

LETTER

Early origin of viviparity and multiple reversions to oviparity in squamate reptiles

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Abstract

Viviparity has putatively evolved 115 times in squamates (lizards and snakes), out of only ~ 140 origins in vertebrates, and is apparently related to colder climates and other factors such as body size. Viviparity apparently evolves from oviparity via egg-retention, and such taxa may thus still have the machinery to produce thick-shelled eggs. Parity mode is also associated with variable diversification rates in some groups. We reconstruct ancestral parity modes accounting for state-dependent diversification in a large-scale phylogenetic analysis, and find strong support for an early origin of viviparity at the base of Squamata, and a complex pattern of subsequent transitions. Viviparous lineages have higher rates of speciation and extinction, and greater species turnover through time. Viviparity is associated with lower environmental and body temperatures in lizards and amphisbaenians, but not female mass. These results suggest that parity mode is a labile trait that shifts frequently in response to ecological conditions.

Keywords

BiSSE, ecological specialisation, ecophysiology, egg laying, evolutionary reversals, live birth, parity mode, squamata.

Ecology Letters (2014) 17: 13–21

INTRODUCTION

Parity mode is one of the most recognisable and well-studied physiological traits in the animal kingdom. Most amniote clades are characterised exclusively by laying eggs (oviparity; e.g. turtles, birds, monotreme mammals, crocodylians), or giving birth to live young (viviparity; e.g. metatherian and eutherian mammals). However, squamate reptiles [lizards, snakes and amphisbaenians ('worm lizards')] exhibit a complex mosaic of reproductive modes, with both oviparity and viviparity observed in the same families, genera and even different populations of the same species (Tinkle & Gibbons 1977; Blackburn 2006). While the ancestral state of Squamata is generally considered to be oviparity, viviparity has evolved numerous times in different squamate lineages (Blackburn 1999). In some skinks, this has been taken to extremes, with a few species exhibiting placental hypertrophy and essentially mammal-like embryonic development and pregnancy (Blackburn & Vitt 2002; Brandley *et al.* 2012; Murphy *et al.* 2012).

At least 115 different squamate lineages exhibit viviparity, of ~ 141 origins in vertebrates (Sites *et al.* 2011). It is also clear from the fossil record that viviparity evolved in squamates by the late Cretaceous (Wang & Evans 2011), as well as in numerous basal Mesozoic diapsids such as ichthyosaurs and plesiosaurs (O'Keefe & Chiappe 2011). These instances have been characterised extensively (Blackburn 1982, 1985, 1999; Shine 1985), and many squamate groups (genera, families, etc.) are known to be exclusively oviparous or viviparous.

However, lacking a large-scale phylogenetic analysis, it is nonetheless possible that multiple viviparous lineages thought to represent multiple independent origins actually form single larger clades with single origins. Some literature has also suggested that reversal to oviparity is possible (de Fraipont *et al.* 1996; Lee & Shine 1998), but unlikely and infrequent due to complexity of the eggshell (Blackburn 1999; Shine & Lee 1999). The majority of viviparous squamates lack extensive placentation, though, and appear to have arisen from oviparous lineages which shifted to in utero egg-retention (Shine 1983), with reduction in shell thickness and increased gas and nutrient exchange (Shine 1985). Thus, many viviparous lineages may retain at least part of the physiological machinery needed to produce thick-shelled eggs. Support for reversal has been found only in New World (NW) pitvipers and Old World sand boas (Lynch & Wagner 2010; Fenwick *et al.* 2012). Parity mode is also associated with differences in diversification rates between oviparous and viviparous lineages in several NW groups, including pitvipers (Lynch 2009) and phrynosomatid lizards (Lambert & Wiens 2013).

Smaller-scale phylogenetic studies of some squamate groups have also revealed interesting patterns regarding ecophysiological correlates of parity mode. In iguanian lizards, the evolution of viviparity predates Pliocene-Pleistocene glacial cycles, rejecting previous hypotheses that live birth evolved recently in response to climatic shifts (Schulte & Moreno-Roark 2010). There are also numerous ecological studies suggesting that factors such as climate are associated with the evolution of viviparity (see Tinkle & Gibbons 1977; Shine 2004). These hypotheses

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have been supported in a phylogenetic context, but only for the NW iguanian lizards *Liolaemus* (Pincheira-Dinoso *et al.* 2013) and Phrynosomatidae (Lambert & Wiens 2013). In addition, body size may also affect parity mode, since larger females can retain embryos in utero while incurring lower physiological costs (Shine 1985), and body temperatures are positively related to body mass (Meiri *et al.* 2013). Finally, increased survival due to maternal temperature-stability may also explain the success of both tropical and temperate viviparous species (Webb *et al.* 2006). However, these hypotheses have not been tested extensively in a large-scale phylogenetic framework sampling numerous squamate lineages and many species.

