

Reproductive mode evolution in lizards revisited: updated analyses examining geographic, climatic and phylogenetic effects support the cold-climate hypothesis

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Abstract

Viviparity, the bearing of live young, has evolved well over 100 times among squamate reptiles. This reproductive strategy is hypothesized to allow maternal control of the foetus' thermal environment and thereby to increase the fitness of the parents and offspring. Two hypotheses have been posited to explain this phenomenon: (i) the cold-climate hypothesis (CCH), which advocates low temperatures as the primary selective force; and (ii) the maternal manipulation hypothesis (MMH), which advocates temperature variability as the primary selective force. Here, we investigate whether climatic and geographic variables associated with the CCH vs. the MMH best explain the current geographical distributions of viviparity in lizards while incorporating recent advances in comparative methods, squamate phylogenetics and geospatial analysis. To do this, we compared nonphylogenetic and phylogenetic models predicting viviparity based on point-of-capture data from 20 994 museum specimens representing 215 lizard species in conjunction with spatially explicit bioclimatic and geographic (elevation and latitude) data layers. The database we analysed emphasized Nearctic lizards from three species-rich genera (*Phrynosoma*, *Plestiodon* and *Sceloporus*); however, we additionally analysed a less substantial, but worldwide sample of species to verify the universality of our Nearctic results. We found that maximum temperature of the warmest month (and, less commonly, elevation and maximum temperature of the driest quarter) was frequently the best predictor of viviparity and showed an association consistent with the CCH. Our results strongly favour the CCH over the MMH in explaining lizard reproductive mode evolution.

Introduction

The evolution of live-bearing (viviparity) from egg-laying (oviparity) is a complex evolutionary transition that has taken place in most major vertebrate groups (Blackburn, 1999). Squamates (lizards and snakes) exhibit the largest number of transitions to viviparity among extant vertebrates, with viviparity evolving

independently a minimum of 108 times, representing approximately 20% of extant species (Shine, 1985; Guillet, 1993; Blackburn, 2000, 2006). This is particularly striking because viviparity is estimated to have evolved only 33–52 times among all other vertebrates (Blackburn, 1999, 2005). Whereas these numbers may be somewhat inflated due to reversions from viviparity to oviparity (Pyron & Burbrink, 2014), the relative frequency and phylogenetically widespread occurrence of this reproductive strategy among squamate reptiles provide a compelling system for studying the evolution of viviparity (Blackburn, 2006). We compare two primary hypotheses for the evolution of viviparity in squamates, the cold-climate hypothesis (CCH; Tinkle & Gibbons,

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1977; Shine, 1985) and the maternal manipulation hypothesis (MMH; Shine, 1995, 2004).

The CCH asserts that viviparity arose in squamates as a response to low temperatures because gravid females are able to behaviourally maintain warmer temperatures that are more conducive to embryonic development (Shine, 1985). Andrews (2000) later postulated that this transition is likely preceded by the shift to shallower nests by the female to take advantage of elevated surface temperatures and that the selective force behind the transition to viviparity is the increased mortality associated with predation of shallower nests and the accelerated embryonic development within the female, as opposed to in the nest. In contrast, the MMH (Shine, 1995, 2004) states that the more constant temperature provided *in vivo*, and not necessarily warmer temperatures, confers an adaptive advantage and the CCH can be viewed as a special case of the broader MMH. The MMH therefore predicts a higher probability of viviparity in locations where there is a high degree of variation in temperatures. Previous empirical studies reportedly support both the CCH and the MMH. For example, the CCH is supported by documented differences in maternal behaviour and deleterious effects on hatch rate, morphology and physiology at colder temperatures (Mathies & Andrews, 1995; Qualls & Andrews, 1999; Shine, 2004). By contrast, the MMH is supported by evidence of changes in thermoregulation of gravid females given experimental variation in laboratory conditions (Webb *et al.*, 2006; Li *et al.*, 2009; Gao *et al.*, 2010). However, the existence of oviparous species at high latitudes and tropical viviparous species (Tinkle & Gibbons, 1977; Hodges, 2004) as well as differences in thermoregulatory behaviour between viviparous and oviparous species or gravid/nongravid viviparous females is at odds with both of these hypotheses (Blackburn, 2000). Recently, updated distributional assessments using larger, more representative data sets and modern quantitative techniques lend support to the CCH (Lambert & Wiens, 2013; Pyron & Burbrink, 2014), although neither study explicitly compares competing hypotheses against one another.

In this study, we compare the CCH and MMH using phylogenetic comparative methods and GIS data. Our study provides the first large-scale quantitative comparison of predominant hypotheses that incorporates geographic and climatic variables to test for relationships among these predictors and reproductive mode in lizards, while also statistically testing and accounting for the effects of phylogeny. By considering only reproductively bimodal genera, we assume that we are more likely to consider those ecological parameters that select for the evolution of viviparity rather than those that may be favourable for its maintenance (Shine & Bull, 1979). We interpret an inverse relationship between temperature and viviparity and a direct, positive

relationship between both latitude and elevation with viviparity as support for the CCH. In contrast, we interpret a direct, positive relationship between variation in environmental variables and viviparity as support for the MMH. Our results generally support the CCH over the MMH, and we propose that factors correlated with higher elevations may selectively favour extended egg retention and ultimately viviparity in lizards.

Materials and methods

Taxonomic sampling

We examined eight lizard families containing 13 genera for which we obtained locality data for 20 994 of these lizards from museum databases (see Fig. 1, Supporting Information). Table 1 provides a summary of species sampling ($n = 215$) and patterns of oviparity and viviparity within the lizard families and genera included in our study. We excluded genera that contained many species with unknown reproductive modes (i.e. *Diploglossus*) as well as three reproductively bimodal species (*Lerista bougainvillii*, *Saiphos equalis* and *Zootoca vivipara*; Smith *et al.*, 2001). Three qualifying Nearctic genera, *Phrynosoma*, *Plestiodon* (formerly *Eumeces*) and *Sceloporus*, are well represented in museum collections. These three genera comprise the majority of our data set (19 879 specimens) and are thus the focus of most of our analyses, whereas the remaining worldwide data set serves to validate the generality of our Nearctic findings. *Phrynosoma* and *Sceloporus* are both members of the family Phrynosomatidae, and the *Plestiodon* is a member of the family Scincidae (Brandley *et al.*, 2010; Wiens *et al.*, 2010). Viviparity is thought to have arisen at least four times within *Sceloporus* (Mendez de la Cruz *et al.*, 1998); at least twice within *Phrynosoma* (Hodges, 2004); and at least twice within North American *Plestiodon* (Blackburn, 2000). All three of these genera occur from sea level to elevations of over 3000 m across a range extending from just south of Mexico northward to Canada (Smith, 1946; Campbell, 1998; Conant & Collins, 1998; Sherbrook, 2003; Stebbins, 2003). Therefore, these widespread genera not only present multiple transitions from oviparity to viviparity, but also provide large sample sizes along both latitudinal and elevation gradients.

