



EVOLUTION OF VIVIPARITY: A PHYLOGENETIC TEST OF THE COLD-CLIMATE HYPOTHESIS IN PHRYNOSOMATID LIZARDS

Shea M. Lambert^{1,2} and John J. Wiens¹

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

²E-mail: sheamaddocklambert@gmail.com

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The evolution of viviparity is a key life-history transition in vertebrates, but the selective forces favoring its evolution are not fully understood. With >100 origins of viviparity, squamate reptiles (lizards and snakes) are ideal for addressing this issue. Some evidence from field and laboratory studies supports the “cold-climate” hypothesis, wherein viviparity provides an advantage in cold environments by allowing mothers to maintain higher temperatures for developing embryos. Surprisingly, the cold-climate hypothesis has not been tested using both climatic data and phylogenetic comparative methods. Here, we investigate the evolution of viviparity in the lizard family Phrynosomatidae using GIS-based environmental data, an extensive phylogeny (117 species), and recently developed comparative methods. We find significant relationships between viviparity and lower temperatures during the warmest (egg-laying) season, strongly supporting the cold-climate hypothesis. Remarkably, we also find that viviparity tends to evolve more frequently at tropical latitudes, despite its association with cooler climates. Our results help explain this and two related patterns that seemingly contradict the cold-climate hypothesis: the presence of viviparous species restricted to low-elevation tropical regions and the paucity of viviparous species at high latitudes. Finally, we examine whether viviparous taxa may be at higher risk of extinction from anthropogenic climate change.

KEY WORDS: Climate, comparative methods, life-history evolution, phylogeny, reproductive mode, squamates.

The transition from oviparity (egg laying) to viviparity (live bearing) is a major evolutionary shift in life history with many potential consequences for the morphology, physiology, behavior, and ecology of an organism (Shine and Bull 1979; Guillelte 1993; Blackburn 2006; Thompson and Blackburn 2006; Thompson and Speake 2006). The evolution of viviparity may have disadvantages (e.g., loss of the entire brood if the mother dies; Neill 1964; Tinkle and Gibbons 1977). However, the repeated evolution of viviparity across many vertebrate clades (e.g., sharks, ray-finned fish, frogs, salamanders, caecilians, mammals, lizards, snakes; Amoroso 1968; Vitt and Caldwell 2009) suggests that viviparity can provide a strong selective advantage under some conditions (Tinkle and Gibbons 1977; Blackburn 1999a). Nevertheless, the particular conditions that favor the evolution of viviparity are not

yet fully understood (Hodges 2004; Blackburn 2005; Sites et al. 2011).

Squamate reptiles (lizards and snakes) offer one of the best model systems for understanding the evolution of viviparity in vertebrates. This is because in squamates, viviparity is estimated to have originated at least 100 times, more than twice the number in all other vertebrate clades combined (Blackburn 2000). These repeated origins facilitate the use of phylogenetic analyses to identify the ecological correlates of these transitions in reproductive mode. As a counterexample, determining the selective forces that led to the evolution of viviparity is difficult or impossible in mammals, in which viviparity seems to have evolved only once among extant lineages and >100 million years ago (Blackburn 2005). The single origin makes statistical analysis difficult, whereas the

ancient timing makes it problematic to infer the ecological conditions that were associated with this transition.

Over a century of research has focused on viviparity in squamates, and numerous hypotheses have been proposed to explain its evolution (reviews in Tinkle and Gibbons 1977; Shine 1985; Sites et al. 2011). The “cold-climate” hypothesis is one of the first hypotheses proposed (Mell 1929; Weekes 1935; Sergeev 1940), and has emerged as one of the most important (e.g., Tinkle and Gibbons 1977; Shine 1985; Sites et al. 2011). The cold-climate hypothesis posits that viviparity evolves as an adaptation to cold temperatures during the egg-laying season (Tinkle and Gibbons 1977; Shine 1985). Cold conditions are problematic because they can slow development (Packard et al. 1977), and are potentially lethal to developing eggs. According to this hypothesis, retention of eggs in utero and behavioral thermoregulation by pregnant females allows for faster development and avoids lethally cold temperatures for developing embryos (Shine 1985). The cold-climate hypothesis is also appealing because intermediate stages on the path toward viviparity (i.e., longer egg retention) should still provide fitness benefits (Shine 1985; Andrews 2000). Specifically, offspring that stay within the mother longer should hatch sooner, giving them an advantage in the form of increased time to build up energy reserves and seek shelter before temperatures reach lethally low levels in autumn and winter (Tinkle and Gibbons 1977; Shine and Bull 1979; Shine 1985).

A number of field and laboratory studies support many of the predictions of the cold-climate hypothesis. For example, studies have shown that the body temperatures of gravid lizards are higher than relevant soil temperatures (Shine 1983), that viviparous populations and species occur in colder climates relative to closely related oviparous populations and species (Shine 1985, 1987; Qualls and Shine 1998), that higher-elevation populations are associated with longer egg retention in oviparous species (Calderón-Espinosa et al. 2006; Rodríguez-Díaz and Braña 2012), and that offspring of oviparous species experimentally incubated at cooler temperatures are less fit and take longer to develop (e.g., Qualls and Andrews 1999; Shine 2002; Parker and Andrews 2007; for an example in a viviparous species, see Li et al. 2009). However, not all studies have found support for the cold-climate hypothesis. For example, Andrews (2000) and Shine et al. (2003) both found that nests of oviparous species and body temperatures of pregnant females show only slight differences in average temperature, and Li et al. (2009) found that females of the viviparous lacertid *Eremias przewalskii* actually selected lower body temperatures when pregnant. Also, García-Collazo et al. (2012) found that in the oviparous lizard *Sceloporus aeneus*, females from lower and warmer environments unexpectedly retain their eggs for longer than those from higher and colder localities.

Nevertheless, the most basic prediction of the cold-climate hypothesis, that viviparity is more likely to evolve in cold cli-

mates, remains untested using the necessary combination of phylogenetic and climatic information. Shine and Berry (1978) examined the distributions of viviparous squamates in North America and Australia, and used stepwise multiple regression to test for an association with broad-scale climatic variables. Surprisingly, they found that their measures of temperature (including mean temperatures of individual summer months) were no more correlated with the proportion of live-bearing species than were other variables such as precipitation and evaporation. Shine and Bull (1979) found that “recent” origins of viviparity (i.e., within genera, subgenera, or species groups) are associated with relatively cold environments, but did not use a phylogeny or quantitative measures of climate. In a landmark paper, Shine (1985) used available phylogenetic information to estimate the number of origins of viviparity in squamates and tested for associations with ecological factors. However, the statistical analyses did not account for phylogeny, and only qualitative measures of climate (e.g., “colder” vs. “warmer”) were used. Other studies have shown that within a single genus (*Pseudechis*; Shine 1987) and species (*Lerista bougainvillii*; Qualls and Shine 1998), viviparous species and populations inhabit significantly colder environments than their oviparous counterparts, but neither study’s statistics accounted for phylogeny. Hodges (2004) used phylogenetic comparative methods and found that viviparity is associated with high elevations in the phrynosomatid lizard genus *Phrynosoma* but did not include explicit data on climate. Similarly, Schulte et al. (2000) used the phylogeny-based concentrated changes test (Maddison 1990), and did not find a significant relationship between viviparity and occurrence in high elevations (>2500 m) or latitudes (>40° south) in the lizard genus *Liolaemus*, but again did not include data on climate. Finally, Schulte and Moreno-Roark (2010) used a time-calibrated phylogeny to estimate the number of origins of viviparity in iguanian lizards and showed that the majority of these origins likely occurred prior to Pliocene-Pleistocene glaciations, but they did not test for associations between transitions to viviparity and climate.

Here we investigate the evolution of viviparity in the lizard family Phrynosomatidae. We use a relatively complete, time-calibrated phylogeny, GIS-based climatic data (Hijmans et al. 2005), and recently developed comparative methods that are explicitly designed to test for a relationship between an independent continuous variable (e.g., climate) and a dependent discrete variable (e.g., reproductive mode). These methods are phylogenetic logistic regression (Ives and Garland 2010) and a Bayesian implementation of Wright’s threshold model from quantitative genetics (Wright 1934; Revell 2012; Felsenstein 2012). We also estimate ancestral values for reproductive mode and environmental variables to better understand the ecological context(s) in which viviparity originates. Without inferring the ecological conditions at the time of origin of viviparity, it is difficult to know if, for

example, viviparity evolved in response to cold climates, or evolved in a very different environment and promoted later invasion of cold climates (Blackburn 2005).