Thus, there remain several important and long-standing questions regarding parity mode evolution in squamates. First, how many times has viviparity evolved, and how many reversals from viviparity to oviparity have occurred? Second, how do transitions between oviparity and viviparity affect speciation and extinction rates? Third, what are the ecological correlates of parity mode? Our understanding of the evolutionary origins and ecological consequences of parity mode in squamates has been hampered in part by the lack of a large-scale phylogenetic hypothesis for the group, including all of the > 100 lineages exhibiting viviparity. Previous higher level phylogenies for squamates included no more than 161 species (Wiens *et al.* 2012). A recent study introduced a phylogeny for squamates sampling 4161 species (~ 44% of ~ 9400 extant species) from all families and subfamilies, and 84% of genera (Pyron *et al.* 2013). Crucially, this tree includes nearly all of the > 100 viviparous lineages representing putative origins identified by previous authors (Blackburn 1982, 1985, 1999; Shine 1985). With most squamates (~ 85%) classified for parity mode, we generated a time-calibrated version of this phylogeny to answer the primary questions above related to: (1) number and rate of transitions between parity modes, (2) effects of parity mode on diversification rates and (3) ecophysiological correlates of parity mode (e.g. climate and body size).

Quite unexpectedly, we find using Maximum Likelihood (ML) that the concentration of viviparous lineages near the root (e.g. in Scincoidea; ~ 166 Ma) suggests an early evolution of viviparity at the base of Squamata (~ 174Ma). Transitions from viviparity to oviparity are roughly as frequent as the reverse (de Fraipont *et al.* 1996), and numerous independent gains and losses of both states present a far more complex pattern than previously imagined (Blackburn 1999; Shine & Lee 1999). Viviparous lineages exhibit significantly higher speciation and extinction rates, leading to higher turnover of live-bearing lineages, but lower net diversification rates. In lizards and amphisbaenians, decreasing environmental and body temperatures, but not female mass, are correlated with viviparity, suggesting that trade-offs between fecundity and ecological selection in cooler climates promote live birth (Tinkle & Gibbons 1977; Shine 1985).

MATERIALS AND METHODS

Time-calibrated tree

We produced a time-calibrated tree containing 4161 species (out of ~ 9400 total) and the outgroup *Sphenodon punctatus*,

based on a recent ML estimate of squamate phylogeny (Pyron *et al.* 2013). Using existing temporal frameworks for squamate evolution (Wiens *et al.* 2006), we calibrated the ages of six higher-level nodes using fixed ages (Supporting Information), as well as fixing the root age at 228 Ma in concordance with previous studies (Wiens *et al.* 2006; Mulcahy *et al.* 2012). We used Penalised Likelihood (Sanderson 2002) in the program treePL (Smith & O'Meara 2012) to estimate divergence times for the remainder of the nodes (Supporting Information). The optimal smoothing factor was determined empirically using cross-validation (Sanderson 2002; Smith & O'Meara 2012). The resulting chronogram is highly concordant with both fossil and molecular estimates of squamate origins (Mulcahy *et al.* 2012). This chronogram shows close concordance with recent estimates of squamate divergence times (Wiens *et al.* 2006; Mulcahy *et al.* 2012). We used the estimated topology and branch lengths to reconstruct parity mode in the group using both parsimony and model-based approaches (see below). The tree is provided in Data File S1.

Parity mode

On the basis of extensive literature searches and previous synopses (Fitch 1970; Tinkle & Gibbons 1977; Blackburn 1982, 1985, 1999; Shine 1985), we characterised the parity mode of 8006 species of squamate (Data File S2), ~ 85% of the ~ 9400 known, extant species and the oviparous outgroup *Sphenodon punctatus*. This accounts for essentially all of the > 100 putative origins in the group (Blackburn 1999; Sites *et al.* 2011), with 6658 oviparous, 1336 viviparous and 12 bi-modal species. Of these, 3950 are represented in the 4161-species phylogeny (plus the outgroup), with 3108 oviparous (47% of the total oviparous species) and 843 viviparous taxa (63%). We pruned the tree to include only those species for which we had parity data (3951 species total). We included the outgroup in subsequent analyses, so that the root node is Lepidosauria and the ingroup is Squamata (Mulcahy *et al.* 2012), to adequately capture dynamics at the most recent common ancestor (MRCA) of Squamata with respect to the basal node subtended by *Sphenodon*. As there is only one species of *Sphenodon*, the outgroup is thus fully sampled (no missing taxa).

State-dependent diversification

We tested for state-dependent diversification using the Binary State Speciation and Extinction (BiSSE) model (Maddison *et al.* 2007), in addition to state-independent ML (Mk2) and parsimony methods. As implemented (FitzJohn 2012) in the R package 'diversitree,' BiSSE accounts for state-dependent incomplete taxonomic sampling (FitzJohn *et al.* 2009), which we based on our known sampling of squamate parity modes (47% of oviparous and 63% of viviparous species). Note that BiSSE supports 'NA' for species with unknown states (we thus could have used the 4162-species chronogram), but this would lump bi-modal species with unknown species, and has little effect on the results (Supporting Information).

We fit a series of eight models accounting for state-dependent diversification in BiSSE (Supporting Information). These

include separate speciation and extinction rates for oviparous and viviparous taxa and asymmetrical transitions, as well as models with equal speciation, equal extinction and symmetrical transition rates, and models that allowed no reversals (both with and without state-dependent speciation and extinction rates). We performed model-fitting using ML (selecting the model with $dAIC = 0$). For the best-fit BiSSE model, we estimated parameter uncertainty using Bayesian Markov Chain Monte Carlo Markov Chain Monte Carlo (MCMC), sampled for 10 000 generations with an exponential prior ($1/2 * \text{the state-independent diversification rate } r$). We summarised the post-burnin MCMC samples to assess variation in speciation (λ) and extinction (μ) rates with respect to parity mode. In addition to absolute speciation and extinction rates, we also calculated net diversification rates ($r = \lambda - \mu$) and relative extinction fractions ($\epsilon = \mu/\lambda$; lineage turnover through time) summarised across samples.