We focused on the paraphyletic group of reptiles generally known as lizards ('*Lacertilia*'), purposefully excluding snakes (Serpentes) and worm lizards (Amphisbaenia). Whereas this renders our analysis incomplete at a phylogenetic level, these three groups exhibit a suite of derived ecological, morphological, physiological and behavioural characteristics (Pough *et al.*, 2003) that justify separate consideration (Meiri, 2008). Furthermore, viviparity in snakes tends to be phylogenetically distributed in widespread genera that are reproductively unimodal, such as *Boa*, *Thamnophis* and

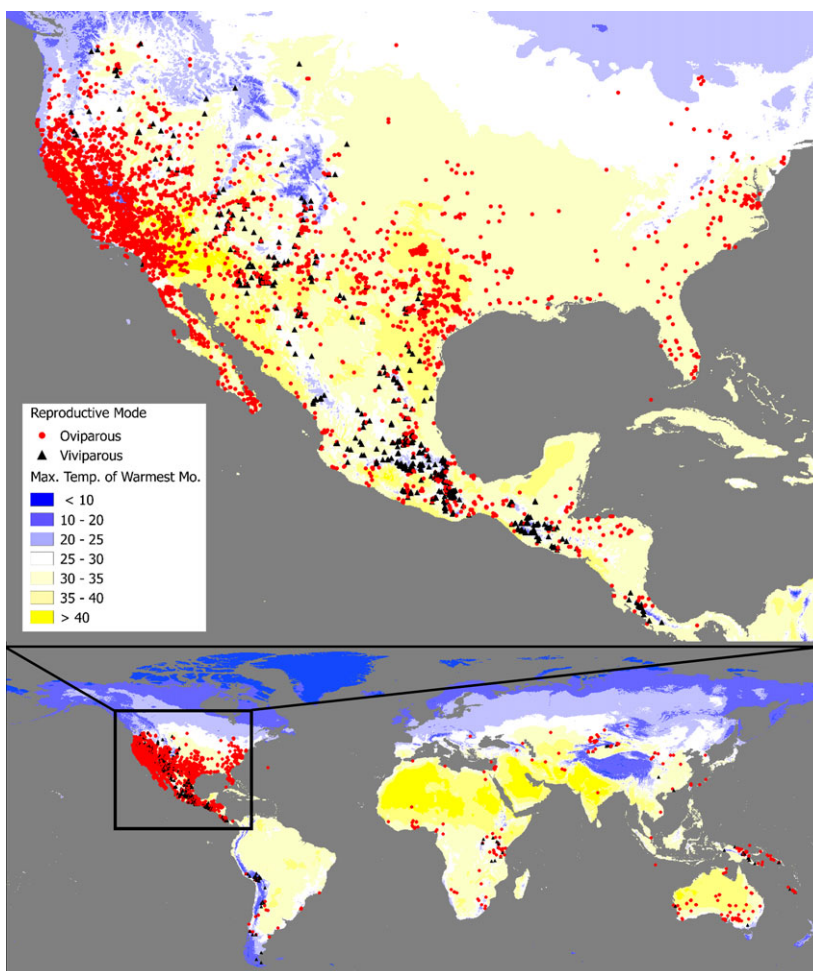


Fig. 1 The distribution of viviparous and oviparous lizard specimens overlaid on the maximum temperature of the warmest month layer ($^{\circ}\text{C}$).

numerous genera within the family Viperidae (Greene, 1997). Whereas we recognize that the taxonomic construct is a subjective delineation, it functionally represents radiations or lineages of very closely related species and therefore serves as a convenient selection criterion for our data set.

Reproductively bimodal genera allow us to model the effects of each climatic and geographic variable within groups that share a more recent common oviparous ancestor. Based upon the theoretical framework of niche conservatism (Peterson *et al.*, 1999; Wiens & Graham, 2005), we assume that the climatic conditions in which viviparity arose in these relatively young, closely related congeners are more similar to current conditions experienced by these species than if we had broadened our criteria to families or even sister genera that share a more ancient common ancestor and are more likely to have evolved under very different historical climatic conditions. Whereas we cannot rule out secondary dispersal in the evolutionary history of the species included in this study as a potential factor

influencing species ranges, we assume that it is less likely among species that share more recent common ancestors.

Climatic and geographical data

Specimens lacking GPS locality information were georeferenced using museum-based locality descriptions. Using DIVA-GIS 7.1.7.2 (<http://www.diva-gis.org>), we extracted climate and elevation data for each point locality from 19 bioclimatic data coverages (Table S1) downloaded from WorldClim (<http://www.worldclim.org>; Hijmans *et al.*, 2005). Variables were natural log-transformed where appropriate to normalize any skewed distributions (Table S1). Temperature variables were divided into two subcategories: those that describe discrete temperatures (annual mean temperature, maximum temperature of warmest month, minimum temperature of coldest month, and mean temperatures of warmest, coldest, wettest, and driest quarters); and those that describe temperature variability (mean

Table 1 Summary of species included in the present analyses.

