**Hypoxia and hypothermia as rival agents of selection driving the evolution of viviparity in lizards**

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**Abstract**

**Aim:** The evolution of key innovations promotes adaptive radiations by opening access to novel ecological opportunity. The acquisition of viviparity (live-bearing reproduction) has emerged as one such innovation explaining reptile proliferations into extreme climates. By evolving viviparity, females provide embryos with internally stable environments to complete development. The classical hypothesis suggests that natural selection for viviparity arises from low temperatures in cold-climates, which promote prolonged egg retention in the mother’s body. An alternative hypothesis proposes that declines in atmospheric oxygen at high elevations create natural selection for embryo retention to provide them with optimal oxygen levels during development. However, although experimental studies support the negative effects of low oxygen on egg development, this ‘hypoxia’ hypothesis has never been tested quantitatively. Here, we compete the hypoxia hypothesis against the ‘cold-climate’ hypothesis, using a highly-diverse lizard genus.

**Location:** South America

**Major taxa:** *Liolaemus* lizards.

**Methods:** We employ a multivariate dataset covering 121-species varying extensively in geographic and climatic distribution (including extreme thermal and oxygen gradients), and parity mode. Based on a new molecular phylogeny for the genus, we use phylogenetic logistic regressions to generate a range of models ranking environmental factors as a function of their effects on parity mode transitions.

**Results:** Elevation and oxygen declines correlate nearly perfectly, and both were identified as the dominant predictors of oviparity-to-viviparity transitions, while the role for temperature (dominated by the coldest winter temperatures and daily fluctuations) is significant but secondary. Overall, we show that oxygen-deprivation and low temperatures both play a role in the evolution of viviparity.

**Main conclusions:** Our findings support the role for selection from declines in oxygen concentrations as the primary driver behind viviparity. However, selection arising from cold temperatures and from reduced fluctuations in daily temperatures contribute to the evolution of these transitions by creating multivariate selection on parity mode.

**Introduction**

Adaptive radiations are triggered by exploitation of ecological opportunity offered by access to novel environments following the evolution of key adaptive innovations ([Schluter, 2000](#_ENREF_47); [Losos, 2010](#_ENREF_27)). The result of such exposure to novel natural selection regimes is the emergence of adaptive variation that can lead to the origin of new species ([Schluter, 2000](#_ENREF_47)). Among animals, squamate reptiles (lizards and snakes) have given rise to some of the most exceptional examples of prolific evolution ([Losos, 2009](#_ENREF_26); [Pincheira-Donoso *et al.*, 2013b](#_ENREF_44)). As a result, these organisms have emerged as attractive models to investigate the causes and consequences of key innovative traits promoting adaptive radiations. As expected, a number of candidate key traits (e.g., toepads, venom) have been implicated with the global diversification of these vertebrates ([Losos, 2009](#_ENREF_26); [Sites *et al.*, 2011](#_ENREF_56)).

The evolution of viviparity (live-bearing reproduction) has increasingly been recognized as a key innovation allowing squamates to access cold climates ([Shine, 2005](#_ENREF_52); [Sites *et al.*, 2011](#_ENREF_56); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). Given that cold temperatures in high latitudes and elevations impose strong natural selection against egg incubation in nests, reptile invasions of cold climates are believed to have been facilitated by egg retention in the maternal body, which provides embryos with a thermally stable incubation environment ([Shine, 2004](#_ENREF_51), [2005](#_ENREF_52)). Therefore, emerging theory predicts that the evolution of reptilian viviparity accelerates diversification rates by facilitating access to the ecological opportunity offered by otherwise inaccessible cold-climate environments ([Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). However, although this prediction has been supported by comparative evidence ([Hodges, 2004](#_ENREF_23); [Shine, 2005](#_ENREF_52); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43); [Pyron & Burbrink, 2014](#_ENREF_45)), this ‘cold-climate’ or ‘hypothermia’ hypothesis ignores the multiple other climatic factors that vary geographically and covary with thermal gradients ([Lambert & Wiens, 2013](#_ENREF_25); [Feldman *et al.*, 2015](#_ENREF_18)). Consequently, the role of temperature as the primary driving force behind transitions to viviparity may have spuriously been overestimated, eclipsing the role of other sources of selection.