The family Phrynosomatidae is an excellent model system to address the evolution of viviparity, for several reasons. Phrynosomatidae includes nine genera and approximately 138 species (review in Wiens et al. 2013) distributed from Canada to Panama (Vitt and Caldwell 2009) with two genera containing both viviparous and oviparous species (*Phrynosoma* and *Sceloporus*), and the other seven genera being entirely oviparous. Previous work suggests that there were four separate origins of viviparity in *Sceloporus* (Mendez-de la Cruz et al. 1998) and two in *Phrynosoma* (Hodges 2004). Thus, there should be enough origins of viviparity to test their environmental correlates with sufficient statistical power. Also, a detailed phylogeny and climatic data for the group are now available (Wiens et al. 2013). Furthermore, because viviparity varies among relatively closely related phrynosomatid species (i.e., congeners) it should be far easier to address the ecological conditions that favor its evolution than in a group where the origins of viviparity are more ancient (e.g., mammals).

The evolution of viviparity in phrynosomatid lizards is particularly interesting because of two patterns seen in *Sceloporus* that seem contradictory to the cold-climate hypothesis. First, a number of lowland, tropical species are viviparous (Guillette et al. 1980; Mendez-de la Cruz et al. 1998). Second, many *Sceloporus* species that inhabit high elevation, cold climates in North America are oviparous (Guillette et al. 1980; Mendez-de la Cruz et al. 1998; this study). We attempt to explain these patterns here.

Finally, a recent analysis has suggested that viviparous species of *Sceloporus* may have a higher extinction risk than oviparous species in the face of recent, anthropogenic climate change (Sinervo et al. 2010). This pattern was suggested because many viviparous species are restricted to high-elevation “islands” in Mexico (with the implicit assumption that these species have narrower elevational ranges), and because climate is changing faster at higher elevation sites. However, the elevational and latitudinal ranges of viviparous and oviparous species were not directly compared. Here we use phylogenetic comparative methods to address how anthropogenic climate change might differently impact the survival of oviparous and viviparous species in Phrynosomatidae, based on their latitudinal and elevational range extents and temperature niche breadths.

Materials and Methods

DATA COLLECTION

For all analyses, we used a time-calibrated phylogenetic tree of 117 species with associated climatic data (from Quintero and Wiens 2013; Wiens et al. 2013). The phylogenetic tree was estimated based on a data matrix combining the data of Wiens et al.

(2010) and Leaché (2010), and includes data from eight nuclear and five mitochondrial loci (but not all species sampled for all genes). Divergence times were estimated using the Bayesian uncorrelated lognormal method in BEAST (Drummond et al. 2006; Drummond and Rambaut 2007) with four fossil calibration points. Environmental data (climatic variables and elevation from the WORLDCLIM database; Hijmans et al. 2005) were extracted from georeferenced museum localities at a ~ 1 km² resolution (Appendix S1; 117 species, mean of ~ 54 localities per species, range from 1 to 1230). Sampled localities were carefully selected and spanned most of each species’ range, based on comparison to published range maps (e.g., Sites et al. 1992; Conant and Collins 1998; Grismer 2002; Stebbins 2003). Although some species are known from few localities, this appears to be related to these species having very restricted geographic ranges (Wiens et al. 2013), especially because phrynosomatid species tend to be conspicuous and common at localities where they occur. Detailed methods for the collection of climatic data and estimation of the time-calibrated phylogeny are provided elsewhere, along with detailed justification for the species-level taxonomy used (Wiens et al. 2013; see also Quintero and Wiens 2013).

We searched the literature to collect information on reproductive mode, and found information for 105 of the 117 species represented in our phylogeny (Appendix S1). For 12 species, definitive information on reproductive mode was not found. We addressed this problem in two ways.

First, parity mode was assumed based on the species’ closest relatives (e.g., many unknown species were only recently recognized as being distinct). Thus, species were assigned to viviparity if they were nested within viviparous clades and to oviparity if they were nested within oviparous clades. In eight out of twelve cases, these unknown species were nested within viviparous clades, and evidence suggests that reversals from viviparity to oviparity are rare in squamates (Shine 1985; Lee and Shine 1998; Shine and Lee 1999; Blackburn 1999b; but see Lynch and Wagner 2009 for an exception in snakes), making viviparity an even safer assumption for these cases.

Second, we repeated all phylogenetic logistic regression, threshold model, and phylogenetic generalized least squares (PGLS) analyses (see below) after removing all 12 species with uncertain reproductive modes from the tree. The results from this dataset were consistent with the larger dataset, and here we present results only from the dataset that used all 117 species and assumed parity mode based on closet relatives (results for the reduced dataset are provided in supplemental Tables S3–S5).

ANCESTRAL STATE RECONSTRUCTIONS: REPRODUCTIVE MODE

We used the binary state speciation and extinction (BiSSE) model (Maddison et al. 2007) from the *diversitree* package (FitzJohn

Table 1. Comparison of BiSSE models using Δ AICc. The six estimated parameters in the full model include transition rates between oviparity (0) and viviparity (1), including both gains of viviparity (0–1) and reversals (1–0), and estimated speciation and extinction rates for each parity mode. Other models hold various parameters constant. The best-fitting model (bold-faced) has different rates of speciation in oviparous and viviparous lineages, equal extinction rates, and no reversals from viviparity to oviparity.

Parameters constrained	Log-likelihood	Parameters	AICc	Δ AICc
Extinction rates equal, no reversals	–448.3487	4	905.0545	0.0000
Extinction and transition rates equal	–449.3546	4	907.0662	2.0117
Extinction rates equal	–448.3487	5	907.2379	2.1834
No reversals	–448.3487	5	907.2379	2.1834
Transition rates equal	–449.3564	5	909.2533	4.1988
None (full model)	–448.3487	6	909.4610	4.4065
Speciation and extinction rates equal, no reversals	–452.7899	3	911.7923	6.7377
Speciation, extinction, and transition rates equal	–453.8471	3	913.9065	8.8519
Speciation rates equal, no reversals	–452.7748	4	913.9067	8.8522
Speciation and extinction rates equal	–452.7888	4	913.9346	8.8801
Speciation and transition rates equal	–453.7789	4	915.9149	10.8604
Speciation rates equal	–452.7737	5	916.0880	11.0335

2012) in R (R Core Team 2012) to reconstruct ancestral states using maximum-likelihood optimization while accounting for the potential impact of parity mode on speciation and extinction (and the potential impacts of speciation and extinction on ancestral reconstructions). To infer possible impacts of parity mode on diversification, we tested the fit of 12 models of discrete trait evolution (including state-dependent speciation and extinction rates) ranging from 3 to 6 parameters and compared models using the differences in the sample-size corrected Akaike information criterion (Δ AICc; Sugiura 1978; Burnham and Anderson 2002). These models are listed explicitly in Table 1. We supplied the BiSSE models with the estimated proportion of oviparous (~87%) and viviparous (~84%) species included in the tree and assumed random sampling, rather than placing unsampled taxa into unresolved clades (Table S6). We reconstructed ancestral states using the BiSSE model with the minimum (best) Δ AICc score in *diversitree* using subplex optimization. Apart from the best-fitting model, no other tested models had a Δ AICc of < 2 , which would indicate “substantial support” for the other model, following Burnham and Anderson (2002). To further determine whether speciation and extinction parameters were significantly different for oviparous and viviparous lineages, we used *diversitree* to run a 10,000 generation Bayesian Markov chain Monte Carlo (MCMC) analysis with all six parameters of the BiSSE model unconstrained, and examined the 95% credible intervals of the posterior distributions for the speciation and extinction parameters. Using the maximum-likelihood estimates of each parameter as starting values and tuning parameters for each parameter derived from a preliminary MCMC of 1000 generations, several replicate analyses appeared to converge immediately, and we used the entire posterior distribution from one MCMC of 10,000 generations for calculating the credible intervals.