Family	Genus	Nonphylogenetic analysis (<i>n</i> = 215)		Phylogenetic analysis (<i>n</i> = 183)	
		Oviparous	Viviparous	Oviparous	Viviparous
Agamidae	<i>Phrynocephalus</i>	13	1	9	1
Chamaeleonidae	<i>Chamaeleo</i>	12	8	12	8
Corytophanidae	<i>Corytophanes</i>	1	1	1	1
Diplodactylidae	<i>Rhacodactylus</i>	3	0	3	0
Lacertidae	<i>Eremias</i>	8	2	5	2
Liolaemidae	<i>Liolaemus</i>	10	12	9	9
Phrynosomatidae	<i>Phrynosoma</i>	7	6	7	6
	<i>Sceloporus</i>	37	24	37	21
Scincidae	<i>Leiolopisma</i>	0	1	0	0
	<i>Lepidothyris</i>	1	0	1	0
	<i>Lerista</i>	20	1	20	0
	<i>Lobulia</i>	1	0	0	0
	<i>Lygosoma</i>	4	0	2	0
	<i>Plestiodon</i>	16	6	16	4
	<i>Prasinohaema</i>	2	4	1	0
	<i>Scincella</i>	5	3	1	2
	<i>Tribolonotus</i>	5	1	4	1
Total species		145	70	128	55

diurnal range, temperature annual range, temperature seasonality and isothermality). We also analysed several WorldClim precipitation variables as these could be as important as temperature in terms of providing a suitable environment for developing eggs. Higher values for each of the discrete temperature variables are predicted to be negatively associated with the incidence of viviparity under the CCH, especially temperatures during the warmer months when lizard eggs are developing. Additionally, higher values for those variables related to temperature variability are predicted to be positively associated with the incidence of viviparity under the MMH.

Conventional statistical analyses

As noted by other authors (e.g. Felsenstein, 1985; Perry & Garland, 2002; Blomberg *et al.*, 2003), conventional statistical approaches are nonphylogenetic, effectively assuming taxa are related by a 'star phylogeny' with all species being equally related. Whereas results can be similar between such traditional methods and phylogenetic comparative methods, it is important to compare findings from both approaches (Garland & Ives, 2000; Garland *et al.*, 2005). For our first set of analyses, we therefore used nonphylogenetic methods. After removing redundant samples (specimens of the same species from the same locality), we conducted logistic regressions predicting the probability of viviparity with each climatic variable, latitude or elevation. Analyses were conducted using generalized linear models (GLMs; with family = 'binomial' using the 'glm' function) in R

(<http://www.cran.r-project.org>). We then compared predictor variables using Akaike information criterion (AIC) scores (Burnham & Anderson, 2002) from the regressions. The model with the smallest AIC score was the 'best fit' model. We analysed our large Nearctic data set at four separate grouping levels, including each of three genera (*Plestiodon*, *Phrynosoma* and *Sceloporus*) and all Nearctic taxa combined. We divided the remaining specimens by geographical region into Neotropical and Old World groups. This resulted in taxonomic/phylogenetic distinctions as a by-product; for example, South American *Liolaemus* and Mexican-Central American Corytophanidae in the Neotropical group form a lineage that is phylogenetically distinct from the Nearctic species, even though those also occur in the New World (Western Hemisphere). The smallest AIC score was subtracted from all scores within each group, producing a Δ AIC value associated with each model. Δ AIC can be viewed as the number of information units lost between the 'best' relative model and each alternative model.

Phylogeny

Recent estimates of squamate phylogeny provide comprehensive hypotheses of relationships among squamate families based on multilocus molecular phylogenetic analyses and morphological data from an efficient subsampling of squamate diversity (e.g. Townsend *et al.*, 2011; summarized in Sites *et al.*, 2011). Despite this, large-scale phylogenetic comparative studies have been somewhat limited as no phylogeny resolves relationships

among all species. However, in a recent study, Pyron *et al.* (2013) compiled all available molecular data on the group and inferred a new, supermatrix phylogeny of 4161 squamate species – nearly 50% of the > 9400 squamate species described to date. Pyron *et al.*'s (2013) molecular hypothesis of higher level squamate relationships represents an important advance providing maximum-likelihood (ML) nodal support values and branch lengths, as well as a basis for revised squamate classification (e.g. of subfamilies). We generated a starting tree for our analyses by pruning the final ML phylogeny from Pyron *et al.* (2013) to a subset matching our study species ($n = 183$ taxa; Fig. 2). The following phylogenetic analyses used species means for each variable. To ensure data quality, we removed redundant samples prior to calculating each species mean.

Phylogenetic comparative methods

We conducted phylogenetic statistical analyses to account for patterns of evolutionary relatedness among squamate lizards (Fig. 2). Analyses were conducted in the R environment using packages APE (Paradis *et al.*, 2004), PHYLOLM (Ho & Ané, 2014a,b) and CAPER (Orme, 2012). Our starter phylogeny contained branch lengths in units of substitutions/site reflecting levels of expected variance proportional to mutation rates. Thus, we avoided using entirely arbitrary branch lengths, as is customary in phylogenetic comparative studies when branch length data are not available *a priori* (e.g. Grafen, 1989; Garland *et al.*, 1992, 2005; Garland & Ives, 2000). However, we conducted phylogenetic regressions and ancestral reconstructions of reproductive mode using an ultrametric version of our tree topology generated using penalized likelihood ('chronopl' function, APE package) while using *Rhacodactylus* (Diplodactylidae), the most basal clade in our starting tree, as the root (Pyron *et al.*, 2013). The ultrametric tree has branch lengths proportional to time, thus assumes that the tips are contemporaneous species. Diagnostic tests, for example plots examining correlations between standardized independent contrasts (Felsenstein, 1985) and their standard deviations (Garland *et al.*, 1992), revealed that mutation- and time-proportional branch lengths adequately standardized independent contrasts; thus, our starting phylogenies were appropriate for the following phylogenetic comparative analyses. We conducted our phylogenetic analyses at geographical and taxonomic scales identical to our nonphylogenetic analyses, using working phylogenies derived by pruning the starting phylogenies accordingly.

We tested for phylogenetic signal among the climatic and geographic variables by estimating Pagel's (1999) λ in APE. Pagel's λ is a branch length scaling parameter varying from 0, indicating no phylogenetic signal, to a maximum of 1, indicating a close fit to a model of trait evolution by Brownian motion (Pagel, 1999). We

inferred ML estimates of λ for each variable using the initial ML topology and the phylogenetic generalized least squares method (PGLS; Pagel, 1999; Garland & Ives, 2000), testing model significance with a *t*-test (1000 permutations). Likelihood ratio tests were used to evaluate the hypotheses that λ estimates significantly differed from $\lambda = 0$ and $\lambda = 1$. Because viviparity is scored as a binary character, we measured phylogenetic signal strength in parity mode in CAPER using a statistic designed specifically for binary data, Fritz & Purvis's (2010) *D* test, and we assessed its significance using a *z*-test (1000 permutations).