An alternative hypothesis for the evolution of viviparity suggests that egg development is compromised by declines in atmospheric oxygen concentrations towards colder climates ([Hodges, 2004](#_ENREF_23); [Lambert & Wiens, 2013](#_ENREF_25)). This ‘hypoxia’ hypothesis posits that decreasing oxygen availability creates natural selection for prolonged embryo retention within the female’s body, where placental structures provide optimal levels of oxygen to complete development ([Deeming, 2004](#_ENREF_14); [Lambert & Wiens, 2013](#_ENREF_25)). Numerous experimental studies on reptiles ([Warburton *et al.*, 1995](#_ENREF_59); [Andrews, 2002](#_ENREF_3); [Deeming, 2004](#_ENREF_14)) and birds ([Black & Snyder, 1980](#_ENREF_4); [McCutcheon *et al.*, 1982](#_ENREF_28)) have consistently shown that low levels of oxygen concentrations are detrimental to fitness via reduced developmental success. Developmental components such as embryonic differentiation and growth rates, water uptake, duration of incubation, growth of the chorioallantonic membrane, egg survival, and hatchling size are known to be affected negatively by hypoxia ([Andrews, 2002](#_ENREF_3); [Parker *et al.*, 2004](#_ENREF_34)). Among lizards, for example, these effects have been shown in *Sceloporus*, in which successful development strongly depends on high levels of *in utero* oxygen ([Andrews, 2002](#_ENREF_3); [Parker *et al.*, 2004](#_ENREF_34); [Parker & Andrews, 2006](#_ENREF_33)). Therefore, when contrasted with the cold-climate hypothesis, this ‘hypoxia’ hypothesis implicitly assumes that the effects of oxygen gradients on creating selection for egg retention (i.e., viviparity) are masked by thermal clines. However, the role of hypoxia in selecting for evolutionary transitions to viviparity has never been tested quantitatively.

The unique geographic architecture and biodiversity of South America offers the ideal scenario to interrogate the hypotheses of oxygen and temperature as alternative (or interacting) sources of natural selection for viviparity evolution. First, the Andes range extends in a nearly perfect latitudinal line from north to south on the western side of the continent, and the elevation of these mountains decreases with latitude from central Argentina-Chile (from ~35-36°S) southwards. Second, this extensive area is dominated by one of the most prolific vertebrate radiations on Earth, the lizard genus *Liolaemus*. The 250+ species of this lineage have colonized an unusually wide variety of environmental extremes ([Pincheira-Donoso *et al.*, 2008b](#_ENREF_41)), ranging from high-temperature climates in the Atacama Desert and tropical Brazil, to some of the coldest environments invaded by reptiles globally in high Andean elevations and Patagonia ([Schulte *et al.*, 2000](#_ENREF_48); [Pincheira-Donoso *et al.*, 2008b](#_ENREF_41); [Breitman *et al.*, 2011](#_ENREF_8); [Pincheira-Donoso & Tregenza, 2011](#_ENREF_37); [Pincheira-Donoso *et al.*, 2015](#_ENREF_42)). Within *Liolaemus*, viviparity has evolved in multiple independent episodes, and the great majority of these transitions to viviparity are strongly predicted by increasing elevations and latitudes ([Schulte *et al.*, 2000](#_ENREF_48); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). Therefore, these elements combined provide the conditions for investigating factors linked to transitions to viviparity along extreme latitudinal gradients without elevational effects (i.e., declines in temperature but not in oxygen concentrations), and along extreme elevational gradients without latitudinal effects (i.e., declines in both temperature and oxygen concentrations simultaneously). Elevational effects can include not just declines in oxygen concentration, but also changes in the magnitude of temperature fluctuations. Our own previous analyses (Pincheira-Donoso et al., 2013a) linked transitions to viviparity to changes in environmental temperatures. Here, we reappraise this lizard model system to test whether the evolution of viviparity can also be driven by natural selection arising from spatial decreases in concentrations of atmospheric oxygen ([Hodges, 2004](#_ENREF_23); [Lambert & Wiens, 2013](#_ENREF_25)) or from variation in temperature range during the active season of these lizard species.

**Materials and methods**

***Data collection***

We studied 121 *Liolaemus* species spanning the entire supra-species phylogenetic, ecological and geographic diversity known for this clade. We collected data for reproductive modes, and latitudinal and elevational distributions (using Global Positioning System technology, GPS). Reproductive mode data were treated as a binary trait consisting of oviparity for egg-laying species, and viviparity for live-bearing species ([Shine, 2005](#_ENREF_52); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). These data come from multiple sources, including publications where reproductive modes are reported ([Schulte *et al.*, 2000](#_ENREF_48); [Pincheira-Donoso & Núñez, 2005](#_ENREF_36); [Pincheira-Donoso *et al.*, 2008a](#_ENREF_40); [Pincheira-Donoso *et al.*, 2013a; Supplementary Table S1](#_ENREF_43)), and from direct observation of 4,500+ female specimens in the field or from museums ([Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). All species for which no observational data on parity modes are available (e.g., species for which parity mode has only been speculated) were excluded from the analyses. These data have been summarized in two previous studies ([Pincheira-Donoso *et al.*, 2008a](#_ENREF_40); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)), where complete details of the published sources per species were presented.

