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RESEARCH PAPER

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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 new 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 use a multivariate dataset covering 121 species varying extensively in geographical 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 were almost perfectly correlated, and both were identified as the dominant predictors of oviparity-to-viviparity transitions, whereas the role for temperature (dominated by the coldest winter temperatures and daily fluctuations) was significant but secondary. Overall, we showed 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 contributes to the evolution of these transitions by creating multivariate selection on parity mode.

KEYWORDS
homeostasis, hypoxia, life-history evolution, *Liolaemus*, macroecology, reproductive modes, squamates, viviparity

1 | INTRODUCTION

Adaptive radiations are triggered by exploitation of ecological opportunity offered by access to new environments following the evolution of key adaptive innovations (Losos, 2010; Schluter, 2000). 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). Among animals, squamate reptiles (lizards and snakes) have given rise to some of the most exceptional examples of prolific evolution (Losos, 2009; Pincheira-Donoso, Bauer, Meiri, & Uetz, 2013). 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 in the global diversification of these vertebrates (Losos, 2009; Sites, Reeder, & Wiens, 2011).

The evolution of viviparity (live-bearing reproduction) has increasingly been recognized as a key innovation that allow squamates to access cold climates (Shine, 2005; Sites et al., 2011; Pincheira-Donoso, Tregenza, Witt, & Hodgson, 2013). 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, 2005). 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, Tregenza, et al., 2013). However, although this prediction has been supported by comparative evidence (Hodges, 2004; Shine, 2005; Pincheira-Donoso, Tregenza, et al., 2013; Pyron & Burbrink, 2014), this 'cold-climate' or 'hypothermia' hypothesis ignores the multiple other climatic factors that vary geographically and covary with thermal gradients (Lambert & Wiens, 2013; Feldman et al., 2015). 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; Lambert & Wiens, 2013). 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; Lambert & Wiens, 2013). Numerous experimental studies on reptiles (Andrews, 2002; Deeming, 2004; Warburton, Hastings, & Wang, 1995) and birds (Black & Snyder, 1980; McCutcheon, Metcalfe, Metzenberg, & Ettinger, 1982) have consistently shown that low 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; Parker, Andrews, & Mathies, 2004). 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; Parker & Andrews, 2006; Parker et al., 2004). 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 geographical architecture and biodiversity of South America offer the ideal scenario to interrogate the hypotheses of oxygen and temperature as alternative (or interacting) sources of natural selection for the evolution of viviparity. 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 and Chile (from *c.* 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 260+ species of this lineage have colonized an unusually wide variety of environmental extremes (Pincheira-Donoso, Hodgson, & Tregenza, 2008), 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 (Breitman, Avila, Sites, & Morando, 2011; Pincheira-Donoso, Harvey, & Ruta, 2015; Pincheira-Donoso, Hodgson, et al., 2008; Pincheira-Donoso & Tregenza, 2011; Pincheira-Donoso, Tregenza, & Hodgson, 2007; Schulte, Macey, Espinoza, & Larson, 2000). 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 (Pincheira-Donoso, Tregenza, et al., 2013; Schulte et al., 2000). 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 only declines in oxygen concentration, but also changes in the magnitude of temperature fluctuations. Our own previous analyses (Pincheira-Donoso, Bauer, et al., 2013) 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; Lambert & Wiens, 2013) or from variation in the temperature range during the active season of these lizard species.

2 | MATERIALS AND METHODS

2.1 | Data collection

We studied 121 *Liolaemus* species spanning the entire supraspecies phylogenetic, ecological and geographical diversity known for this clade. We collected data for reproductive modes and for latitudinal and elevational distributions [using Global Positioning System (GPS)

technology). Reproductive mode data were treated as a binary trait consisting of oviparity for egg-laying species and viviparity for live-bearing species (Pincheira-Donoso, Tregenza, et al., 2013; Shine, 2005). These data come from multiple sources, including publications where reproductive modes are reported (Pincheira-Donoso & Núñez, 2005; Pincheira-Donoso, Scolaro, & Sura, 2008; Pincheira-Donoso, Tregenza, et al., 2013; Schulte et al., 2000; Supporting Information Table S1), and from direct observation of 4,500+ female specimens in the field or from museums (Pincheira-Donoso, Tregenza, et al., 2013). 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, Scolaro, et al., 2008; Pincheira-Donoso, Tregenza, et al., 2013), where complete details of the published sources per species were presented.