Ancestral states

If different character states result in different rates of speciation and extinction, ordinary reconstructions using Mk2 or parsimony will be biased due to the asymmetric representation of those states among extant taxa (Maddison 2006; Paradis 2008). To account for this, we first reconstructed ancestral states under the best-fit 6-parameter BiSSE model accounting for state-dependence of speciation and extinction rate. For comparison with state-independent models, we then estimated ancestral states under the Mk2 model using both the chronogram and the uncalibrated ML branch-lengths, the latter to ensure that results were not being influenced heavily by temporal calibrations. Next, we estimated ancestral states under a BiSSE sub-model constrained in diversitree to allow no changes from viviparity to oviparity (Mk1 with no reversals), and Maximum Parsimony (MP) in 'APE' (Paradis *et al.* 2004), both using the chronogram. The latter two represent the typical previous interpretations of parity mode evolution in squamates. The full trees showing these estimates are shown in Appendix S1-5.

For model-based ancestral states, we consider > 95% support for one state to be 'strong.' Note that the model-based estimates are marginal reconstructions (the likelihood of the states at each node under the ML parameter estimates), and represent the support for each state having existed at a particular node, rather than a globally optimised character-history of transitions. Future studies interested in exact transition-sequences may explore sampling from the potential character-histories estimated from joint reconstructions or stochastic mapping; these are not currently available for BiSSE models, and were omitted for the other reconstructions (e.g. Mk2) due to computational constraints. The previous study (Pyron *et al.* 2013) also presented 10 other sub-ML estimates (un-calibrated) of the squamate phylogeny generated during the search for the primary ML tree. We reconstructed ancestral states on these trees using the Mk2 model (in Mesquite) to ensure that results were not unique to the single tree analysed here, or excessively sensitive to phylogenetic variation in topology and branch length around the ML estimate (Supporting Information).

Ecophysiology

A recent study showed that body and environmental temperatures are uncorrelated overall in lizards and amphisbaenians, but are significantly related when activity patterns and micro-habitat use are included in models (Meiri *et al.* 2013). From this data set of 861 species, 746 were represented in our phylogeny with parity mode data, which we trimmed to include only those taxa. This does not include snakes, but does include Anguimorpha + Iguania, the sister-group of snakes (Pyron *et al.* 2013). The data are mean annual temperature (degrees centigrade), calculated as the mean of 0.16-degree grid cells within range-maps generated as part of an ongoing effort to map squamate distributions (S. Meiri, *pers. comm.*), mean body temperature (degrees centigrade) of active individuals from the literature and personal observation, and mean female mass (grams), calculated from snout-vent length (SVL) using clade-specific allometric curves, which provides a more robust measure of body size than SVL (Meiri *et al.* 2011).

We tested separately for a correlation between parity mode and each of the three continuous variables using the threshold model, which estimates the correlation between two variables that co-vary with respect to an unobserved underlying liability (Felsenstein 2012). While it would be desirable to include the multiple continuous traits simultaneously (as in a multiple regression) due to possible interactions, and this is theoretically possible in the threshold framework (Felsenstein 2012), current implementations only allow one continuous variable to be tested at a time. We estimate these parameters via Bayesian methods, sampling from the posterior distribution using MCMC. We used threshBayes in the R package 'phytools,' with chains run for 2.5 million generations, discarding the first 500 000 as burn-in, and summarising the remainder to determine whether the estimated correlation coefficients differed significantly from zero (i.e. 95% Highest Posterior Density [HPD] does not include 0).

RESULTS

Diversification rates

Using the BiSSE framework to reconstruct ancestral states while accounting for state-dependent diversification, we find strong support for a model containing six parameters (Table 1). These are state-dependent speciation and extinction rates, as well as asymmetric rates of transition between oviparity and viviparity. This is supported significantly over models without state-dependent diversification (Mk2), and models that do not allow reversions to oviparity (Table 1). The best-fit 6-parameter BiSSE model indicates that speciation and extinction rates vary significantly between oviparous and viviparous lineages (Table 1; Fig. 2). Egg-laying species have significantly lower rates of speciation ($\lambda = 0.063$ lineages $\times \text{my}^{-1}$ [95% HPD = 0.061–0.065]) and extinction ($\mu = 0.00000013$ [0–0.014]) than viviparous lineages ($\lambda = 0.085$ [0.076–0.096]; $\mu = 0.027$ [0.011–0.045]). However, this results in a higher relative-extinction fraction (ϵ) and lower net-diversification rate (r) in viviparous ($r = 0.058$ [0.049–0.066]; $\epsilon = 0.32$ [0.15–0.47]) vs. oviparous ($r = 0.063$

[0.061–0.064]; $\varepsilon = \sim 0$ [0–0.02]) lineages (Figure S1, S2). These values are similar to other analyses (Lynch 2009; Lambert & Wiens 2013), suggesting the results are not heavily skewed or dominated by a single lineage driving rate estimates (Davis *et al.* 2013).