***Environmental predictors***

To investigate the relationships between environmental (ecological, climatic) conditions and oviparity-viviparity transitions, we first extracted fine-scale resolution data on multiple alternative measures of environmental temperature ([Hodges, 2004](#_ENREF_23); [Shine, 2005](#_ENREF_52); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)) and other climatic factors from the Worldclim (www.worldclim.org) database ([Hijmans *et al.*, 2005](#_ENREF_21)), based on a dataset of 8,169+ locality points covering all *Liolaemus* species in the phylogeny. To create a species-level dataset, we extracted data from every locality point per taxon (after removing duplicates to avoid sampling-bias), and calculated an average value per variable per species (see Supplementary Material 1; this table shows the centroid latitude and longitude for the geographic range of each species). Climatic variables include annual mean temperature (all temperature measures are in °C), diurnal thermal range (mean of monthly maximum temp - minimum temperatures), mean temperature in the warmest and in the coldest quarter of the year separately, thermal seasonality (calculated as the standard deviation of the annual mean temperature x 100), isothermality ([Mean Diurnal Range/Temperature Annual Range]x100), and annual precipitation (in mm per year). We also added mean ‘activity season’ (or warm season) temperatures by averaging mean temperatures between October and April, and the thermal range for this period (minimum temperature of the coldest month from the maximum temperature of the warmest month for each individual species), which is the main activity season for *Liolaemus* lizards ([Pincheira-Donoso & Meiri, 2013](#_ENREF_38)). All variables were obtained with a spatial resolution of 30 seconds (0.86 km at the equator). These bioclimatic layers are the result of collections of monthly measurements of multiple bioclimatic variables conducted between 1950 and 2000 by a large number of scattered weather stations around the world ([Hijmans *et al.*, 2005](#_ENREF_21)). To this climatic dataset we added levels of UV-B radiation, known to cause damage to developing eggs in other ectotherms ([Blaustein *et al.*, 1994](#_ENREF_5); [Dethlefsen *et al.*, 2014](#_ENREF_15)), and Normalized Difference Vegetation Index (NDVI) as an indicator of selection emerging from substrate aridity, obtained from Nasa Earth Observations (NEO, available at: http://neo.sci.gsfc.nasa.gov) with the same spatial resolution (Supplementary Material 1). Finally, we estimated air pressure to subsequently calculate the concentrations of available atmospheric oxygen throughout the distributional range of *Liolaemus* species, for which air pressure is calculated with the hypsometric equation P = P0 x (1 – 0.0065 x *h*/T + 0.0065 x *h* + 273.15)–5.257 ([Seinfeld & Pandis, 2016](#_ENREF_49)), where P is atmospheric pressure (in hPa), P0 represents atmospheric pressure at sea-level (1013.25 hPa or 1atm), *h* indicates the altitude above sea level (in metres), and T indicates environmental temperature (in °C). We used this atmospheric pressure (P) values to obtain a measure of the proportion of available atmospheric oxygen relative to the baseline 100% availability of oxygen at sea level with the formula: Oxygen = (P/P0)x100 ([Seinfeld & Pandis, 2016](#_ENREF_49)). These data were then assigned to each *Liolaemus* species in our dataset, and all extractions of variables and their visualization on climatic maps (Fig. 1) were performed using ArcGIS software version 10.0 ([www.esri.com](http://www.esri.com)).