ENVIRONMENTAL VARIABLES

We focused on four environmental variables that are potentially relevant to the cold-climate hypothesis: midpoint of the elevational range, midpoint of the latitudinal range, and the mean across all localities for a given species for the climatic variables Bio1 (annual mean temperature; a standard climatic variable in ecological studies) and Bio10 (mean temperature of the warmest quarter of the year). We used the mean temperature of the warmest quarter because phrynosomatid lizards typically lay their eggs during the warmest months of the year (e.g., Smith 1995; Jones and Lovich 2009), making Bio10 the seemingly most relevant variable for testing the cold-climate hypothesis. For all variables, the mean and midpoint were highly correlated (> 0.95) using Pearson product–moment correlations, implying that our decisions to use mean or midpoint should not influence the results.

PHYLOGENETIC LOGISTIC REGRESSION

Logistic regression allows predictions for a binary-dependent variable (i.e., reproductive mode) to be made from continuous independent variables (i.e., climate). We use the method of Ives and Garland (2010) for phylogenetic logistic regression, implemented using MATLAB version 2009a. The method uses a two-state Markov process for the evolution of discrete traits, and treats continuous variables as known properties of extant species. We generated the necessary variance–covariance matrix from the time-calibrated tree using the PDAP package version 1.16 (Midford et al. 2010) in MESQUITE version 2.75 (Maddison and Maddison 2011). We used 2000 nonparametric bootstrap replicates and a significance threshold of 0.05 to determine significance and estimate confidence intervals. All independent variables were standardized to have a mean of 0 and a variance of 1 prior to analyses, to optimize the performance of the nonparametric bootstrapping

procedure (A. Ives, pers. comm.). We initially performed analyses including all species of Phrynosomatidae, and then conducted separate analyses for the genera *Phrynosoma* and *Sceloporus*, to identify any deviations from the family-level patterns.

CORRELATIONS USING WRIGHT'S THRESHOLD

MODEL

The threshold model for discrete traits was first introduced by Wright (1934). The model assumes that an unobserved quantitative character, termed the liability, determines the state of discrete characters based on whether the liability exceeds a threshold value. Compared to the Markov process used by Ives and Garland (2010), this is potentially a more biologically realistic way to model the evolution of discrete traits, especially those that are complex and likely polygenic, such as viviparity. An important difference is the way each model treats the probability of a state transition in the binary variable. The Markov process used for discrete trait evolution in the approach of Ives and Garland (2010) is “memoryless,” such that the probability of a state transition is the same at any given time, regardless of prior states. In contrast, using the threshold model, transitions are more likely when the liability is closer to the threshold. For example, a reversal from viviparity to oviparity would be more likely if it occurred shortly after the original transition to viviparity.

Under the threshold model, underlying liabilities of discrete traits and continuous characters are assumed to evolve through covarying Brownian motion, allowing correlation between discrete and continuous characters. The threshold model has been recently implemented using MCMC sampling under maximum likelihood (Felsenstein 2012) and Bayesian (Revell 2012) frameworks. We used the Bayesian method implemented in the R package *phytools*. We examined the same independent variables as in the phylogenetic logistic regression, and again performed analyses at both the family (Phrynosomatidae) and genus (*Sceloporus* and *Phrynosoma*) levels. We ran several MCMC simulations of 2 million generations each to ensure consistent estimation of parameters and likelihoods, followed by one simulation of 10 million generations for each variable. We report the mean of r (the correlation between the liability of reproductive mode and the continuous character[s]) from the posterior probability distribution and used the quantile function in R to obtain 95% confidence intervals.

ANCESTRAL RECONSTRUCTIONS OF ENVIRONMENTAL VARIABLES

We used ancestral reconstructions to address the environmental conditions under which viviparity evolved, a key question that is not directly addressed by phylogenetic logistic regression or the threshold model. We reconstructed ancestral values for the three environmental variables that had the strongest statistical relationships with viviparity (midpoints of the elevational and latitudinal

ranges, mean temperature of the warmest quarter; see Results) using maximum likelihood in the R package APE (Paradis et al. 2004). The distribution-based traits we reconstruct here are not technically heritable in the genetic sense, but should be closely related to intrinsic, heritable properties such as physiological tolerances and behavioral habitat choice. Furthermore, each of the reconstructed environmental variables in this study has phylogenetic signal (estimated with Pagel's λ ; Pagel 1999) significantly different from 0 according to likelihood-ratio tests completed using the *phylog* function of the R package *phytools* (Table S2). Thus, the presence of phylogenetic signal suggests that it should be appropriate to do phylogenetic reconstructions of ancestral values for these variables. We compared models of trait evolution using the R package GEIGER version 1.3-1 (Harmon et al. 2008) and selected models based on Δ AICc scores as described earlier for reconstructions of reproductive mode. In the case of multiple models having Δ AICc of < 2 (i.e., statistically indistinguishable support), we performed reconstructions using each model and report results from each. We considered a model using the maximum-likelihood estimate of Pagel's λ (Pagel 1999) for branch-length transformation, a Brownian motion model (equivalent to $\lambda = 1$), a “white noise” model (equivalent to $\lambda = 0$, or a star phylogeny) and an Ornstein–Uhlenbeck (OU) model (Hansen and Martins 1996) with a single optimum.

The key prediction we tested using ancestral reconstructions is whether the conditions in viviparous clades at the time of their origin are significantly different (e.g., colder) than conditions in lineages that did not evolve viviparity. This prediction is distinct from those based on extant conditions experienced by viviparous and oviparous species (i.e., as in our threshold model and phylogenetic logistic regression analyses). This prediction also focuses more directly on the conditions under which viviparity evolves, and to our knowledge, has not been tested before.

To test this prediction, we took a nonparametric approach, sampling from the distribution of ancestral values for all nodes reconstructed as oviparous. Using this distribution enabled us to account for the distribution of conditions experienced by oviparous phrynosomatid species. For example, if a large proportion of oviparous phrynosomatid lineages inhabit cold environments, then the origin of viviparity in such conditions does not offer strong support for the cold-climate hypothesis. For each variable that produced significant relationships with reproductive mode, we first calculated the mean of the ancestral values from the nodes at which viviparity originated. For *Sceloporus aeneus* and *S. bicanthalis*, the immediately ancestral node is reconstructed as oviparous; we addressed this by using: (1) the extant values for these species and (2) the mean of the immediate ancestral node and the extant value. Next, we randomly sampled six values (equal to the number of origins of viviparity in Phrynosomatidae; Results) from the distribution of reconstructed values for oviparous nodes.

We repeated this sampling 100,000 times using R, and for each sample, took the mean of the six values. We then calculated 95% one-sided confidence intervals from the resulting distribution of sample means, and compared the mean value from the nodes associated with origins of viviparity to these confidence intervals. If the mean from nodes where viviparity originated fell outside of the one-sided confidence intervals in the predicted direction, we concluded that there was a significant difference between reconstructed environmental conditions at the origins of viviparity and those for oviparous lineages.

We also used the ancestral reconstructions to address the origins of viviparous species living in lowland tropical environments. We first identified viviparous species that live primarily in low-elevation habitats (here arbitrarily defined as having an elevational midpoint of < 500 m) and at latitudes below 23.4°. For each such species, we plotted the estimated values for elevational midpoint and mean summer temperature for each ancestral node in a straight-line path from the root of Phrynosomatidae to the extant values for that species. In addition, we plotted the reconstruction of reproductive mode at each node, allowing for a straightforward visualization of the estimated environmental conditions at the evolution of viviparity and any subsequent adaptation to differing climates (Fig. 2).

VIVIPARITY AND RANGE SIZE

We first quantified for each species their elevational and latitudinal range sizes (obtained by subtracting the maximum and minimum recorded values for each species) and temperature niche breadth (defined here as the difference between the minimum value of Bio6, the minimum temperature of the coldest month, and the maximum value of Bio5, the maximum temperature of the warmest month, across all the sampled localities within the range of each species, following Quintero and Wiens 2013). We then compared these values for oviparous and viviparous species using PGLS regression (Grafen 1989) implemented using the R package NLME (Pinheiro et al. 2012). For these analyses, parity mode was considered to be a categorical independent variable with two possible states, and range sizes and niche breadth as the dependent variables. For each variable, we compared GLS models using ΔAICc as described earlier. We evaluated GLS models with phylogenetic correlation structures (one using the maximum likelihood estimate of λ , one using Brownian motion [$\lambda = 1$], and one using the OU correlation structure of Hansen and Martins 1996), as well as a model that lacked phylogenetic correlation structure ($\lambda = 0$). In the case of a GLS model that lacks phylogenetic correlation structure, the model gives the same results as a *t*-test comparing the means for viviparous and oviparous species. As with phylogenetic logistic regression, we performed analyses at the family (Phrynosomatidae) and genus (*Sceloporus* and *Phrynosoma*) levels.