Next, we conducted phylogenetic logistic regression analyses using a novel method recently developed by Ives & Garland (2010; hereafter, 'IG10' method). The IG10 method modifies a GLM framework for binomial distributions to incorporate a correlation matrix representing phylogenetic relatedness among taxa. It does this based on a model in which a binary trait evolves up a phylogenetic tree while switching between values of 0 and 1. Hence, this method is similar to phylogenetic applications of generalized estimating equations (GEEs; Paradis & Claude (2002)). However, the IG10 method improves upon previous phylogenetic logistic regressions (e.g. Pagel, 1994) and GEE models by: (i) accommodating continuous and discrete independent variables; (ii) giving a correlation structure that is correct for binary data; (iii) estimating α , a parameter that governs the strength of phylogenetic signal in the dependent variable, that is the rate at which phylogenetic correlations among species are lost; and (iv) using a Firth-style penalized likelihood estimation to reduce the known bias of ML logistic regression estimates away from zero (discussed in Ives & Garland, 2010). We conducted IG10 model analyses using the 'phyloglm' subroutine of the PHYLOLM package and subsets of our ultrametric tree. For each grouping level in our analysis, we compared phylogenetic GLM results and determined the best fitting model using AIC model selection based on AIC and Δ AIC scores within group/sample levels. Others have advocated considering phylogenetic tests significant at the 0.05 α level when analyses incorporated > 20 taxa, a sampling threshold shown to yield ≥ 0.8 power to detect phylogenetic signal in continuous traits (Blomberg *et al.*, 2003). However, to account for potentially elevated Type II error rates (i.e. false negatives) due to lower power, we modified the significance level to $\alpha = 0.10$ for continuous variables for analyses of clades with ≤ 20 taxa (Nearctic, *Phrynosoma* and *Plestiodon* groups). Also, Ives & Garland (2010) show that phylogenetic signal is inherently more difficult to detect in binary traits due to low power stemming from lower information content of binary rather than continuous variables, as well as strong independent variable effects. Power to detect phylogenetic signal in residual variation of binary traits is also paradoxically lowered when phylogenetic signal of that trait is high (Ives &

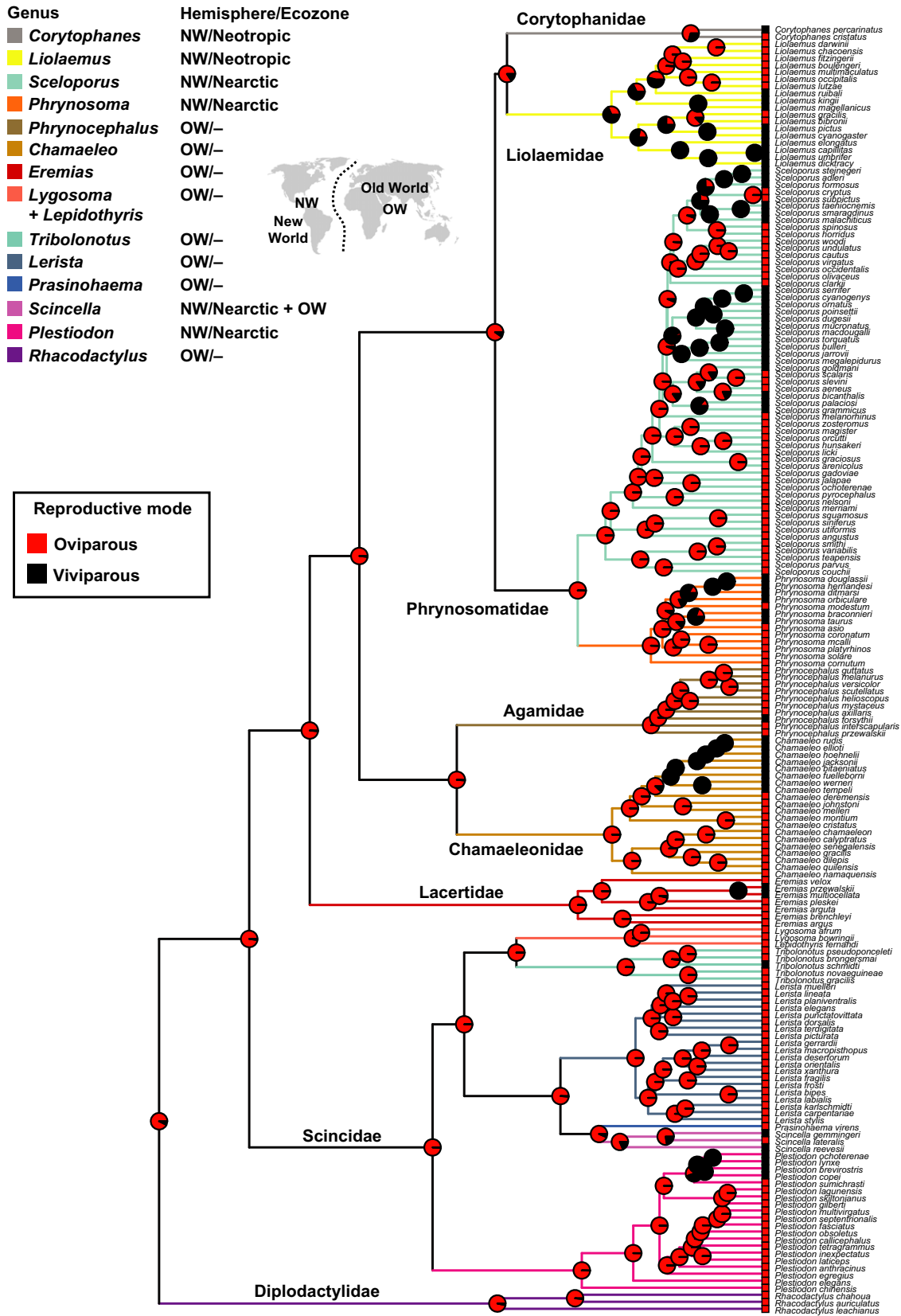


Fig. 2 Hypothesized phylogenetic relationships among 183 species of lizards in this study based on maximum-likelihood (ML) analysis of seven nuclear loci and five mitochondrial genes (Pyron *et al.*, 2013). ML ancestral character reconstructions of reproductive mode are shown along the tree, and tip boxes show observed reproduction mode of species. Reproductive mode probabilities on internal nodes were estimated using maximum likelihood under an equal-rates model. Branches are coloured by family, and legends at left summarize the taxonomic and geographical distributions of each taxon.