To test this further, we fit a 2-state, 12-parameter model ('splitBiSSE'), decoupling state-dependent diversification between Scincoidea (which appears to contribute strongly to our results; see below), and the rest of Squamata. Results from this model are similar for non-scincoidean squamates, though scincoideans actually show slightly increased speciation rates in oviparous lineages, and little difference in extinction rates (Supporting Information). Thus, effects of parity mode on diversification are not limited to a single group, though dynamics (direction and magnitude of state-dependence) may differ among subclades (Lynch 2009; Lambert & Wiens 2013).

Ancestral states

Ancestral-state reconstructions using the state-dependent BiSSE models are thus expected to differ significantly from the Mk and MP estimates, due to the correlation of diversification and parity mode (Goldberg & Igc 2008). However, both the BiSSE and Mk2 models reconstruct an early evolution of viviparity in Squamata, with strong support for the MRCA of the group (174.1 Ma) as live bearing from the 6-parameter BiSSE model (Fig. 1), and moderate support under the Mk2 model (66%). The Mk2 analyses of the ML branch-lengths (including the 10 sub-ML trees) are similar to the chronogram reconstructions, but with more ambiguously reconstructed branches and an ambiguous root-state for Squamata (41% support for viviparity). The model with no reversal predictably yields an oviparous Squamata with multiple independent origins of viviparity (see below). Finally, MP also estimates an oviparous Squamata, but an early potential evolution of viviparity on the stem lineage of Scincoidea (166.6 Ma).

In both the model-based and parsimony reconstructions, there are numerous origins of viviparity and strongly supported reversions to oviparity (Appendix S1–S5), in groups that are strongly supported in the ML phylogeny (Pyron *et al.* 2013). Thus, there are likely significantly fewer than 115 unique origins of viviparity, as numerous putatively independent origins actually form single larger clades. We consider strong support as > 95% probability for a state, and conservatively count multiple clades connected by ambiguously reconstructed branches as single gains or losses. In the

6-parameter BiSSE estimate, there are 34 origins including the root node, and 59 reversals to oviparity. The Mk2 analyses of the chronogram and ML branch-lengths yield highly similar patterns (but with more ambiguously reconstructed branches), though groups such as Scincoidea and Alethinophidia are still strongly supported as ancestrally viviparous. There are 121 origins under the model with no reversions, counting singleton lineages (i.e. single viviparous species in oviparous clades), but many of these are part of single larger clades (e.g. 29 in Iygosomine skinks) that represent many fewer independent origins under both model-based and parsimony estimates. There are 73 origins under the ordinary maximum-parsimony estimate, with 12 unambiguously reconstructed reversals to oviparity. Rates of transition from oviparity to viviparity are far lower than the reverse in both the BiSSE (0.00071 [0.00052–0.00094] lineages \times my⁻¹ vs. 0.0059 [0.0044–0.0075]) and Mk2 (0.001 vs. 0.007) models.

Ecophysiological correlates

From a previous analysis (Meiri *et al.* 2013), we were able to obtain mean annual and mean body temperature (degrees centigrade), and mean female mass (g) for 746 lizard and amphibian species in our tree for which we also had parity mode data. Using the threshold model to link continuous traits to discrete variables via their unobserved underlying liability (Felsenstein 2012), we find a strong negative correlation between parity mode and both mean annual temperature ($r_p = -0.63$ [–0.70 to –0.55]; ESS = 66; Fig. 3) and mean body temperature (–0.40, [–0.54 to –0.21]; ESS = 35), indicating that viviparity is associated with both cooler climates and bodies (Meiri *et al.* 2013). There is no correlation with mean female mass (0.09 [–0.04–0.21]; ESS = 296). The likelihood profiles of the MCMC chains indicate convergence with good mixing after burnin of 500 000 generations (Figure S3–S5).

DISCUSSION

Unexpectedly, ancestral-state reconstructions using model-based estimates suggest an early evolution of viviparity in Squamata, with the ancestral node reconstructed as live bearing (Fig. 1). Support for this is very strong when the best-fit trait-dependent model is used to estimate ancestral states, and moderate under a two-rate model with trait-independent diversification. Even under parsimony, viviparity is estimated to have evolved relatively early in Unidentata, on the stem

Table 1 Comparison of likelihoods for alternative ancestral-reconstruction models constructed in BiSSE

Model	DF	dAIC	AICw	λ_0	λ_1	μ_0	μ_1	q01	q10
Full	6	0	0.97	0.0628	0.0848	0.0000	0.0270	0.0007	0.0059
No Reversal	5	147	0.00	0.0625	0.0726	0.0000	0.0000	0.0015	Fixed (0)
Equal λ	5	18	0.00	0.0640	–	0.0000	0.0000	0.0006	0.0072
Equal μ	5	7	0.03	0.0624	0.0719	0.0000	–	0.0006	0.0069
Equal q	5	54	0.00	0.0636	0.1024	0.0000	0.0599	0.0017	–
Mk2	4	16	0.00	0.0640	–	0.0000	–	0.0006	0.0072
Mk1	3	67	0.00	0.0640	–	0.0000	–	0.0013	–
Mk1, No Reversal	3	158	0.00	0.0640	–	0.0000	–	0.0015	Fixed (0)

All submodels were generated by constraining the full model in BiSSE (Supporting Information).