Results

The preferred model for the evolution of reproductive mode contains four parameters, including different speciation rates for oviparous and viviparous clades (Table 1). In this preferred model, extinction rates for oviparous and viviparous clades were constrained as equal, and state transitions were constrained to happen unidirectionally from oviparity to viviparity (i.e., no reversals). Our reconstructions using this model support six origins of viviparity in Phrynosomatidae: four in *Sceloporus* and two in *Phrynosoma* (Fig. 1), in agreement with previous studies (Méndez-de la Cruz et al. 1998; Hodges 2004). Bayesian MCMC analysis of the unconstrained BiSSE model recovered 95% credible intervals for the extinction parameter that overlapped for oviparous and viviparous lineages, but the credible intervals for the speciation parameter did not, with viviparous lineages having a significantly higher estimated speciation rate (maximum likelihood estimate = 0.12 lineages/Million years) than oviparous lineages (maximum likelihood estimate = 0.06).

Using phylogenetic logistic regression, reproductive mode is best predicted by the mean temperature of the warmest quarter (estimated slope coefficient $B_1 = -1.06$, $P = 0$) and the midpoint of the elevational range ($B_1 = 0.91$, $P = 0$) across Phrynosomatidae (Table 2). The signs of these relationships are as predicted by the cold-climate hypothesis: cooler climates and higher elevations predict viviparity. Reproductive mode is also predicted by midpoint of the latitudinal range ($B_1 = -0.72$, $P = 0.02$), but this relationship is opposite to what might be expected under the cold-climate hypothesis: viviparity is associated lower latitudes, rather than the higher latitudes that are typically correlated with cooler temperatures (Tinkle and Gibbons 1977; Hodges 2004). This pattern might be partially explained by the positive correlation between the midpoint of the latitudinal range and the mean temperature of the warmest quarter (Bio10) across all species of Phrynosomatidae (nonphylogenetic Pearson product-moment correlation $r = 0.23$, $P = 0.01$). This indicates that higher-latitude phrynosomatid species inhabit areas with higher maximum summer temperatures, such as deserts (for *Sceloporus*, see also Oufiero et al. 2011). Interestingly, in no cases did Bio1, the mean annual temperature, significantly predict reproductive mode.

Results from Wright's threshold model are consistent with the results from phylogenetic logistic regression, in terms of the rank order of the importance of the independent variables and statistical significance, at least for the family-level analyses (Tables 2 and S3). For the genus-level analyses, the threshold model provided wider confidence intervals, all of which overlapped with 0 (indicating nonsignificance), perhaps owing to the smaller sample sizes of these analyses.

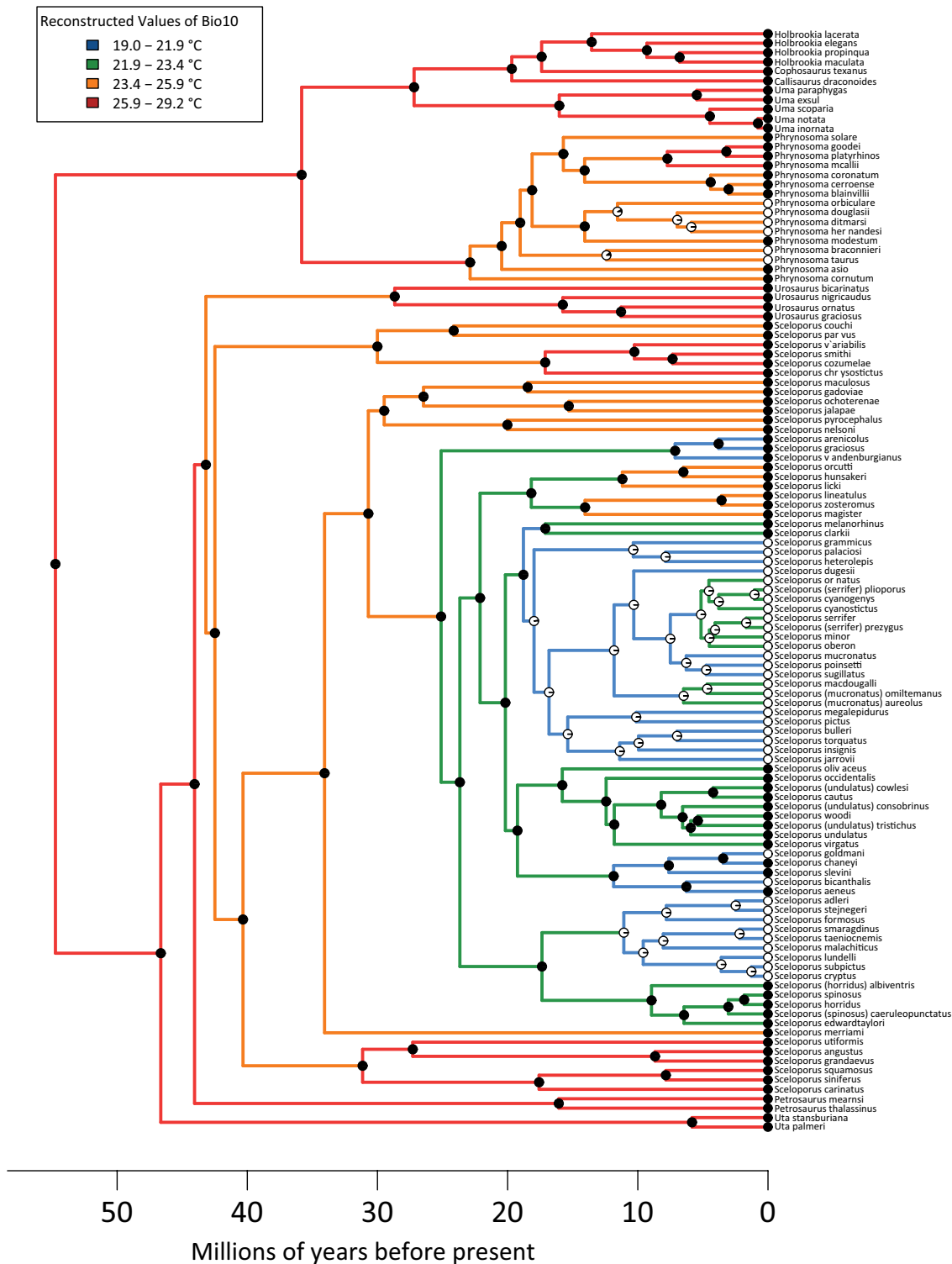


Figure 1. Time-calibrated phylogeny of Phrynosomatidae based on Bayesian analysis of nuclear and mitochondrial genes (from BEAST). Tip labels indicate reproductive mode (black = oviparous, white = viviparous). Node labels show the maximum-likelihood estimate of reproductive mode under the best-fitting model of binary trait evolution (Table 1). Branch colors indicate the reconstructed value for mean temperature of the warmest quarter (Bio10) for the ancestral node of each branch, based on maximum likelihood reconstruction as a continuous character. For illustrative purposes in this figure, these continuous reconstructions are then binned into four categories according to the quantiles of the reconstructed values (blue = 0–25%, green = 26–50%, orange = 51–75%, red = 76–100%; see figure legend for exact values). Some species are treated as separate lineages here that were traditionally recognized as subspecies. In these cases, the traditional species designation is indicated with parentheses.

Table 2. Results of statistical tests of the relationships between environmental variables and reproductive mode. Estimates of B_1 and P are from phylogenetic logistic regression and estimates of r are from Wright's threshold model. Bolded results are significantly different from 0 (i.e., 95% confidence intervals do not overlap with 0). Bio10.mean is the mean across all localities for a given species for Bio10, the mean temperature of the warmest quarter; Elev.mid is the midpoint of the elevational range for each species (calculated using the minimum and maximum values in our dataset); Lat.mid represents the midpoint of the latitudinal range; Bio1.mean is the mean across all localities for a given species for Bio1, the mean annual temperature.

