and Mccauley 1999). In this study, we measured population differentiation by inferring the slope of IBD for each species. This approach poses three primary limitations. First, for most of the species considered in this study, a simple IBD model explained much of the variation in pairwise F_{ST} estimates among individuals (fig. S6). Yet, for other species, IBD fails

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Table 1: Results across the two taxonomies used in this study: the revised taxonomy as defined by the generalized mixed Yule-coalescent (GMYC) approach and the existing taxonomy

	Revised taxonomy	Existing taxonomy
Number of tips	104	98
Phylogenetic signal for β_{IBD}	.34	.35
Are β_{IBD} and within-population diversity correlated?	Yes, $r = .34$	Yes, $r = .28$
β_{IBD} and within-population diversity correlation P value	.01917	.016
Are β_{IBD} and limb reduction correlated?	Yes, $r = .16$	Yes, $r = .16$
β_{IBD} and limb reduction correlation P value	3.4×10^{-6}	6.0×10^{-6}
Number of rate shifts inferred by BAMM	1	1
Are β_{IBD} and λ_{DR} correlated?	No, $r = .004$	No, $r = .04$
β_{IBD} and λ_{DR} correlation P value	.47	.39

Note: Shown is population structure (β_{IBD}) and its predictors, speciation rate, and correlations between population structure and speciation rate across two taxonomies: the revised taxonomy as defined by GMYC and the existing taxonomy. Results are qualitatively and quantitatively similar across both taxonomies. Correlation values are estimated using pairwise correlations between independent contrasts and are included for reference only. The P values are estimated from the full phylogenetic linear model. BAMM = Bayesian analysis of macroevolutionary mixtures; DR = diversification rate; IBD = isolation by distance.

to adequately describe within-species patterns. For some of these species, environmental or geographic barriers might be more strongly structuring genetic diversity. Second, we can measure only current patterns of differentiation, but these patterns might be decoupled from historical rates of differentiation that underlie species diversity in the focal group. For example, demographic shifts such as range expansion can reset equilibrium patterns of genetic variation (Peter and Slatkin 2013). Third, just as species continuously evolve greater levels of divergence (Darwin 1859; Mallet 1995), so do populations. IBD captures the earliest stage of population differentiation, but this early stage need not necessarily correlate with the formation of progressively more isolated population clusters or phylogeographic lineages (Avise 2000). Increased population differentiation has been hypothesized to result in an increase in the rates at which population isolates both form and extirpate (Levin 2000). If so, later stages of differentiation are more likely to reflect clusters that survived these initial stages of isolation and differentiation (Futuyma 1987; Dynesius and Jansson 2014). Defining the threshold between highly differentiated population and species remains both a practical and a theoretical challenge (Sukumaran and Knowles 2017), which can bias our understandings of this iterative process.

Another potential explanation for our results is that we are posing this question at the wrong phylogenetic scale. A single clade, as considered here, may simply be inadequate to detect such a weak correlation, and our power analysis confirms this intuition (fig. S12). Analyses across broader phylogenetic scales, including more independent shifts in differentiation and diversification rates, would have greater power to detect weaker relationships. Indeed, studies at broader scales have recovered modest, albeit significant, correlations between differentiation and diversification (Haskell and Adhikari 2009; Riginos et al. 2014; Harvey et al. 2017; but see Kisel et al. 2012). However, the sphenomorphine clade shows variation in dispersal-related traits, levels of population structure, and speciation rates. If population structure were a strong predictor of variation in speciation rate, we should have recovered it in this system: minimally, we should have observed faster speciation rates for *Lerista* (low dispersal; high β_{IBD}) relative to its sister clade *Ctenotus* (high dispersal; low β_{IBD}), but no such pattern was noted (fig. 6).

Perhaps most interestingly, both the weak correlations found in previous studies and the lack of relationship in our study might reflect a more fundamental reality about how species form. The prediction that the extent of population differentiation and diversification are correlated assumes that population differentiation is a rate-limiting step in species formation. If population splitting is not a rate-limiting step, then speciation might be limited primarily by the evolution of reproductive isolation or population persistence (Allmon 1992). Few studies have directly tested how the rate at which reproductive isolation evolves predicts broadscale patterns of speciation, and results from these studies are equivocal. In birds and drosophilid fruit flies, the rates at which intrinsic reproductive isolation evolves and species form appear to be uncorrelated (Rabosky and Matute 2013). In plants, shifts in mating system evolution, which can lead to reproductive isolation, do not predict diversification (Sabath et al. 2016). Nonetheless, the data are limited and so we cannot draw general conclusions about the extent to which the evolution of reproductive isolation limits the rate of speciation in nature. We note, with some optimism, that it is possible—albeit time-consuming—to infer reproductive isolation among scincid lizard species (Richmond and Jockusch 2007; Singhal and Moritz 2013) and to assemble comparative data sets on premating and postmating isolation across diverse clades (Sasa et al. 1998; Mendelson 2003; Stelkens et al. 2010).

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Alternatively, speciation might be limited more by population persistence, which is perhaps the most difficult aspect of the speciation process to quantify. Both species-level traits (i.e., range size, degree of ecological specialization) and historical events (i.e., stability of climate and geography through time) can affect population persistence. Further, many of these traits that promote population persistence are expected to have inverse effects on other stages of the speciation process. For example, while ecological specialization potentially increases the risk of population extinction, it may also increase the rate of population isolation. Much like measuring extinction on phylogenies is difficult (Rabosky 2016*a*), so is identifying cases where populations failed to persist. Researchers typically observe populations at a single point in time (the present) and are thus limited in their ability to observe population extirpation. Despite the complexity of population persistence, we suspect it plays an important role in generating extant diversity (Dynesius and Jansson 2014), especially given that isolated populations typically form more quickly than species (Rosenblum et al. 2012; Rabosky 2016*b*; Harvey et al. 2017). Our results suggest that, at least in Australian scincid lizards, the rate at which reproductive isolation evolves, along with factors that affect the longevity of incipient species, may be more important in determining the tempo of speciation than rates of population differentiation.

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