***Phylogeny and divergence time analysis***

We created a phylogenetic tree for 160 *Liolaemus* species (the genus’ largest phylogeny to date; Supplementary Material 4), and three species of the sister genus *Phymaturus* (*P. indistinctus, P. patagonicus* and *P. vociferator*) as outgroups. The concatenated matrix was performed with four mitochondrial gene sequences for each individual (*Cytochrome b*, 12S ribosomal RNA, NADH dehydrogenase subunit 2 [ND2], NADH dehydrogenase subunit 4 [ND4]), obtained from the GenBank database (Supplementary Material 2). The alignment of the genetic data was performed in BioEdit version 7.0 ([Hall, 1999](#_ENREF_20)) and subsequently confirmed with GeneDoc ([Nicholas *et al.*, 1997](#_ENREF_30)). Only three gene sequences (Cytb, ND2 and ND4) were translated into amino-acids to check for premature stop codons or other nonsense mutations, which would have indicated the amplification of nuclear mitochondrial translocations ([Triant & De Woody, 2007](#_ENREF_57)). Best-fit models of evolution were estimated for each dataset using MrModeltest ([Nylander, 2004](#_ENREF_31)) and were chosen based on an Akaike Information Criterion (AIC) approach. The selected model was GTR+I+Γ for all the genes. Analyses were run for 60 million generations, with samples retained every 1,000 generations. Results were displayed in Tracer to confirm acceptable mixing and likelihood stationarity of the Markov Chain Monte Carlo (MCMC) analyses, appropriate burn-in, and adequate effective sample sizes (>200) for each estimated parameter. We summarized parameter values of the samples from the posterior probability on the maximum clade credibility tree using TreeAnnotator v1.8.0, with the posterior probability limit set to 0.5, and mean node heights summarized.

We estimated divergence dates using a Bayesian relaxed molecular clock method with uncorrelated lognormal rates among branches ([Drummond *et al.*, 2006](#_ENREF_17)), assuming a Yule tree prior to the speciation model as implemented in Beast v.1.8.0 ([Bouckaert *et al.*, 2014](#_ENREF_7)). To time-calibrate the tree, we constrained the nodes based on data from the *Liolaemus* fossil record ([Albino, 2008](#_ENREF_2)) with lognormal distributions to estimate divergence dates throughout the phylogeny. Based on this evidence, for the tree’s calibration point, we set the origin of the *Liolaemus* crown group radiation (beginning with the latest common ancestry between the subgenera *Eulaemus* and *Liolaemus* *sensu stricto*) at 19.25 million years ago (Mya) ([Pincheira-Donoso *et al.*, 2015](#_ENREF_42)). This time estimate represents the average between paleontological and molecular estimates, which places the origin of the crown group radiation, respectively, at 18.5 and 20 Mya.

For the analysis we used a lognormal prior for the treeModel.root Height parameter, and the following additional constraints ([Breitman *et al.*, 2012](#_ENREF_9)): the stem of *Eulaemus* origin was constrained with a zero offset (hard upper bound) of 18.5 million years ago (Ma), a lognormal mean of 1.0, and a lognormal standard deviation of 1.5. This produced a median age centered at 21.22 Ma and a 95% prior credible interval (PCI) at 50.55 Ma.

***Quantitative analyses***

To test the hypothesis that oviparity-to-viviparity transitions have evolved in association with species’ occupations of low-oxygen, low-temperature and/or thermally variable environments, we conducted phylogenetic regressions of parity mode (as a binary response) against a set of environmental drivers (Supplementary Table 1). We also included decimal latitude in our models to absorb any influence of North-South radiation of the *Liolaemus* lineage. We then performed phylogenetically-controlled generalized linear models using the *phyloglm* function of the package ‘phylolm’ ([Ho *et al.*, 2015](#_ENREF_22)) in R ([R Development Core Team, 2016](#_ENREF_46)). This function replicates the phylogenetic logistic regression algorithm of Ives & Garland ([2010](#_ENREF_24)). To conduct these analyses, parity mode was modelled as a binary response variable with logit link function. Contribution of phylogenetic signal was determined by iterative fitting of the phylogenetic distance matrix against the residual covariance matrix, yielding a phylogenetic correlation *α*. Greater phylogenetic correlation in the transition between parity modes is signaled by *α*-values closer to zero. We determined the importance and information content of environmental predictors of parity mode by comparing multiple regression models according to their AIC values and the significance of Wald-like Chi-square tests of the predictors’ slopes. With 14 environmental drivers to be analysed (Supplementary Material 1), a full dredge of all model structures was not possible (there exist 214 = 16,384 possible combinations of main effects), and indeed, it is not recommended due to inflation of Type I error rates ([Burnham & Anderson, 2002](#_ENREF_10)). Instead, we used a hybrid model-building approach ([Davies *et al.*, 2011](#_ENREF_13)). We ranked the AIC values of all fourteen single-predictor regressions. We found that Elevation of the species’ range centroid was the most informative predictor of parity mode. We chose this simple regression model as our baseline model, but we recognize that elevation is not a direct agent of selection – instead, elevation acts as a proxy for correlated environmental drivers (i.e., actual selection pressures) that influence the relative success of oviparous and viviparous strategies under a given set of environmental factors via their effects on fitness. Therefore, we aimed to identify which environmental drivers best explained the influence of elevation on parity mode. We chose the most informative of the single predictors, and built two-predictor regression models based on each of them. We retained interest in models if they lay within six AIC units of the most informative model ([Burnham *et al.*, 2011](#_ENREF_11)), contained only significant predictors, and did not contain elevation as a predictor. We continued to build multiple regression models with this approach until all new models failed to join the top model set because they either had AIC > six units higher than the baseline model, or contained non-significant predictors. This approach risks missing a “best” model if one exists that contains many predictors, however, we favour our approach since it reduces the risk of false significance caused by model dredging.