	Independent variable	B_1	P	B_1 95% CI	Mean r	r 95% CI
Phrynosomatidae	Bio10.mean	-1.06	0.00	(-1.89, -0.40)	-0.44	(-0.72, -0.09)
	Elev.mid	0.91	0.00	(0.38, 1.72)	0.38	(0.08, 0.63)
	Lat.mid	-0.72	0.02	(-1.66, -0.12)	-0.30	(-0.56, -0.01)
	Bio1.mean	-0.07	0.50	(-0.73, -0.23)	-0.18	(-0.51, -0.21)
<i>Sceloporus</i>	Bio10.mean	-0.80	0.01	(-1.82, -0.10)	-0.38	(-0.71, 0.07)
	Elev.mid	0.93	0.01	(0.23, 2.01)	0.31	(-0.03, 0.60)
	Lat.mid	-0.80	0.03	(-2.03, -0.06)	-0.38	(-0.69, 0.01)
	Bio1.mean	-0.09	0.75	(-0.84, 0.36)	-0.14	(-0.53, 0.32)
<i>Phrynosoma</i>	Bio10.mean	-1.47	0.01	(-3.65, -0.31)	-0.45	(-0.92, 0.42)
	Elev.mid	1.39	0.02	(0.20, 3.02)	0.43	(-0.13, 0.84)
	Lat.mid	-0.29	0.67	(-1.65, 1.05)	-0.10	(0.67, 0.54)
	Bio1.mean	-0.58	0.26	(-1.98, 0.43)	-0.23	(-0.82, 0.53)

Table 3. Results of model comparisons for the evolution of continuous characters used in ancestral reconstructions. Values shown are for $\Delta AICc$, with 0 being the best model and models having $\Delta AICc < 2$ also considered to have strong support following Burnham and Anderson (2002). Bolded values indicate models with strong support.

Model	Elevation (midpoint)	Latitude (midpoint)	Bio10 (mean)
Lambda	0.00	1.75	0.00
Brownian motion	61.57	5.29	46.56
White noise	363.90	62.31	27.71
OU	136.20	0.00	8.92

Table 4. Results from phylogenetic generalized least squares (PGLS) comparisons of elevational and latitudinal ranges of viviparous and oviparous species. Under the best-fitting model, the correlation structure from the model with the lowest $\Delta AICc$ is given (with "no correlation structure" referring to a standard GLS model without any phylogenetic correlation structure). The intercept is interpretable as the estimated mean of oviparous species, with the slope coefficient representing the difference in the means of oviparous and viviparous species. Bolded P -values indicate statistically significant differences between oviparous and viviparous species using a threshold of 0.05.

Taxon	Variable	Best-fitting model	Intercept	Slope coefficient	P
Phrynosomatidae	Latitudinal range	Lambda	6.36	-2.63	0.03
	Elevational range	Lambda	1257.08	-143.78	0.39
	Temperature niche breadth	Lambda	37.52	-5.91	0.03
<i>Sceloporus</i>	Latitudinal range	Lambda	4.60	-2.80	0.01
	Elevational Range	No correlation structure	1269.45	-31.11	0.90
	Temperature niche breadth	Lambda	30.73	-6.74	0.02
<i>Phrynosoma</i>	Latitudinal range	No correlation structure	7.12	-0.89	0.74
	Elevational range	No correlation structure	1499.50	-302.83	0.54
	Temperature niche breadth	No correlation structure	40.99	-1.87	0.75

For ancestral reconstructions, model-testing for the environmental variables favors the model using the maximum-likelihood estimate of λ for the midpoint of the elevational range and for the mean of Bio10 (Table 3). For the midpoint of the latitudinal range, the OU and maximum-likelihood estimate of λ models were indistinguishable (Table 3). Ancestral reconstructions support the

hypothesis that viviparity generally evolves in cold climates and high elevations (Fig. 1; Table 5); we find no origins of viviparity in ancestors reconstructed as occurring in relatively warm or low-elevation environments. Although the two origins in *Phrynosoma* are supported as occurring in the upper 50% of all reconstructed Bio10 values across Phrynosomatidae, both origins occur in

Table 5. Results from nonparametric test comparing the mean ancestral values for the six origins of viviparity with the distribution of means from 100,000 random samples of six nodes from all oviparous nodes. "Test statistic" refers to the relevant tail of the one-sided 95% confidence interval from the distribution of sampled means from the oviparous reconstructions. The values used for *Sceloporus bicanthalis* and *Sceloporus aeneus* are the midpoint between the extant values and the immediately ancestral (oviparous) nodes. Results from two models of continuous character evolution for latitude are presented for latitude because both had ΔAICc values of < 2 .

Origin	Mean of Bio10	Midpoint latitude (λ)	Midpoint latitude (OU)	Midpoint elevation
<i>S. poinsetti</i> group	21.58	23.14	23.33	1556.36
<i>P. orbiculare</i> group	23.83	30.66	30.21	1177.13
<i>P. braconneri</i> group	24.50	23.06	23.54	1122.78
<i>S. formosus</i> group	20.54	18.03	18.51	1537.56
<i>S. bicanthalis</i>	16.91	20.53	20.41	2297.14
<i>S. aeneus</i>	16.75	22.02	21.97	2559.47
Mean (origins)	20.86	22.91	23.00	1708.41
Test statistic	23.15	23.31	23.52	1324.72

relatively low temperatures when compared to the reconstructed values within *Phrynosoma* (third and fifth lowest out of the 14 reconstructed values within the genus, with the first and second coldest reconstructed values also found within viviparous clades). In *Sceloporus*, each of the four origins is reconstructed to have occurred in environments with reconstructed summer temperatures in the lowest 25% of all reconstructed values (Fig. 1). The nonparametric test we used to compare estimated conditions at the six origins of viviparity with the estimated conditions in oviparous lineages across Phrynosomatidae indicates that the conditions at the origins of viviparity were significantly colder, at higher elevations, and at lower latitudes than those reconstructed for oviparous lineages (Table 5).

Another pattern we addressed using ancestral reconstructions is the presence of viviparous species that are restricted to tropical, low-elevation environments. Three species in particular fit this pattern: *Sceloporus macdougalli* (maximum recorded elevation 53 m, latitudinal midpoint $\sim 16^\circ$), *Sceloporus (serrifer) serrifer* (maximum recorded elevation 32 m, latitudinal midpoint $\sim 21^\circ$), and *Sceloporus lundelli* (maximum recorded elevation 92 m, latitudinal midpoint $\sim 20^\circ$). Ancestral reconstructions indicate that these species invaded their warm, low-elevation environments after their ancestors evolved viviparity in high-elevation, cool environments (Fig. 2; because the reproductive mode of *S. lundelli* is unknown and assumed based on closest relatives, we present results only for the former two species). In contrast, the two most recent origins of viviparity in Phrynosomatidae (i.e., in *S. bicanthalis* and *S. aeneus* of the *scalaris* group) are associated with the first and third coldest extant mean summer temperatures and the first and second highest elevational midpoints, respectively, in the entire dataset (Appendix S1).

Our comparisons of the elevational range sizes, latitudinal range sizes, and temperature niche breadths of oviparous and viviparous species resulted in several instances where multiple GLS models had ΔAICc scores of < 2 (Table S3). However,

the GLS results using these models never disagree regarding the statistical significance of the difference between oviparous and viviparous species (using $P < 0.05$; Table S4), and for simplicity, we report the estimated means and P -values from the model with the minimum ΔAICc in these cases. The elevational range sizes of oviparous and viviparous species are not significantly different at any taxonomic level examined (Table 4). Across Phrynosomatidae and within *Sceloporus*, we find that the latitudinal range sizes and temperature niche breadths of viviparous species are significantly smaller than those of oviparous species. Within *Phrynosoma*, we find no significant differences in any of the examined variables.

Discussion

The origin of viviparity is a major transition in life-history evolution in vertebrates, but the selective factors that lead to this transition are not fully understood. In this study, we provide the first direct test of the most basic prediction of the cold-climate hypothesis (that viviparity evolves in colder climates) by combining explicit data on climate and phylogeny. Our results support the cold-climate hypothesis, but also suggest an intriguing twist: cold climates favor the evolution of viviparity, but mostly in the tropics.