Garland, 2010). Therefore, we considered tests for phylogenetic signal in parity mode and phylogenetic logistic regressions significant at the 0.10 α level.

Ancestral state reconstruction

To reconstruct ancestral character states of reproductive mode in lizards, we performed stochastic character mapping in `MESQUITE 2.75` (Maddison & Maddison, 2011). We also performed ML reconstructions in `R` using the 'ace', or ancestral character estimation, subroutine of the `APE` package, specifying equal rates of transition between the two character states (Felsenstein, 1985; Pagel, 1994; Schluter *et al.*, 1997). The $n = 183$ taxa data set and the ultrametric time tree were used for all ancestral state reconstruction analyses.

Results

Conventional statistical analyses

Consistent with the predictions of the CCH, absolute value of latitude and elevation produce the 'best' models (with the lowest Δ AIC values) among our nonphylogenetic taxonomic/regional models (Table 2). Moreover, the absolute value of latitude and elevation usually show associations as predicted by the CCH (Table 2). Surprisingly, however, absolute value of latitude is negatively correlated with the probability of viviparity across five analyses, and annual mean temperature exhibits a positive association in the *Sceloporus* group (Fig. 2); these associations are opposite to CCH predictions. By contrast, temperature and precipitation variables perform very poorly when compared with latitude and elevation. The models involving MMH temperature variables as predictors also perform very poorly compared to the models incorporating latitude or elevation. Finally, precipitation-related variables exhibit inconsistent relationships across taxonomic groups. To test whether the two major Nearctic high-elevation geographic features (the Sierra Madre to the South and Rocky Mountains to the North) exhibit similar trends, we further split the Nearctic data set into two parts: one low latitude group ($< 31^\circ\text{N}$) and an upper latitude group ($> 31^\circ\text{N}$). The results were the same as for the combined analysis (data not shown). When Pearson correlations are calculated for each climatic variable compared to elevation, we find that the

mean temperature of warmest quarter (-0.665), annual mean temperature (-0.635), maximum temperature of warmest month (-0.595), minimum temperature of coldest month (-0.506) and mean temperature of driest quarter (-0.502) exhibit the strongest associations. Despite this, none of these variables independently approached the performance of the elevation model.

Phylogenetic comparative analyses

Using likelihood ratio tests, we discover statistically significant phylogenetic signal ($\lambda > 0$) for the majority of study variables within *Sceloporus* (54.5%), Nearctic taxa combined (81.8%), Neotropical taxa combined (72.7%), Old World taxa combined (90.9%) and all taxa combined (100%) (Tables 3 and S2). In contrast, only 18.2% of *Plestiodon* traits show significant phylogenetic structuring, and likelihood ratio test results are 63.6% equivocal among *Phrynosoma* traits (Table S2). Nonetheless, permutation tests of Fritz & Purvis' *D* reveal that lizard parity mode exhibits significant phylogenetic signal and *D* estimates < 0 at all group levels of our analysis, indicating greater phylogenetic conservatism of parity mode than expected under a Brownian motion model (Table 4). *D* tests also supported significant phylogenetic signal in parity mode at the level of our entire data set (Table S3). Consequently, the phylogenetic logistic regressions specified using IG10 GLMs are justified.

Similar to our nonphylogenetic analyses, our phylogenetic modelling results also strongly support the CCH over the MMH. However, AIC comparisons between the phylogenetic models indicate that the CCH-related variable maximum temperature of the warmest month (Fig. 1), and not the geographical variables, is the best predictor of parity mode (Table 5). Indeed, maximum temperature of the warmest month is the best predictor of viviparity based on model selection, or at least as good a predictor as other best fit models (i.e. indistinguishable from other perfectly predicting models; see below), in five of the six groups we analysed. The exception to this pattern occurred in the Old World lizards group, where the best predictor of viviparity was the mean temperature of the driest quarter (Table 5). Nevertheless, best fit models corresponded to CCH-related variables and showed associations with viviparity consistent with CCH predictions. In *Plestiodon*, multiple variables are perfect separators of reproductive

Table 2 Δ AIC ($AIC_{\text{model}(i)} - AIC_{\text{best model}}$) comparisons of geographic and climatic variables for each taxonomic group, as used to construct our nonphylogenetic models. Lower Δ AIC scores indicate better models, and results for the 'best fit' models are presented in boldface. Signs given in parentheses are associated with logistic regression parameter estimates and signify whether an increase (+) or decrease (–) in the predictor variable was associated with an increase in the probability of viviparity.

Variable	Nearctic				Neotropics All	Old World All
	<i>Plestiodon</i>	<i>Phrynosoma</i>	<i>Sceloporus</i>	All		
Geography						
Absolute value of latitude	0 (–)	326(–)	0 (–)	16 (–)	57 (+)	51 (–)
Elevation	169 (+)	0 (+)	412 (+)	0 (+)	11 (+)	0 (+)
Cold-climate hypothesis						
Annual mean temperature	480 (–)	131 (–)	1310 (+)	1544 (–)	3 (–)	63 (–)
Max. temperature of warmest month	280 (–)	102 (–)	749 (–)	634 (–)	1 (–)	8 (–)
Mean temperature of warmest quarter	312 (–)	81 (–)	989 (–)	882 (–)	0 (–)	10 (–)
Mean temperature of wettest quarter	466 (+)	327 (–)	1143 (+)	1402 (+)	15 (–)	66 (–)
Mean temperature of driest quarter	450 (–)	246 (–)	1139 (–)	1272 (–)	8 (–)	63 (–)
Mean temperature of coldest quarter	364 (+)	188 (–)	1061 (+)	1396 (+)	11 (–)	68 (+)
Min. temperature of coldest month	415 (+)	181 (–)	1126 (+)	1462 (+)	8 (–)	68 (+)
Maternal manipulation hypothesis						
Mean diurnal range	477 (–)	317 (+)	1250 (–)	1525 (–)	28 (+)	67 (–)
Temperature annual range	239 (–)	326 (+)	513 (–)	731 (–)	46 (+)	59 (–)
Temperature seasonality	37 (–)	326 (–)	237 (–)	354 (–)	59 (+)	58 (–)
Isothermality	28 (+)	321 (+)	131 (+)	157 (+)	60 (+)	46 (+)
Precipitation						
ln (precipitation of warmest quarter)	323 (+)	159 (+)	90 (+)	138 (+)	50 (–)	51 (+)
ln (precipitation of wettest month)	344 (+)	263 (+)	664 (+)	897 (+)	46 (–)	58 (+)
ln (precipitation of wettest quarter)	360 (+)	266 (+)	717 (+)	956 (+)	46 (–)	58 (+)
ln (precipitation of coldest quarter)	351 (–)	326 (+)	1096 (–)	1214 (–)	26 (–)	66 (+)
ln (precipitation of driest month)	448 (+)	225 (+)	1017 (+)	1222 (+)	22 (–)	59 (+)
ln (annual precipitation)	406 (+)	259 (+)	803 (+)	1079 (+)	39 (–)	58 (+)
ln (precipitation of driest quarter)	456 (+)	227 (+)	1055 (+)	1268 (+)	26 (–)	58 (+)
Precipitation seasonality	432 (+)	325 (–)	1144 (+)	1385 (+)	33 (+)	68 (–)
Number of specimens	<i>n</i> = 1431	<i>n</i> = 1218	<i>n</i> = 4054	<i>n</i> = 6899	<i>n</i> = 105	<i>n</i> = 299