**Results**

*Liolaemus* species are distributed across most latitudes of South America, and persist across a wide range of elevations (Fig. 1). Visual assessment suggests that viviparous species are found predominantly in southerly latitudes and at high elevations, and hence, at cold-climates regardless of levels of oxygen. At extreme high elevations and latitudes, no oviparous species exist (Fig. 1). The *Liolaemus* phylogeny (Fig. 1; Supplementary Material 5) confirms that parity mode has switched from oviparity to viviparity on several independent evolutionary events during the radiation of these lizards. Importantly, however, while multiple independent oviparity-viviparity transitions followed invasions of higher Andean elevations, the large diversity of viviparous species at high latitudes (Patagonia) is likely to be the result of *in situ* diversification of two ancestrally viviparous clades whose species retained viviparity (Fig. 1; also, figure in Supplementary Material 5).

Our logistic regression analyses with parity mode as binary response produced a range of models ranked according to AIC values (Table 1; Supplementary Material 2, for additional models). These analyses show that the most informative predictor of parity mode is elevation itself (Table 1). Only four regression models fully satisfied our full set of criteria that they should lie within six AIC units of the elevation model, should contain only significant predictors, and should not contain elevation as a predictor (Table 1; Fig. 2). Oxygen concentration was a significant contributor to all three models below the preferred oxygen-only based model (Table 1), and consistently predicted an increase in the probability of viviparity in environments with decreasing oxygen concentrations (Fig. 2). Mean diurnal thermal range contributed to two of the rival models, and consistently predicted an increase in the probability of viviparity with decreasing daily range in temperature, over and above any influence of oxygen concentration (Table 1). Mean temperature of the coldest quarter of the year contributed significantly to one model in the candidate set, predicting an increase in the probability of viviparity for species occupying ranges with colder winters. Latitude contributed significantly (and replaced measures of environmental temperature) in the least-preferred candidate model, predicting an increase in the probability of viviparity as species’ ranges move further south of the equator.

Phylogenetic correlation in parity mode was strong in all the candidate models, as expected based on the distribution of viviparity across the phylogeny (Fig. 1; see also Supplementary Material 5). The *α*-parameter estimated by phylogenetic logistic regression lay between 0.022 and 0.031 across candidate models, with bootstrapped confidence intervals spanned by 0.0008 and 2.59. This is suggestive of very strong phylogenetic correlation, but relatively high levels of uncertainty suggesting that more species, or more parity transitions, would help to clarify predictions of phylogenetic signal. It is worth noting that strong phylogenetic signal reduces power to detect environmental predictors of trait values, and hence, the conclusions that derive from these findings are robust and credible.

**Discussion**

Our study provides the first empirical investigation of the hypothesis that natural selection for evolution of reptilian viviparity arises from declines in oxygen availability, rivalling the traditional ‘cold-climate’ hypothesis ([Hodges, 2004](#_ENREF_23); [Shine, 2005](#_ENREF_52); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). As predicted by theory, our analyses suggest that oxygen-deprived environments play a primary role in increasing the likelihood of transitions from oviparity to viviparity, while cold environmental temperatures (especially the coldest winter temperatures) remain identified as an important driver. Interestingly, our study also identified ‘homeostasis’ as a potential third source of selection: viviparity is promoted by environments where daily thermal fluctuations are narrower – this observation conflicts with the hypothesis that selection for embryo retention emerges not only from low, but also from fluctuating temperatures ([Shine, 2004](#_ENREF_51), [2005](#_ENREF_52)). Consequently, our findings support both the ‘hypoxia’ and ‘hypothermia’ hypotheses at the macroevolutionary level. Further investigation of the role of fluctuating environmental temperatures, is warranted.