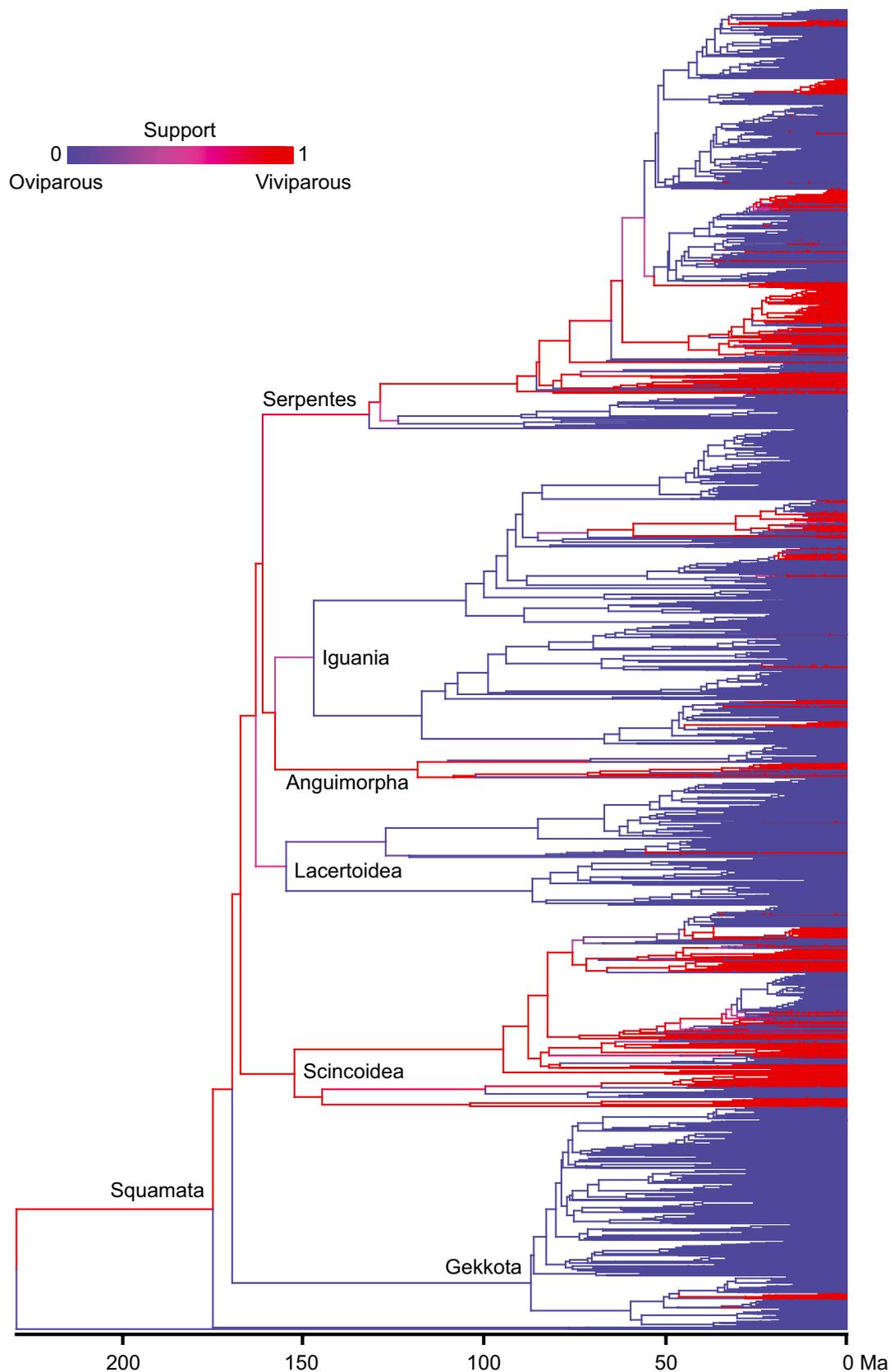


Figure 1 Marginal reconstruction of ancestral states under the best-fit 6-parameter BiSSE model (fully labelled tree in Appendix S1). Branches are coloured continuously according to support for each state. Major clades are labelled to illustrate the location of early transitions. The colour assigned to a branch connecting two nodes is based on the ancestral-state inference for the node to the right (towards the present); e.g. the ancestral state of Iguania is uncertain, as indicated by a purple sub-tending branch.

lineage of Scincoidea. Indeed, several live-bearing lizard taxa are known from the mid to late Cretaceous (Wang & Evans 2011), supporting an earlier evolution of viviparity than recovered when reversals are disallowed, but consistent with the time-frame suggested by the model-based analyses (Appendix S1–S5). To our knowledge, this is the first study to suggest an early origin of viviparity in Squamata, rather than multiple, more recent origins (Blackburn 1999). Support for viviparity at the root of Squamata and the presence of numerous viviparous basal diapsids (O’Keefe & Chiappe 2011) suggests that viviparity as a life-history strategy may have a more ancient history in amniotes than previously believed.