AIC, akaike information criterion.

status, including two CCH-related temperature variables (maximum temperature of warmest month, mean temperature of warmest quarter) and elevation. Unfortunately, logistic regression cannot produce a likelihood score in such a situation, and it is therefore impossible to compare the remaining predictors in a Δ AIC framework. Still, whereas we only report the Δ AIC scores with respect to the best score that converged, it should be remembered that the perfect separators are clearly the best predictors. Thus, we consider each of the non-converging models to represent indistinguishable best fit models for *Plestiodon* (Table 5), and all patterns in these models fit the predictions of the CCH, not the MMH.

Ancestral state reconstruction

To understand the evolutionary history of reproductive mode in lizards, we mapped the reproductive states of all lizards with phylogenetic information in our analysis, using phylogenetic relationships from a supermatrix phylogeny with ML branch lengths (Pyron

et al., 2013) transformed into units of time using penalized likelihood. Ancestral reproductive mode was then reconstructed using stochastic character mapping, and ancestral reproductive mode probabilities were estimated using maximum likelihood. Results are similar for both stochastic and ML methods; thus, results are only presented for ML-estimated ancestral reproductive mode probabilities. In our phylogeny, the last common ancestor of all lizards was oviparous, and viviparity evolved separately approximately 15 times, including one origin of viviparity within *Plestiodon*; two origins within *Scincella*; single origin within *Tribolonotus*, *Eremias*, *Chamaeleo* and *Phrynocephalus*; two origins of viviparity within *Phrynosoma* (with one loss in *P. modestum*); four origins, with one subsequent loss (*cryptus* + *subpictus* clade), in *Sceloporus*; one origin followed by two losses in the Neotropical *Liolaemus*; and one origin within Corytophanidae (Fig. 2). Thus, some clades such as *Sceloporus* and *Liolaemus* exhibited much higher variation in reproductive mode than others such as *Lerista*, which showed no parity mode transitions.

Table 3 Results of tests for phylogenetic signal for geographic and environmental predictor variables across all 183 taxa in the phylogenetic comparative analysis. Results are based on analyses using the maximum-likelihood phylogeny of Pyron *et al.* (2013). The null hypothesis of no phylogenetic signal ($\lambda = 0$) was rejected for each variable ($P < 0.0001$; not shown).

Variable	ML λ (predictor var.)	λ 95% CIs	$\lambda = 1$ (Brownian motion)
Geography			
Absolute value of latitude	0.996	0.979, NA	0.533
Elevation	0.933	0.848, 0.971	< 0.001
Cold-climate hypothesis			
Annual mean temperature	0.899	0.786, 0.955	< 0.001
Max. temperature of warmest month	0.930	0.841, 0.969	< 0.001
Mean temperature of warmest quarter	0.914	0.801, 0.963	< 0.001
Mean temperature of wettest quarter	0.603	0.186, 0.856	< 0.001
Mean temperature of driest quarter	0.781	0.573, 0.905	< 0.001
Mean temperature of coldest quarter	0.948	0.884, 0.979	< 0.001
Min. temperature of coldest month	0.957	0.901, 0.984	< 0.001
Maternal manipulation hypothesis			
Mean diurnal range	0.943	0.849, 0.984	< 0.001
Temperature annual range	0.995	0.978, NA	0.106
Temperature seasonality	0.995	0.980, NA	0.088
Isothermality	0.996	0.984, 1.000	0.042
Precipitation			
ln (precipitation of warmest quarter)	0.960	0.886, 0.987	< 0.001
ln (precipitation of wettest month)	0.970	0.923, 0.990	< 0.001
ln (precipitation of wettest quarter)	0.974	0.932, 0.992	< 0.001
ln (precipitation of coldest quarter)	0.895	0.690, 0.964	< 0.001
ln (precipitation of driest month)	0.967	0.916, 0.989	< 0.001
ln (annual precipitation)	0.975	0.935, 0.992	< 0.001
ln (precipitation of driest quarter)	0.946	0.867, 0.980	< 0.001
Precipitation seasonality	0.959	0.820, 0.995	0.009

CIs, confidence intervals; ML, maximum likelihood. Bold denotes statistical significance at $\alpha = 0.05$.

Discussion

Many studies have documented transitions from oviparity to viviparity in squamates, or potential ecological factors associated with such transitions (e.g. Blackburn, 1982, 1999, 2006; Shine, 1985, 1995, 2004; Andrews, 2000; Webb *et al.*, 2006; Gao *et al.*, 2010; Lambert & Wiens, 2013; Pyron & Burbrink, 2014). Still, we know relatively little about the factors, including selective processes, that work singly or in concert to shape the evolution of squamate viviparity. Here, we test the two most popular hypotheses that make different predictions about the evolution of viviparity in squamates – the CCH (e.g. Shine, 1985) and the MMH (Shine, 1995, 2004). The CCH predicts that the distribution of viviparous lizard species should be associated with lower temperatures, and (by their natural correlation with temperature) also higher elevations and latitudes. By contrast, the MMH predicts higher instances of viviparity in environments with greater temperature variability, with the female serving to stabilize the temperature fluctuations *in vivo*. The results of our non-phylogenetic and phylogenetic analyses indicate that the present-day occurrence of viviparity in lizards is best explained by the CCH (Figs 1 and 2; Tables 2 and 5).