We support the cold-climate hypothesis in that we find that low summer temperatures (Bio10) are strongly related to viviparity, according to both phylogenetic logistic regression and correlations from Wright's threshold model (Table 2). We find no significant relationships between viviparity and mean annual temperature, but this result is also consistent with the cold-climate hypothesis. The selective pressures that favor viviparity should result from the temperature during the egg-laying and incubation season, which occurs during the summer for oviparous phrynosomatid lizards (e.g., Smith 1995; Jones and Lovich 2009). High and low temperatures during other parts of the year may be of limited relevance. In fact, it might be more accurate to call the

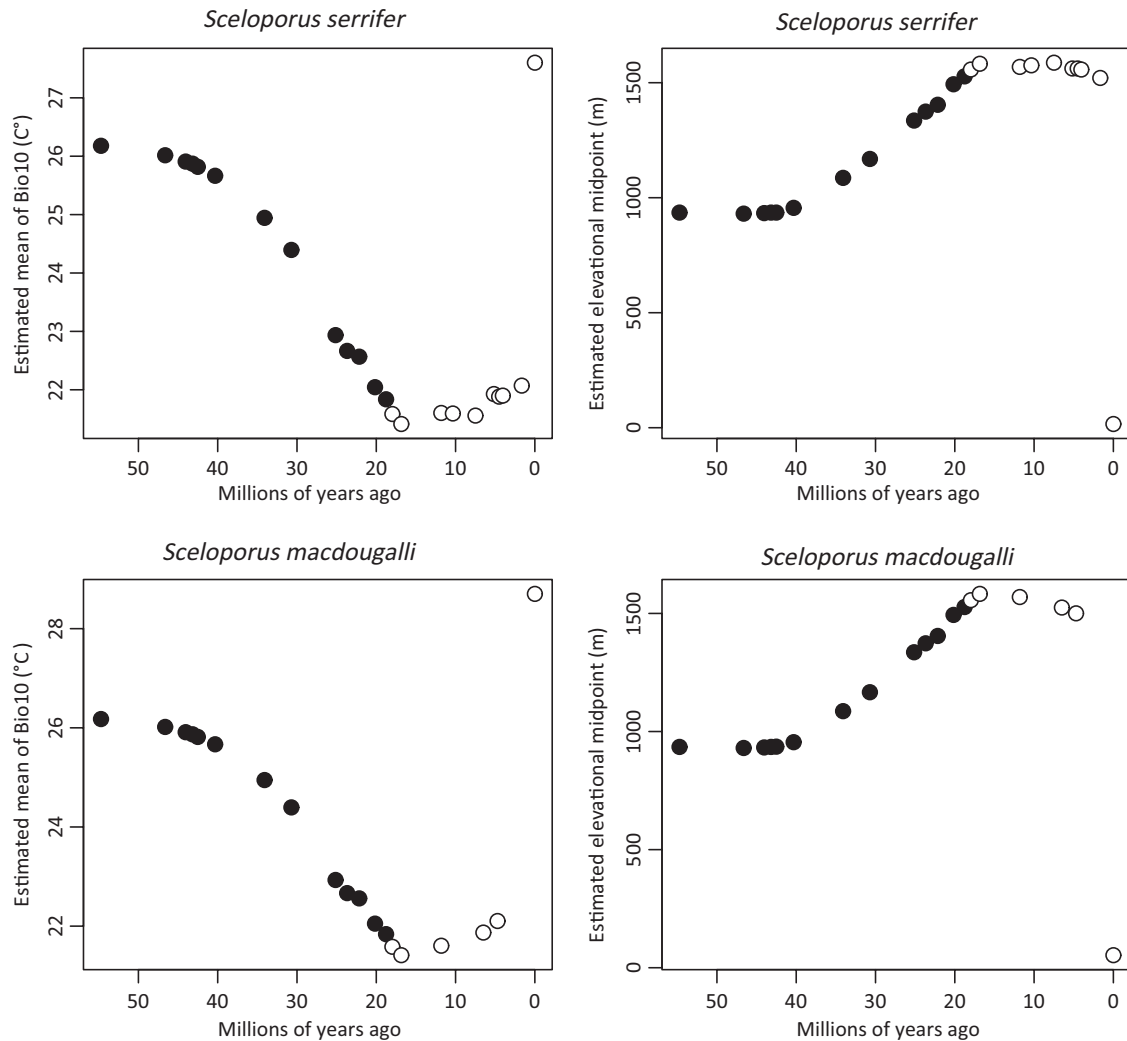


Figure 2. Diagram showing the reconstructed values of temperature and elevation for two viviparous species that inhabit exclusively tropical, low-elevation sites (< 100 m elevational midpoint), showing the values at each node in a straight-line path from the root of the Phrynosomatidae to the extant values. Temperature is the mean temperature of the warmest quarter (Bio10) and elevation is the midpoint of the elevational range. The figure shows that the warm, low-elevation climates inhabited by these species represent secondary invasions after the evolution of viviparity in an ancestor that inhabited relatively cool, high-elevation environments. Colors of each circle indicate the reconstruction of reproductive mode (black = oviparity, white = viviparity) at the corresponding node.

“cold climate hypothesis” the “cool summer hypothesis,” especially given the demonstrated prevalence of viviparity in tropical regions.

Our results are generally concordant with those of Hodges (2004), who examined the evolution of viviparity in *Phrynosoma* only. In agreement with Hodges (2004), we find that elevation significantly predicts reproductive mode within *Phrynosoma*. However, Hodges (2004) further suggested that high-elevation environments have characteristics beyond cold temperatures that also favor viviparity. Our results show that summer temperatures generally have a stronger relationship with parity mode than elevation (Table 1), but summer temperatures and elevation are highly correlated (Table S7), making their effects difficult to distinguish.

Hodges (2004) highlighted three other factors at high elevations that might favor viviparity. First, lower oxygen concentrations at higher elevations can slow embryonic development (Andrews 2002), and placental structures can allow viviparous mothers to better supply their young with oxygen. Second, diel temperature fluctuations at high elevations may present another difficulty that viviparous females can mitigate through behavioral thermoregulation. Unfortunately, these hypotheses are difficult to address with the data available for phrynosomatids. Third, Hodges (2004) stated that higher elevations are drier, and more arid conditions are problematic for developing eggs. However, we find that elevation and annual precipitation (Bio12) are actually positively correlated for the environments inhabited by Phrynosomatidae (Table S7).

Our analyses also help to explain three patterns seen in Phrynosomatidae that seem to contradict the cold-climate hypothesis: the presence of viviparous species in lowland tropical habitats, the scarcity of viviparous species at high latitudes, and the tendency of viviparity to evolve in tropical regions. First, our ancestral reconstructions indicate that the viviparous species of *Sceloporus* inhabiting primarily tropical, low-elevation environments (e.g., *S. [serrifer] serrifer* and *S. macdougalli*) secondarily invaded these areas and are descended from high-elevation, cool-climate dwelling viviparous ancestors (Fig. 2). These results highlight the importance of considering phylogenetic history when testing hypotheses of adaptation: in phrynosomatids, viviparity appears to have originated exclusively in cold climates, even if the descendant species have subsequently invaded other environments. Similar information is needed for other viviparous squamates that occur in warm environments to determine if they represent in situ origins of viviparity or secondary invasions of such environments. For example, the exclusively viviparous and lowland Neotropical skink genus *Mabuya* (sensu lato) potentially challenges the generality of the cold-climate hypothesis (Shine 1985, 2004).

Furthermore, our results suggest that viviparous species are able to rapidly adapt to warm climates without reverting to oviparity. This pattern might occur because these lineages have lost the genetic and developmental (and possibly behavioral) features needed for oviparity to re-evolve. Alternately, viviparity might still provide advantages even in environments that are not cold. The maternal manipulation hypothesis (Shine 1995) posits that viviparous females can enhance offspring fitness by maintaining conditions for the embryo that are advantageous relative to those in nesting sites. For example, in warm environments, viviparous females might provide more stable temperatures for developing embryos (Webb et al. 2006; Ji et al. 2007). However, it is unclear whether such an advantage could facilitate evolution of viviparity in lowland tropical climates, invasion of these environments by viviparous species, or allow maintenance of viviparity under these conditions.