***Selection from oxygen, temperature, or both?***

Evidence has historically shown that reptile viviparity is predominantly associated with cold-climates regardless of elevation ([Schulte *et al.*, 2000](#_ENREF_48); [Shine, 2005](#_ENREF_52); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43); [Pincheira-Donoso & Hunt, 2017; but see Lambert & Wiens, 2013, below](#_ENREF_39)). This strong link between viviparity and low-temperatures poses the major challenge to the hypoxia hypothesis. Fundamentally, for the hypoxia hypothesis to emerge as a theoretically-sound alternative, a link between spatial gradients of oxygen and temperature should be validated (i.e., selection for viviparity arises from oxygen, but has been confounded by temperature). In fact, a strong correlation between declines in both temperature and oxygen is broadly known to hold along elevations ([Nagy & Grabherr, 2009](#_ENREF_29)). However, given that oxygen declines with decreasing atmospheric pressure ([Nagy & Grabherr, 2009](#_ENREF_29)), it follows that oxygen availability only declines with elevation but not with latitude. In contrast, environmental temperatures decrease with both increasing elevation and latitude ([Bonan, 2008](#_ENREF_6); [Nagy & Grabherr, 2009; see Fig. 1](#_ENREF_29)), which strongly matches the macroevolutionary patterns of viviparity in reptiles in general ([Shine, 2005](#_ENREF_52)), including *Liolaemus* ([Schulte *et al.*, 2000](#_ENREF_48); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)).

Although our findings suggest the role of cold-climates in promoting and maintaining viviparity, selection from reduced oxygen availability emerges as a primary agent driving this parity mode. Therefore, how can this low-oxygen effect be reconciled with the observation that viviparity still increases with latitudes, and in the absence of oxygen declines? Interestingly, although viviparity in *Liolaemus* increases with increasing latitude ([Schulte *et al.*, 2000](#_ENREF_48); [Pincheira-Donoso, 2011](#_ENREF_35); [Pincheira-Donoso *et al.*, 2013a; our results](#_ENREF_43)), our results revealed that the role of latitude in explaining viviparity is marginal, and dependent on decreasing oxygen concentrations creating selection for embryo retention. In fact, the high frequency of viviparous *Liolaemus* in high, cold latitudes (Patagonia) is the result of only two ancestrally viviparous species that are likely to have invaded these climates and only subsequently proliferated across these environments – therefore, the predominantly viviparous species in Patagonia seem to have retained the ancestral parity mode as they diversified within this biome (Fig. 1; Supplementary Material 5). Yet, importantly, this scenario does not contradict a role for low temperatures as promoters of viviparity, given that the success of these clades and their consistent retention of live-bearing parity ([Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)) suggests the advantage of viviparous reproduction under these conditions of thermal selection on life history traits ([e.g., see Shine, 2015](#_ENREF_53)). However, whether this scenario is the norm or the exception for reptiles across the tree of life remains an open question.

At a more conceptual level, we suggest two alternative possibilities. First, transitions to viviparity are unlikely to be driven by a single factor (e.g., either temperature or oxygen). Instead, a combination of factors may simultaneously play strong and independent roles as sources of selection. That is, as our results suggest, the combined effects of oxygen and declining temperatures may promote viviparity along elevational gradients, while transitions to viviparity may also evolve in response to the individual effect of temperature along latitudinal gradients. Therefore, because this scenario suggests that fitness is enhanced by egg retention in the mother’s body, the exposure of a species to either, or multiple, selective pressures will impose selection for viviparity. Second, in the absence of selection arising from low oxygen, temperature may in fact play a predominant role. This alternative is supported by observations on oviparous species inhabiting cold-climates (including high elevations). In fact, cold-climate oviparous species have consistently been observed to adapt in the way eggs deal with low temperatures, whereas no links to selection arising from reduced oxygen appear similarly obvious. Three such adaptations that replace the benefits of a thermally viable maternal body in cold-climates can support this scenario: (*i*) many cold-climate oviparous species select exceptionally warm nesting sites, in which embryo development can be rapidly completed within a single, short, warm season ([Shine & Harlow, 1996](#_ENREF_54); [Shine, 1999](#_ENREF_50); [Shine *et al.*, 2002](#_ENREF_55)). This phenomenon relies on increasing incubating temperatures to accelerate embryogenesis, and hence, is directly linked to the cold-climate model ([Shine, 1999](#_ENREF_50)); (*ii*) other cold-climate oviparous adapt their physiology via adjustments of their thermal tolerances and rates of egg development, which replace strategies that *avoid* low temperatures via modification of developing environments, such as viviparity ([Shine, 1999](#_ENREF_50)). Some cold-climate reptiles, for example, have evolved significantly shorter incubation periods than their warm-climate conspecifics ([Olsson *et al.*, 1996](#_ENREF_32); [Shine, 1999](#_ENREF_50), [2005](#_ENREF_52)). Likewise, the physiological ability to retard freezing has been observed in species that overwinter in the nest ([Cagle *et al.*, 1993](#_ENREF_12)); finally (*iii*) cooperative nesting strategies, such as (intraspecific or interspecific) communal nesting, may improve the thermal conditions experienced by developing eggs in cold-climates ([Doody *et al.*, 2009](#_ENREF_16); [Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). For example, it has often been found that nest temperature is higher in communal than in solitary nests, especially towards their centre ([Hakansson & Loman, 2004](#_ENREF_19); [Doody *et al.*, 2009](#_ENREF_16)). Also, this phenomenon has been regarded as an adaptation to prevent freezing and to accelerate incubation times in cold climates ([Waldman & Ryan, 1983](#_ENREF_58); [Doody *et al.*, 2009](#_ENREF_16)). Likewise, the only oviparous *Liolaemus* species known to inhabit an extreme high elevation (2800-3300m) nests communally ([Pincheira-Donoso *et al.*, 2013a](#_ENREF_43)). The same strategy has been observed in other oviparous lizards from cold-climates that exhibit other adaptations to deal with low-incubation temperatures ([Shine, 1999](#_ENREF_50)). Regarding oxygen, interestingly, it has been observed that embryonic development can be compromised in larger reptile clutches given that oxygen concentrations decline and CO2 increases towards the centre of the nest ([Ackerman & Lott, 2004](#_ENREF_1)). Therefore, this effect entirely opposes the thermal advantages of a nest hosting more eggs, thus selecting against communal nesting. Yet, given that temperature is a major selective agent, the thermal benefits of nesting communally may outweigh the detrimental effects of oxygen on fitness.