Our findings based on conventional statistical analyses generally support the CCH for the evolution of viviparity, while at the same time providing strong support against the MMH. Elevation performs very well as a predictor of viviparity for most groups, but the temperature variables overall did not, and latitude exhibits an opposite association than was predicted (except in *Phrynosoma*; Table 2). All MMH variables, as well as the precipitation variables, are relatively poor predictors across groups. Additionally, we do not observe any consistent and expected relationship between the MMH-related temperature variables or any precipitation variables, with the exception of isothermality (Table 1). In this case, an increase in isothermality, which is measured by taking the ratio of mean diurnal range and temperature annual range (Table S1), was associated with an increase in the predicted probability of viviparity.

As was the case with the conventional statistical analyses, our phylogenetic comparative analyses support the CCH over the MMH as the better explanation for the evolution of viviparity in lizards (Tables 5 and S4). However, these fundamentally different approaches yield notably different results (Tables 2 and 5). The principal difference between these two sets of results is that elevation and the absolute value of

Table 4 Measures of phylogenetic signal strength for parity mode across all group levels in the analysis. Results are based on analyses using the maximum-likelihood phylogeny of Pyron *et al.* (2013). Negative D values indicate that patterns of viviparity in a group were more phylogenetically conserved (clumped) than expected under a Brownian motion model of random evolutionary change.

	Nearctic				Neotropics All	Old World All
	<i>Plestiodon</i>	<i>Phrynosoma</i>	<i>Sceloporus</i>	All		
Fritz & Purvis' (2010) D	-3.223	-3.193	-1.222	-0.310	-0.790	-0.108
$P(D = 1)$, probability of random trait distribution across the tree (no phylogenetic signal)	0.000	0.005	0.000	0.000	0.001	0.000
$P(D = 0)$, probability of Brownian structure, that is phylogenetic signal	0.985	0.969	1.000	0.809	0.881	0.610
Number of species	$n = 20$	$n = 13$	$n = 58$	$n = 91$	$n = 20$	$n = 72$

Bold denotes statistical significance at $\alpha = 0.05$.

latitude are the best predictors of viviparity in most of the conventional analyses, whereas maximum temperature of the warmest month is the best predictor in the majority of taxonomic/regional groups in our phyloge-

netic analyses. Here, we place our confidence in the phylogenetic model results for at least two reasons. First, in the light of evidence for significant phylogenetic signal in the dependent and independent variables

Table 5 ΔAIC scores ($AIC_{\text{model}(i)} - AIC_{\text{best model}}$) comparisons of geographic and climatic variables for each taxonomic group based on phylogenetic comparative analyses using the IG10 method. Lower ΔAIC scores are associated with better models, and 'best' models are shown in boldface. We inferred indistinguishable best fit models for *Plestiodon* in which estimates diverged, leading to no convergence but perfect prediction (PP) of parity mode. Signs given in parentheses are associated with logistic regression parameter estimates and signify whether an increase (+) or decrease (-) in the predictor variable was associated with an increase in the probability of viviparity.

Variable	Nearctic				Neotropics All	Old World All
	<i>Plestiodon</i>	<i>Phrynosoma</i>	<i>Sceloporus</i>	All		
Geography						
Absolute value of latitude	3.5 (-)	8.0 (-)	6.2 (-)	21.5 (-)	6.3 (+)	3.4 (-)
Elevation	PP (+)	0.1 (+)	4.8 (+)	8.2 (+)	2.9 (+)	4.3 (+)
Cold-climate hypothesis						
Annual mean temperature	7.1 (-)	6.5 (-)	6.2 (-)	20.4 (-)	3.9 (-)	3.0 (-)
Max. temperature of warmest month	PP (-)	0 (-)	0 (-)	0 (-)	0 (-)	5.4 (-)
Mean temperature of warmest quarter	PP (-)	0.1 (-)	5.5 (-)	8.1 (-)	1.6 (-)	6.3 (-)
Mean temperature of wettest quarter	7.8 (-)	7.9 (-)	7.2 (-)	21.2 (-)	6.3 (-)	0.9 (+)
Mean temperature of driest quarter	7.7 (-)	7.0 (-)	5.7 (-)	19.9 (-)	3.9 (-)	0 (-)
Mean temperature of coldest quarter	8.0 (+)	8.6 (-)	5.3 (-)	19.1 (-)	5.0 (-)	3.8 (-)
Min. temperature of coldest month	7.6 (-)	8.5 (-)	5.2 (+)	18.6 (-)	4.9 (-)	3.3 (-)
Maternal manipulation hypothesis						
Mean diurnal range	11.8 (-)	8.2 (-)	6.9 (-)	16.6 (+)	6.5 (+)	2.0 (-)
Temperature annual range	6.0 (-)	7.0 (-)	6.5 (-)	16.9 (-)	6.3 (+)	3.0 (-)
Temperature seasonality	0.0 (-)	6.8 (-)	7.6 (-)	16.7 (-)	6.4 (-)	4.0 (-)
Isothermality	0.1 (+)	7.0 (+)	4.8 (+)	16.6 (+)	5.7 (+)	3.0 (+)
Precipitation						
ln (precipitation of warmest quarter)	8.3 (+)	7.3 (+)	5.8 (+)	20.2 (+)	6.2 (-)	1.9 (+)
ln (precipitation of wettest month)	8.4 (+)	5.9 (+)	8.8 (+)	20.9 (+)	5.7 (-)	3.2 (+)
ln (precipitation of wettest quarter)	8.5 (+)	5.8 (+)	8.4 (+)	21.1 (+)	6.7 (-)	2.7 (+)
ln (precipitation of coldest quarter)	7.7 (-)	8.5 (+)	3.7 (+)	18.4 (-)	6.1 (-)	2.5 (-)
ln (precipitation of driest month)	7.7 (-)	6.0 (+)	5.7 (+)	19.0 (+)	6.3 (-)	2.6 (-)
ln (annual precipitation)	8.7 (+)	4.9 (+)	8.4 (+)	21.2 (+)	5.9 (-)	2.4 (+)
ln (precipitation of driest quarter)	7.7 (-)	6.0 (+)	5.8 (+)	19.3 (+)	6.4 (-)	2.6 (-)
Precipitation seasonality	6.8 (+)	8.3 (+)	6.4 (-)	18.7 (+)	7.3 (+)	2.3 (+)
Number of species	$n = 20$	$n = 13$	$n = 58$	$n = 91$	$n = 20$	$n = 72$