Both model-based and parsimony reconstructions strongly support multiple transitions between oviparity and viviparity, in contrast to most previous hypotheses (Blackburn 1982, 1985, 1999; Shine 1985; Shine & Lee 1999; Sites *et al.* 2011), though increasingly supported by phylogenetic analyses of numerous subclades (de Fraipont *et al.* 1996, 1999; Lynch & Wagner 2010; Fenwick *et al.* 2012). Previous analyses have suggested that reversals to oviparity may be more common than previously suspected (de Fraipont *et al.* 1996), but were criticised for focal clade choice and assumptions regarding ancestral states (Shine & Lee 1999). We address those concerns here by conducting a large-scale, time-calibrated phylogenetic analysis containing representatives from essentially all viviparous and oviparous lineages, and accounting for confounding effects of state-dependent diversification.

Previous authors have cautioned that ancestral-state estimates of traits such as reproductive mode may be misleading, and should be assessed using data from other organismal traits or climatic data (Wiens *et al.* 2007). However, the evolutionary patterns observed here are corroborated by our eco-physiological data; species inhabiting cooler climates are more likely to give birth to live young, as expected (Shine 1985; Meiri *et al.* 2013). Furthermore, there are few hypothesised physiological constraints on reversion to oviparity (Blackburn 1999), and thus no well-defined intermediate stages are being skipped in transition between parity modes (i.e. necessarily ordered states). Squamates with simple placentae (the vast majority) are essentially retaining shell-less eggs in utero (Thompson & Speake 2006), and the reversion to oviparity apparently only involves the re-introduction of shell deposition during embryonic development.

Finally, ancestral states under parsimony and likelihood can be strongly misled if character states affect diversification rates, as found here (Maddison 2006; Goldberg & Igc 2008; Paradis 2008). Indeed, the correlation between parity mode and evolutionary rates (Fig. 2) suggests that ancestral-state estimates generated via parsimony or likelihood methods have a high probability of error, due to the biased representation of states in the phylogeny resulting from asymmetric diversification of oviparous and viviparous lineages (Maddison 2006; Paradis 2008). Reassuringly, our estimates from both the state-dependent BiSSE and state-independent Mk2 models (using both time-calibrated and molecular branch-lengths) are actually very similar. Thus, there is no apparent reason to suspect that these results are being strongly misled by any statistical artefacts. That early evolution of viviparity and multiple reversions to oviparity is also recovered in the state-

independent likelihood analyses using the ML branch lengths (Pyron *et al.* 2013) suggests that variation in divergence-time estimates does not strongly affect our results.

The basal placement of Scincoidea, with its many ancient viviparous lineages (as well as the basal viviparous lineages of snakes and anguimorphs), appears to strongly influence estimation of an early evolution of viviparity, but this phylogenetic relationship has been supported by > 44 loci in other phylogenetic analyses (Wiens *et al.* 2012; Pyron *et al.* 2013). The exact number of origins and losses of oviparity may shift based on species-level phylogenetic hypotheses (e.g. in Lygosominae), but this is unlikely to affect the strongly supported findings of an early origin of viviparity, and multiple reversions to oviparity. Our results indicate that while viviparity has evolved far less than 115 times, parity mode is an evolutionarily labile trait in squamates, and bi-directional transitions between oviparity and viviparity are both possible and frequent. Other traits, such as venom and limb structure are also highly labile in squamates compared to other groups, with multiple transitions and reversions (Sites *et al.* 2011).

This also mirrors recent results for amphibians showing that life-history strategies change often, with frequent transitions between different modes (Gomez-Mestre *et al.* 2012). We point out that these extreme rates of change in squamates are contrasted against other extant amniotes, where a single transition from oviparity to viviparity is recorded in mammals, and oviparity is fixed in the clade consisting of turtles, crocodilians, and birds. In fish, there have been as many as 29 transitions from oviparity to viviparity (Blackburn 2005), but this group also has not been subject to a large-scale analysis, and the physiological pathways are poorly known.

While the effect of climate on parity mode evolution is well-established (Tinkle & Gibbons 1977), this is also the first large-scale phylogenetic study to find a widespread association between ecological conditions, parity mode, and diversification rates in squamates. In agreement with the expectations and findings of previous ecological and phylogenetic analyses, colder climates appear to promote the evolution of viviparity (Fig. 3), presumably as environmental pressures on egg mortality promote retention of embryos in utero (Shine 1985, 2005; Meiri *et al.* 2013; Pincheira-Dinoso *et al.* 2013). Interestingly, body mass does not seem to have a strong effect; species with larger females do not preferentially exhibit viviparity, even though larger individuals typically have higher body temperatures (Meiri *et al.* 2013). The strength of the relationship between temperature and viviparity explains the appearance of viviparous temperate subclades of otherwise oviparous groups from warm regions, such as the geckos of New Zealand (Shine 1985), and the liolaemid iguanians of Patagonia (Schulte *et al.* 2000), derived from tropical Australian and South American faunas respectively.