**Conclusion**

Our study reveals the multivariate nature of selection promoting the evolution of reptilian viviparity. Our analyses combining a multitude of environmental sources of selection support the ‘hypoxia’ hypothesis that oxygen-deprivation favours prolonged embryo retention that leads to viviparous reproduction, adding a substantial conceptual layer to the longstanding ‘cold-climate’ hypothesis that oviparity-viviparity transitions are driven by decreasing temperatures. Interestingly, our phylogenetic analyses also reveal a role for reduced daily fluctuations in temperatures as a potential promoter of viviparity – this finding opens the possibility of a ‘homeostasis’ hypothesis that warrants empirical investigation. Finally, the comparative, macroecological nature of our study provides empirical evidence compatible with the mechanistic basis of the tested theories. Yet, the risk of some unmeasured environmental variables that could spatially correlate with oxygen concentrations (and which could create selection by preventing completion of embryo development in eggs) exists, which could also contribute to the multivariate selection for viviparity.

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**Biosketch**

Daniel Pincheira-Donoso is a Senior Lecturer in Evolutionary Biology. His research investigates the role of selection as a driver of adaptive diversity, with a primary focus on the interplay between the emergence of adaptive traits and their impact on global patterns of diversity.

**Data Accessibility**

All data used in this study are fully available in the Supplementary Material 1.