AIC, akaike information criterion.

across groups (Tables 3, 4 and S2), the assumption made by nonphylogenetic logistic regressions that species represent independent data points is not met. Thus, we expect that accounting for the phylogenetic signal in the data should produce more accurate statistical models (Garland *et al.*, 2005; Ives & Garland, 2010). Second, we feel confident in our tests of phylogenetic signal based on Pagel's λ (with PGLS) and Fritz & Purvis' D , because these are recommended (Fritz & Purvis, 2010; Revell, 2010) over other methods such as that of Blomberg *et al.* (2003). Thus, whereas elevation remains a good predictor at least in *Plestiodon*, the 'new-found' superiority of maximum temperature of warmest month in the phylogenetic results is likely due to the accounting for phylogenetic information in the data in these models. By comparison, the conventional results are likely confounded by pseudoreplication caused by not factoring species relatedness into the models to differing degrees for each predictor variable.

Overall, the only subanalysis for which we find relatively weak evidence for the CCH over the MMH during the phylogenetic comparative analyses is in the Old World. In this case, although the best model was given by mean temperature of the driest quarter, mean diurnal range has an AIC score similar enough (within two information units) to conclude that both models were equally supported by the data. Given the relatively similar AIC scores across models in the Old World subanalysis (the largest Δ AIC was 6.3), two conclusions seem appropriate. First, many of the variables we evaluated are equally important in driving the evolution of reproductive mode, or, second, the evolution of viviparity within the Old World taxa is primarily driven by factors not represented by the variables in our analysis.

Whereas it may be assumed that MMH variables were of some importance during the evolution of viviparity within the Old World taxa given their relatively low Δ AIC scores, it is important to note that the estimated effect was negative for three of the four MMH variables, meaning that as variability increased, the probability of viviparity decreased. Isothermality, a measure of the temperature variability a point may experience on a monthly basis compared to the yearly variability, was within 3.0 information units of the best model (Table S1) and did show a positive association, although it is not clear to us whether this is necessarily predicted by the MMH. Whereas the MMH-relevant bioclimatic variables in our study certainly measure temperature variability, we feel that one shortcoming of the data is that they are not as ideal with regard to time as the CCH variables. Specifically, the CCH-relevant temperature variables not only measure temperature but also do so at or close to the time periods when eggs would be developing (e.g. warmest month, warmest quarter). By contrast, MMH-relevant temperature variables available from WorldClim, such as isothermality, only reflect temperature variability throughout the

year. Therefore, AIC scores associated with the less temporally specific MMH variables are likely biased towards larger values, although the validity or extent of this bias cannot be determined at present.

A possible exception to our MMH variables being less time appropriate would occur if precipitation variables were also considered part of the relevant predictors of the MMH. Whereas the MMH is generally considered to make specific predictions in relation to temperature, precipitation (moisture) and associated environmental changes are known to have profound effects on maternal behaviour and embryos in developing eggs (e.g. Stamps, 1976; Ackerman, 1992; Vleck, 1992). Additionally, several of the precipitation variables represented in the bioclimatic database we analysed are more temporally appropriate. Regardless, we found little support for any of the precipitation variables analysed in this study. Specifically, a precipitation model was only indistinguishable from a best fit temperature variable model in a single case; in analyses of Old World taxa combined, \ln (precipitation of warmest quarter) was < 2 information units from the best fit model (Table 5). This suggests that, even if precipitation variables were shown to be relevant to the MMH, our results would still strongly support the CCH.

Between our two geographic variables, elevation consistently performs well across most groups as a predictor of viviparity. There are unmeasured factors associated with elevation, such as decreased land area, decreased atmospheric pressure (and associated partial pressures of the atmospheric gasses) and increased solar radiation, that could potentially work in concert with lower temperatures to generate or accentuate the actual selective pressure(s) driving the evolution of viviparity in lizards. Whereas the potential effects of these factors are primarily speculative in nature, future research should empirically explore these climatic factors that covary with elevation, particularly decreasing partial pressures of oxygen.

In conclusion, our results provide strong support for the CCH over the MMH for explaining the evolution of viviparity in lizards. Our conclusions must be tempered, however, by weighing two potential sources of uncertainty in our results. First, although our evolutionary models (i.e. working phylogenies) adequately fit the data according to diagnostic tests and we used the most comprehensive and recently published molecular phylogeny for squamates (Pyron *et al.*, 2013), we must acknowledge the limitation of potential inaccuracies in the phylogenetic hypotheses and branch lengths employed. However, as phylogenetic data continue to improve for squamates, it will be possible to test adaptive hypotheses with more accurate and explicit reference to time across an ever more complete squamate phylogeny. Secondly, whereas information-theoretical approaches provide powerful tools for directly comparing multiple competing hypotheses, our observational

data set does not allow AIC, nor any other statistic, to test causality. Instead, our analyses are designed to determine which model best fits the observed data, while penalizing models with more parameters. Future analyses may benefit from conducting model selection analyses similar to ours, but on a broader universe of potential models predicting viviparity, perhaps from a multivariate perspective. Because we find that variables that capture variation in temperatures during the time embryos develop (warmest day of the warmest month, mean temperature during driest quarter) serve as good predictors of the distribution of viviparity, we suggest that a potentially fruitful direction for future research will also be to focus on elucidating the physiological mechanisms that are affected by these and associated environmental-climatic variables.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Bioclimatic environmental data variables used in this study.

Table S2 Estimates of Pagel's (1999) λ and likelihood ratio tests for phylogenetic signal using PGLS, for all environmental variables in this study.

Table S3 The measure of phylogenetic signal in reproductive mode across all of the taxa in the phylogenetic comparative analyses, based on Fritz & Purvis' (2010) *D*.

Table S4. Full results and parameter estimates from phylogenetic logistic regressions (GEE models) of reproductive mode (oviparity, viviparity) against each environmental variable in this study.

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