Our results may also help to explain the absence of viviparous *Sceloporus* at the highest latitudes and elevations in temperate regions, despite our overall support for the cold-climate hypothesis. Although there are phrynosomatid species with ranges that extend to relatively high latitudes (to 47.7°, *Phrynosoma douglassi*) and high elevations (to 4067 m, *Sceloporus graciosus*) in temperate North America (Appendix S1; Smith 1995; Jones and Lovich 2009), these belong to one of two categories. First, there are viviparous “invaders” to high latitudes (i.e., *Phrynosoma douglassi* and *P. hernandesi*), which are reconstructed as having evolved from an ancestor that evolved viviparity at lower latitudes (~30°; Table 5). Second, there are oviparous species of *Sceloporus* and oviparous species of other genera (e.g.,

Holbrookia, *Urosaurus*, *Uta*). We find that viviparous *Sceloporus* do not occur above a maximum of ~33° latitude in North America, even though 28 oviparous phrynosomatid species do.

We suggest that the scarcity of high-latitude viviparous species may be explained by the same factor that underlies the tendency for viviparity to evolve at tropical latitudes. We find that five of the six origins of viviparity in the family seemingly occurred below 24° latitude (using the midpoint of the latitudinal range of species, Table 5), and the relationship between low latitudes and viviparity is also supported by our phylogenetic comparative analyses (Table 2) and nonparametric test of latitudinal origins (Table 5). Although other studies have noted higher numbers of viviparous species in the tropics (e.g., Tinkle and Gibbons 1977), they have not suggested that viviparity is more likely to originate in the tropics, and they actually found a greater proportion of viviparous species at high latitudes. In contrast, we find that both the proportion and absolute number of viviparous species in Phrynosomatidae is highest at tropical latitudes (Fig. 4).

We propose that latitudinal variation in temperature seasonality might explain the paradoxical tendency for viviparity to evolve in the tropics and be largely absent at high latitudes in Phrynosomatidae. Specifically, seasonal temperature stability in tropical regions might facilitate the evolution of cool-climate, high-elevation specialists. These cool-climate specialists may then be more likely to evolve viviparity, as the selective pressure (cool summer temperatures) is present throughout their geographic range, whereas climatic generalists may have ongoing gene flow with warm-climate populations where oviparity is favored instead. This hypothesis recalls that of Janzen (1967), who hypothesized that seasonal stability in temperature at tropical localities will result in selection for narrower physiological tolerances. In turn, these narrow physiological tolerances will reduce the dispersal ability of individuals across elevations and may facilitate the evolution of species that are high-elevation versus low-elevation specialists (Ghalambor et al. 2006). At more northern latitudes, each locality will experience a relatively broad range of temperatures regardless of elevation, potentially reducing specialization to both high and low elevations.

In support of this hypothesis, we find that many high-latitude phrynosomatids occur at both low and high elevations (e.g., *S. consobrinus*, *S. graciosus*, *S. occidentalis*, *S. tristichus*, *Urosaurus ornatus*, *Uta stansburiana*, each with elevational ranges of > 2200 m). Of the 28 oviparous species found above 33° latitude, 17 have elevational ranges > 2000 m, and only six have elevational ranges < 1000 m. Importantly, those species with ranges < 1000 m are primarily confined to lower elevations and latitudes in temperate North America (the maximum recorded elevation among these species is 1249 m for *Sceloporus arenicolus*, and the average midpoint of their elevational ranges is ~340 m). We hypothesize that

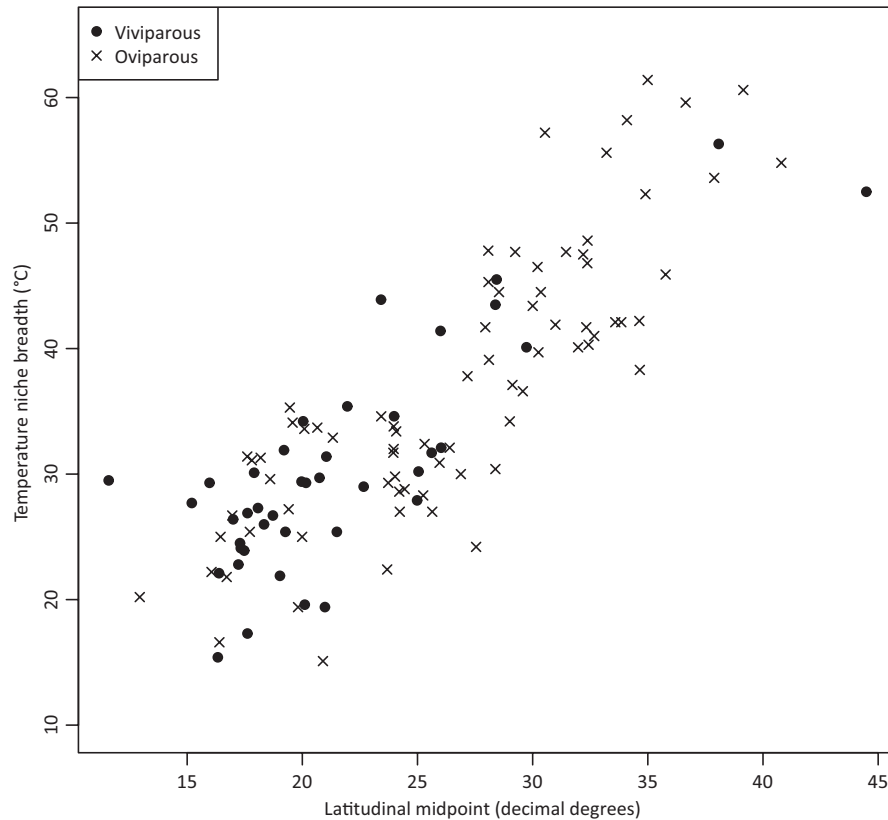


Figure 3. Scatter plot of temperature niche breadths (y-axis) and midpoint of the latitudinal range (x-axis) for oviparous and viviparous phrynosomatid species (dark circles are viviparous species, crosses are oviparous species). The viviparous species at high latitudes are *Phrynosoma hernandesi* and *Phrynosoma douglassi*.

viviparity fails to evolve in high-latitude species because of gene flow between low-elevation populations (where viviparity may not be favored because high summer temperatures allow for rapid egg development) and high-elevation populations (where viviparity might otherwise be favored). However, intrinsic physiological constraints of certain species or clades might also be a factor. For instance, it has been shown that species of *Sceloporus* vary in their ability to continue embryonic development during egg retention, with in utero oxygen availability implicated as an important constraint (Andrews and Rose 1994; Andrews 2002; Parker and Andrews 2006). Nevertheless, the high-latitude, oviparous, climatic generalists do belong to diverse clades across phrynosomatids, arguing against obvious clade-specific constraints.

In further support of the idea that there are fewer cool-climate specialists at temperate latitudes, there is a strong positive relationship between thermal niche breadth and latitude (Fig. 3; $R^2 = 0.712$, $P = 0.000$ from PGLS; Quintero and Wiens 2013), where thermal niche breadth is the difference between the minimum value of Bio6 (the minimum temperature of the coldest month) and the maximum value of Bio5 (the maximum temperature of the warmest month) across sampled localities. Quintero and Wiens (2013) also found that wide thermal niche widths in

temperate phrynosomatids are caused primarily by within-locality seasonal variation, rather than between-locality differences in temperature values, an important assumption in Janzen's (1967) hypothesis.