The converse of this hypothesis, implied by our data suggesting an early evolution of viviparity in squamates, is that the costs incurred by females promote reversion to oviparity when environmental conditions are stable enough for egg survival (Tinkle & Gibbons 1977; Shine 1985). Viviparous females incur physiological costs related to metabolism (energetic demands of embryos, etc.) and behaviour (thermoregulation and predator avoidance, etc.), among others (Shine

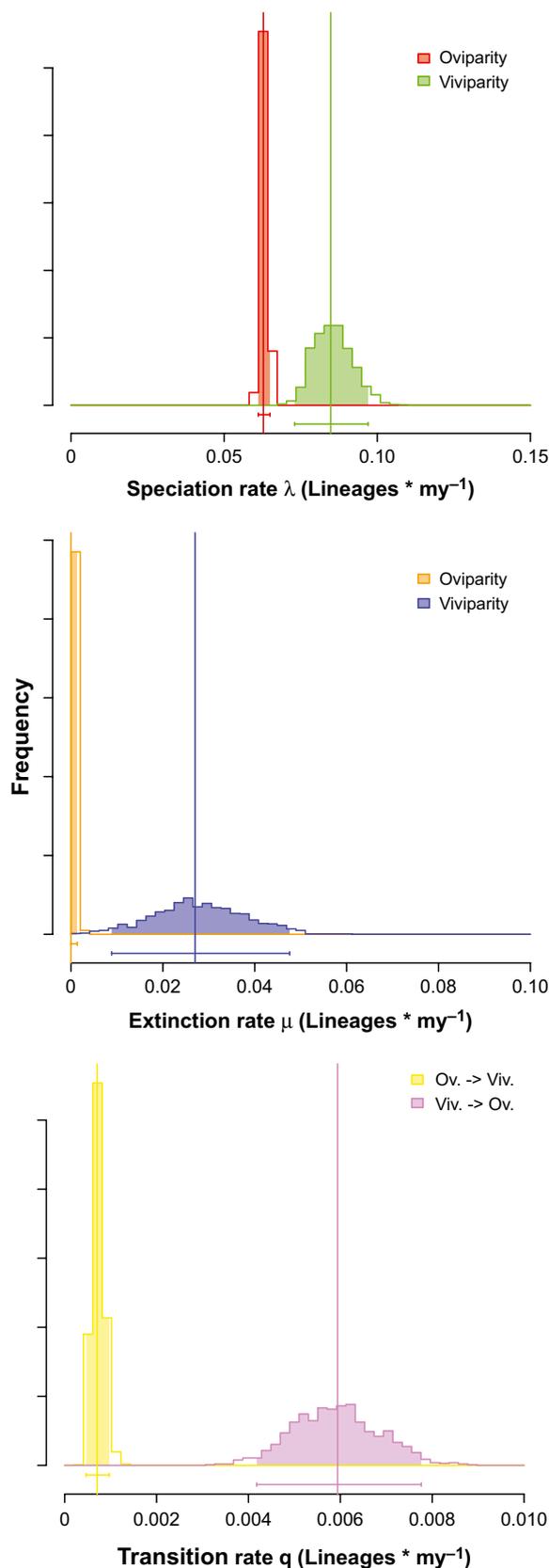


Figure 2 Parameter estimates from best-fit BiSSE model, showing differences in speciation (λ), extinction (μ) and transition (q) rates between oviparous and viviparous taxa. Net diversification rates (r) and relative extinction fractions (ϵ) also differ between modes (Figures S1 and S2).

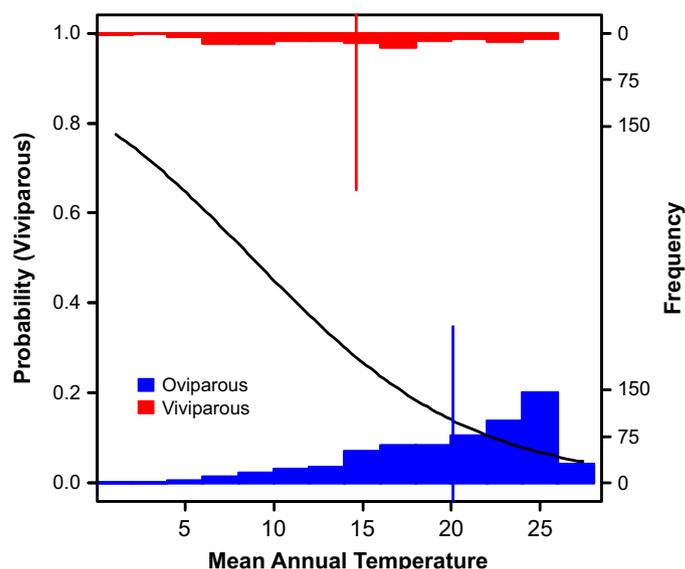


Figure 3 Histogram of mean annual temperature (degrees centigrade) throughout the range of oviparous and viviparous taxa. Logistic regression curve is for illustrative purposes to show difference between parity modes; the phylogenetically corrected threshold model (Felsenstein 2012) indicates that the correlation is significant ($r = -0.63$; $P < 0.00001$; Supporting Information).

1985). This touches on the key novelty of our results in contrast with traditional hypotheses about parity mode evolution: are there physiological constraints for re-gaining the eggshell (Blackburn 1999; Shine & Lee 1999; Thompson & Speake 2006)? Our results imply that oviparity is easily re-gained in lineages occurring in warmer environments. As viviparity in squamates is almost uniformly uterine retention of eggs with reduction in shell thickness (Shine 1985), it is unclear that the physiological mechanisms needed to produce thick-shelled eggs are actually lost in oviparous lineages (Blackburn 1999). Thus, viviparity and oviparity seem to represent two relatively similar physiological states (Fitch 1970; Shine 1985; Thompson & Speake 2006). These states vary primarily in the thickness and calcification of the shell and the location of embryonic development (Shine 1983), rather than physiologically distinct development pathways, and are apparently highly labile in both directions.