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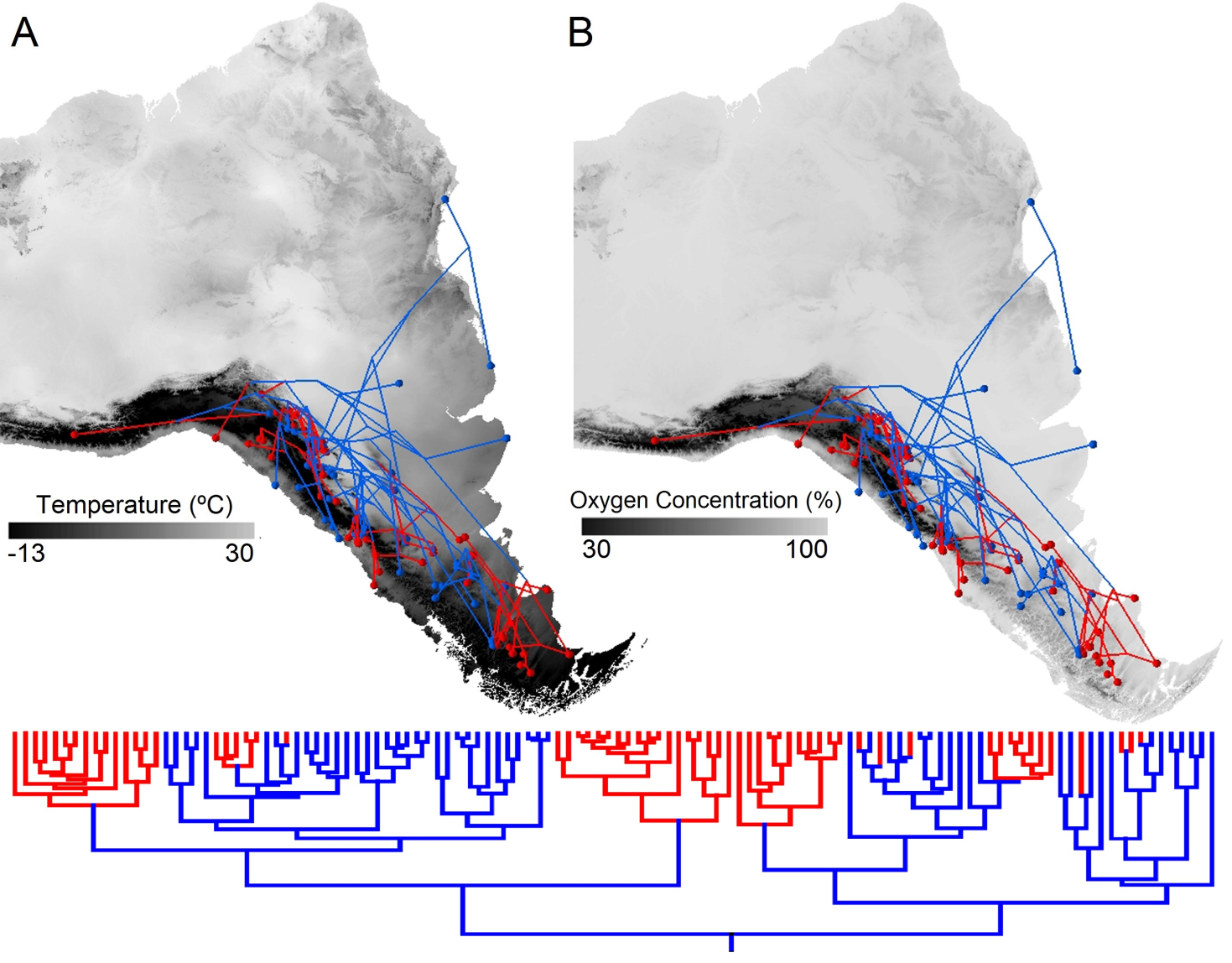
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**Table 1.** Ranking of best candidate phylogenetic models for the evolution of viviparity in *Liolaemus* lizards above the ΔAIC = 6 threshold. Analyses identified elevation and concentration of oxygen in the atmosphere (%O2) as the dominant factors driving oviparity-viviparity transitions. The other factors found to have minor significant effects are latitude (Latit, in degrees South), mean diurnal thermal range (DRTe, in °C), and mean temperature in coldest quarter of the year (MTeCQ, in °C). See Supplementary Material 2 for a range of additional models, including secondary significant models below the ΔAIC = 6 threshold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** | **Term** | ***α*** | **Bootstrap *α*** | **Slope** | **AIC** | **ΔAIC** | ***P*** |
| Elevation  %O2  %O2+ DRTe + MTeCQ  %O2+ DRTe  %O2+Latitude | Elevation  %O2  MTeCQ  DRTe  Latitude | 0.02  0.03  0.03  0.03  0.02 | 2.57  2.58  2.59  2.58  2.59 | 0.94  -0.91  -0.89  -0.57  -0.68 | 76.16  78.78  79.13  80.67  81.29 | 0  2.62  2.97  4.51  5.14 | <0.001  <0.001  0.002  0.035  0.044 |

**Figure 1.** Phylogenetic frequency and distribution of oviparous (blue) and viviparous (red) *Liolaemus* species on the genus phylogenetic tree (botton) and on climatic maps showing spatial gradients of annual temperatures (A) and oxygen concentrations (B).



**Figure 2.** Transitions in parity mode (0 = oviparous, 1 = viviparous) within the *Liolaemus* genus as functions of environmental predictors scaled to mean zero and standard deviation one. Each plot includes line of best-fit calculated using phylogenetic logistic regression. Plots (a) to (c) include fitted lines calculated from a model containing all three predictors: % Oxygen, mean diurnal range in temperature, and mean temperature of the coldest quarter of the year. Each fitted line is plotted against a single predictor based on the average value of the other two predictors. Plot (d) fits the impact of latitude given a mean value of % Oxygen.