This hypothesis of cool-climate, high-elevation specialization favoring the evolution of viviparity in the tropics will require further testing, perhaps including analyses of gene flow and local adaptation to different elevations in tropical versus temperate regions (with higher gene flow and less local adaptation over similar elevational ranges predicted for temperate species). This hypothesis also predicts broader physiological tolerances to temperature in temperate versus tropical species (e.g., Janzen 1967; Ghalambor et al. 2006), a prediction that has been confirmed using several species of *Sceloporus* (van Berkum 1988). Future studies should also address why viviparous *Phrynosoma* species were able to invade high-latitude regions from the south, whereas viviparous *Sceloporus* have not done so. Another challenge is to compare these patterns in temperate North American phrynosomatids to those in other clades and regions (e.g., Eurasia, austral South America, temperate Australia). For instance, the Eurasian lacertid *Zootoca vivipara* contains multiple viviparous lineages that appear to have originated recently at high latitudes, possibly

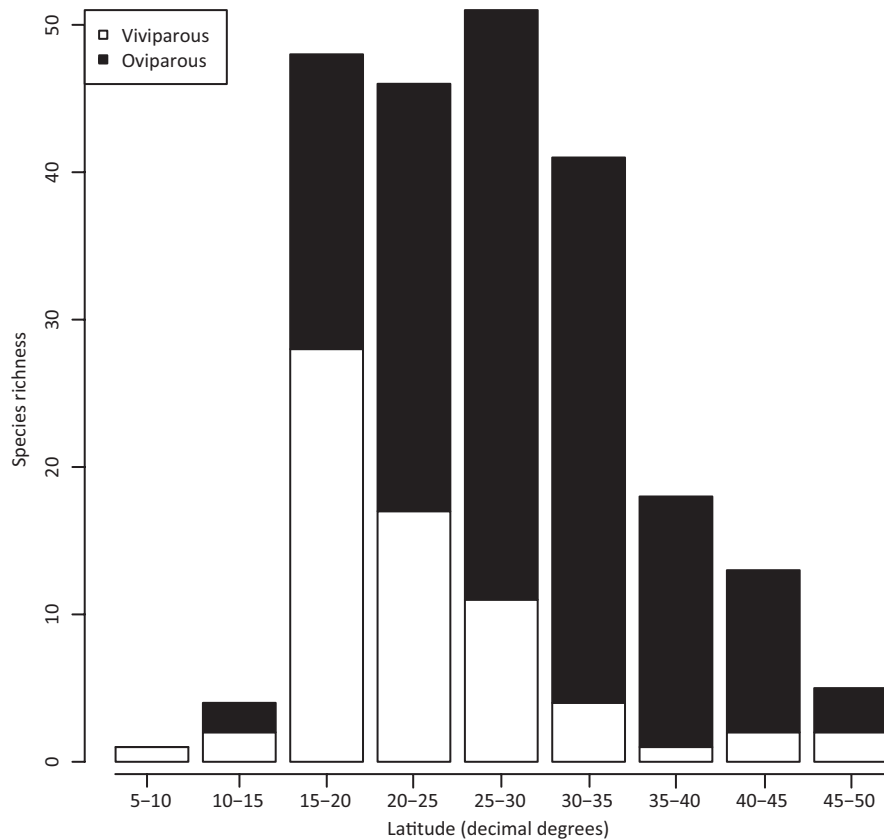


Figure 4. Species richness of oviparous species (black) and viviparous species (white) within latitudinal bins encompassing the entire range of Phrynosomatidae (based on data in Appendix S1). Any species whose latitudinal range overlaps a bin was counted toward the species richness of that bin (even if there were no localities sampled in that particular range of latitudinal values).

related to climate change during the Pleistocene (Surget-Groba et al. 2001, 2006).

In summary, despite our support for the cold-climate hypothesis, we find that viviparity evolves more often at tropical latitudes than in temperate regions, and that viviparity is relatively uncommon in high-latitude phrynosomatids. This pattern seems to be explained by the presence of high-elevation, cool-climate specialists in tropical regions, and the dominance of elevational generalists and low-elevation specialists in temperate regions. These patterns are in turn explained by greater temperature seasonality in the temperate zone, leading to wider climatic niche breadths and a lack of elevational specialists.

VIVIPARITY, RANGE SIZE, AND CLIMATE CHANGE

A previous study suggested that viviparous species of *Sceloporus* have a higher risk of extinction from anthropogenic climate change because they tend to be restricted to high-elevation habitats, where climate is changing most rapidly (Sinervo et al. 2010). Our results from phylogenetic logistic regression and Wright's threshold model clearly support the assumption that viviparous species inhabit higher elevations (Table 2). To further explore the hypothesis that viviparous species may be more at risk from cli-

mate change, we compared the latitudinal range sizes, elevational range sizes, and temperature niche breadths of viviparous and oviparous phrynosomatid species. Across Phrynosomatidae and within *Sceloporus*, we find that viviparous species have significantly smaller latitudinal ranges and temperature niche breadths, as expected. However, we find similar elevational range sizes in oviparous and viviparous species. Climate change may modify local conditions such that populations will go extinct if they cannot disperse and/or adapt (e.g., Holt 1990). A larger range size should allow a species to persist despite some localized extinctions. The smaller latitudinal ranges and temperature niche breadths of viviparous species place them at a disadvantage in this respect, but their relatively large elevational range sizes may help them persist.

VIVIPARITY AND DIVERSIFICATION RATE

Our results also suggest that viviparous phrynosomatid lineages have a significantly higher speciation rate than oviparous lineages. A previous study (Lynch 2009) also found that viviparous clades of vipers (Viperidae) had higher speciation rates than their oviparous counterparts, suggesting the possibility that this might be a relatively broad evolutionary pattern. However, Lynch (2009)

made the important caveat that an increased speciation rate in viviparous lineages could be due to traits that are associated with viviparity, rather than viviparity itself.

One such trait for viviparous phrynosomatid species might be their presence in tropical montane regions. As climate changes through time, tropical montane species may be more likely to become isolated on mountaintops because of the increased climatic zonation in tropical regions (Janzen 1967), potentially leading to increased rates of allopatric speciation (e.g., Ghalambor et al. 2006). An ancillary prediction is that viviparous species will show signatures of speciation via climatic niche conservatism (Wiens 2004; Hua and Wiens 2013), with similar climatic niches in allopatric sister species that differ markedly from the lowlands that separate them (e.g., Kozak and Wiens 2006). This isolation at high elevations through time may ultimately help explain both the evolution of viviparity itself and the faster rate of diversification estimated for viviparous lineages.

However, viviparity itself might increase diversification rates. One proposed mechanism is the viviparity-driven conflict hypothesis (Zeh and Zeh 2000, 2008). Briefly, this hypothesis suggests that the intensified physiological interactions between a viviparous mother and her offspring create greater selective pressure for genomic compatibility that is not present in oviparous species. This selection then leads to a faster build-up of postzygotic isolation in viviparous species. One potential prediction of this hypothesis is that pairs of viviparous species that show sympatry without introgression will be younger than similar pairs of oviparous species because of more rapid evolution of postzygotic isolation. This hypothesis has been proposed as an explanation for the apparent faster speciation rate of other viviparous lineages (e.g., mammals; Zeh and Zeh 2000). However, the effects of viviparity-driven conflict may be absent in squamate reptiles, where interactions between mother and embryo are typically more limited than in mammals (Yaron 1985; Stewart and Blackburn 1988; Blackburn 1998; Stewart and Thompson 2000). Nevertheless, both the climate and conflict-based hypotheses should be explored in future studies.

Conclusions

Our study is the first to integrate phylogeny and climatic data to address the origin of viviparity in squamate reptiles, and we strongly support the cold-climate hypothesis. Using phylogenetic comparative methods, we find that the parity mode of extant phrynosomatid species is most strongly related to the mean temperature of the warmest (egg-laying) season. Using ancestral reconstructions, we found that viviparity evolves in cooler climates, and that viviparous species inhabiting exclusively tropical, low-elevation areas represent secondary invasions of these environments. In addition, we found that reproductive mode is also significantly

related to latitude within Phrynosomatidae, with viviparity more likely to evolve in lower (tropical) latitudes. We hypothesize that this surprising latitudinal pattern could be due to greater seasonal temperature stability in the tropics facilitating the evolution of cool-climate, high-elevation specialists. This hypothesis also explains the paradoxical scarcity of viviparous phrynosomatid species at high latitudes. Finally, our results suggest that viviparous lineages have higher speciation rates than oviparous lineages.

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Supporting Information

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Appendix S1. List of taxa used in this study with information on reproductive mode (0 = oviparous, 1 = viviparous) and the continuous variables used in comparative analyses.

Table S2. Results of likelihood ratio tests for significant phylogenetic signal for all continuous traits used in ancestral reconstructions.

Table S3. Results from phylogenetic logistic regression and the threshold model, using the dataset with taxa having uncertain reproductive modes removed.

Table S4. Δ AICc scores for all models tested for phylogenetic generalized least squares comparisons of range sizes and temperature niche breadths.

Table S5. Full results from phylogenetic generalized least squares comparisons of range sizes and temperature niche breadths, for all models having Δ AICc scores of < 2 .

Table S6. Reproductive mode for species not included in the phylogenetic tree (0 = oviparous, 1 = viviparous).

Table S7. Pearson product-moment correlations between independent variables at each taxonomic scale, calculated using the "cor.test" function in R.