The dependency of speciation and extinction rate offers another interesting perspective on this issue. For viviparous lineages in cold climates, previous ecological and phylogenetic analyses either implicitly (Shine 1985) or explicitly (Pincheira-Dinosa *et al.* 2013) assume that the cold climates inhabited by viviparous lineages are, for the most part, inherently unstable, and that those taxa are subject to more intense environmental pressures. Thus, we would expect viviparous lineages in cold climates to have access to open niche spaces (i.e. relatively free of competitors) promoting diversification, but also to experience higher extinction pressures as a result of climatic instability (Pincheira-Dinosa *et al.* 2013). These changes in diversification rate may be independent of or a result of the physiological costs imposed by viviparity. This is supported by our results; viviparous lineages have significantly higher rates of both speciation and extinction, but lower net-diversification

rates, indicating increased turnover of lineages through time (Fig. 2). Thus, the evolution of viviparity seemingly represents a significant trade-off, rather than an overall selectively advantageous or evolutionarily stable strategy.

These hypotheses remain to be tested in snakes, for which body and environmental temperatures were unavailable, and patterns are not nearly as clear-cut. Viviparity is relatively common in tropical snakes (e.g. anilioids, boids, elapids, homalopsids, crotalines), and not restricted to temperate clades. Many of the viviparous tropical groups are also relatively species-poor (Pyron & Burbrink 2012). There may be alternative mechanisms driving the evolution of viviparity in warmer climates, explaining the frequency of tropical viviparous species, and the early evolution of this mode in squamates during warmer time-periods (i.e. the Jurassic). Alternatively, previous studies have suggested that the same mechanism (maternal manipulation of uterine temperatures) can explain the success of both tropical and temperate viviparous species, as the selective advantage of viviparity outweighs the physiological costs (Webb *et al.* 2006). Thus, the invasion of temperate areas (and subsequent evolution of viviparity) may be a more recent development in the history of squamates, but driven by the same mechanism as tropical viviparity. Whether early viviparous squamates also had elevated turnover is unknown.

As a final word of caution, the threshold model assumes that diversification is state-independent, which the BiSSE results suggest is not the case, though the strength and biological plausibility of the results suggest that the observed correlations are not entirely artifactual. It is also possible that other variables related to the climatic or ecophysiological differences between oviparous and viviparous species actually drive differences in speciation and extinction rates, and that parity mode and environmental temperature are simply correlates of this (Pincheira-Dinoso *et al.* 2013). Further study will be needed to elucidate the ecological mechanisms of these differences. Finally, when state-dependent dynamics are decoupled in Scincoidea, rates change less in scincoids (and in the opposite direction) than in other squamates (Supporting Information). Differences in the direction and magnitude of state-dependent diversification rates mediated by reproductive strategies are also observed in Iguania (Lambert & Wiens 2013) and Serpentes (Lynch 2009). Determining how these dynamics vary among squamate subclades with respect to factors such as climate and biogeography will likely be a fruitful area for future research.

CONCLUSIONS

In close concordance with theoretical expectations and previous results, we find that colder climates are strongly associated with the evolution of viviparity in squamate reptiles (Tinkle & Gibbons 1977; Shine 1985). For the first time using phylogenetic analyses, we show that this is associated with increased rates of both speciation and extinction, leading to lower net-diversification rates and increased turnover (lineage replacement through time) for live-bearing lineages. However, this means that ancestral-state reconstructions using ordinary parsimony or likelihood methods are likely to be strongly biased due to the asymmetric representation of character states among

extant species (Maddison 2006; Paradis 2008). Using methods that account for this explicitly (Maddison *et al.* 2007), we find the surprising result that viviparity is the ancestral state of Squamata (Fig. 1). Rather than 115 independent origins of viviparity, we find a complex pattern of multiple transitions between egg laying and live birth. This echoes recent work in amphibians showing that reproductive modes shift frequently (Gomez-Mestre *et al.* 2012), and in squamates showing that traits such as venom and body form are similarly labile (Sites *et al.* 2011). In most squamate lineages, oviparity and viviparity derive from similar underlying physiological mechanisms (Shine 1983), and our data suggest that transitions between them are less constrained than previously thought. These results should provide a platform to resolve questions such as the causes and effects of variation in gestation period and clutch and litter size, the physiological mechanisms of transition between egg laying and live birth, and the ecological mechanisms that relate these strategies to speciation and extinction.

ACKNOWLEDGEMENTS

We thank D. Blackburn and M. Brandley for early discussions of parity mode, L. Revell for assistance with R code, D. Thompson and L. Hughes for help with literature on fish, and P. Thrall, L. Harmon, S. Otto, and two anonymous reviewers for comments on the MS. This research was funded in part by US NSF grant DBI-0905765 to R.A.P.

STATEMENT OF AUTHORSHIP

RAP and FTB conceived the article, RAP collected data and performed the analyses, RAP and FTB wrote the manuscript.

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Editor, Luke Harmon

Manuscript received 2 May 2013

First decision made 6 June 2013

Second decision made 9 July 2013

Manuscript accepted 22 July 2013