2.2 | Environmental predictors

To investigate the relationships between environmental (ecological and climatic) conditions and oviparity-to-viviparity transitions, we first extracted fine-scale-resolution data on multiple alternative measures of environmental temperature (Hodges, 2004; Pincheira-Donoso, Tregenza, et al., 2013; Shine, 2005) and other climatic factors from the Worldclim (www.worldclim.org) database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005), 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 Supporting Information Material 1; this table shows the centroid latitude and longitude for the geographical range of each species) Supporting Information Material 2. Climatic variables include annual mean temperature (all temperature measures are in degrees Celsius), diurnal thermal range (mean of monthly maximal temperature minus minimal temperature), mean temperature in the warmest and in the coldest quarter of the year separately, thermal seasonality (calculated as the *SD* of the annual mean temperature $\times$ 100), isothermality [(mean diurnal range/temperature annual range) $\times$ 100] and annual precipitation (in millimetres 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 (minimal temperature of the coldest month from the maximal temperature of the warmest month for each individual species), which is the main activity season for *Liolaemus* lizards (Pincheira-Donoso & Meiri, 2013). 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). To this climatic dataset we added levels of ultraviolet-B radiation, known to cause damage to developing eggs in other ectotherms (Blaustein et al., 1994; Dethlefsen, von Westernhagen, Tüg, Hansen, & Dizer, 2014), and the 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 (Supporting Information Material 1). Finally, we estimated air pressure in order subsequently to calculate the concentrations of available atmospheric oxygen throughout the distributional range of *Liolaemus* species, for which air pressure is calculated with the hypsometric equation, $P = P_0 \times (1 - 0.0065 \times h/T + 0.0065 \times h + 273.15)^{-5.257}$ (Seinfeld & Pandis, 2016), where P is atmospheric pressure (in hectopascals), P_0 represents atmospheric pressure at sea level (1013.25 hPa or 1 atm), h indicates the altitude above sea level (in metres), and T indicates environmental temperature (in degrees Celsius). We used these 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 following formula: Oxygen $= (P/P_0) \times 100$ (Seinfeld & Pandis, 2016). These data were then assigned to each *Liolaemus* species in our dataset, and all extractions of variables and their visualization on climatic maps (Figure 1) were performed using ArcGIS software version 10.0 (www.esri.com).

2.3 | Phylogeny and divergence time analysis

We created a phylogenetic tree for 160 *Liolaemus* species (the genus' largest phylogeny to date; Supporting Information Material 3,4), 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) and NADH dehydrogenase subunit 4 (ND4)], obtained from the GenBank database (Supporting Information Material 2). The alignment of the genetic data was performed in BioEdit version 7.0 (Hall, 1999) and subsequently confirmed with GeneDoc (Nicholas, Nicholas, & Deerfield, 1997). 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). Best-fit models of evolution were estimated for each dataset using MrModeltest (Nylander, 2004) and were chosen based on an Akaike information criterion (AIC) approach. The selected model was General Time Reversible-Invariant $+ I + \Gamma$ for all the genes. Analyses were run for 60 million generations, with samples retained every 1,000 generations. Results were displayed in Tracer (Available at http://beast.bio.ed.ac.uk/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, (Available at http://beast.bio.ed.ac.uk) 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, Ho, Phillips, & Rambaut, 2006), assuming a Yule tree before the speciation model as implemented in Beast v.1.8.0 (Bouckaert et al., 2014). To time calibrate the tree, we constrained the nodes

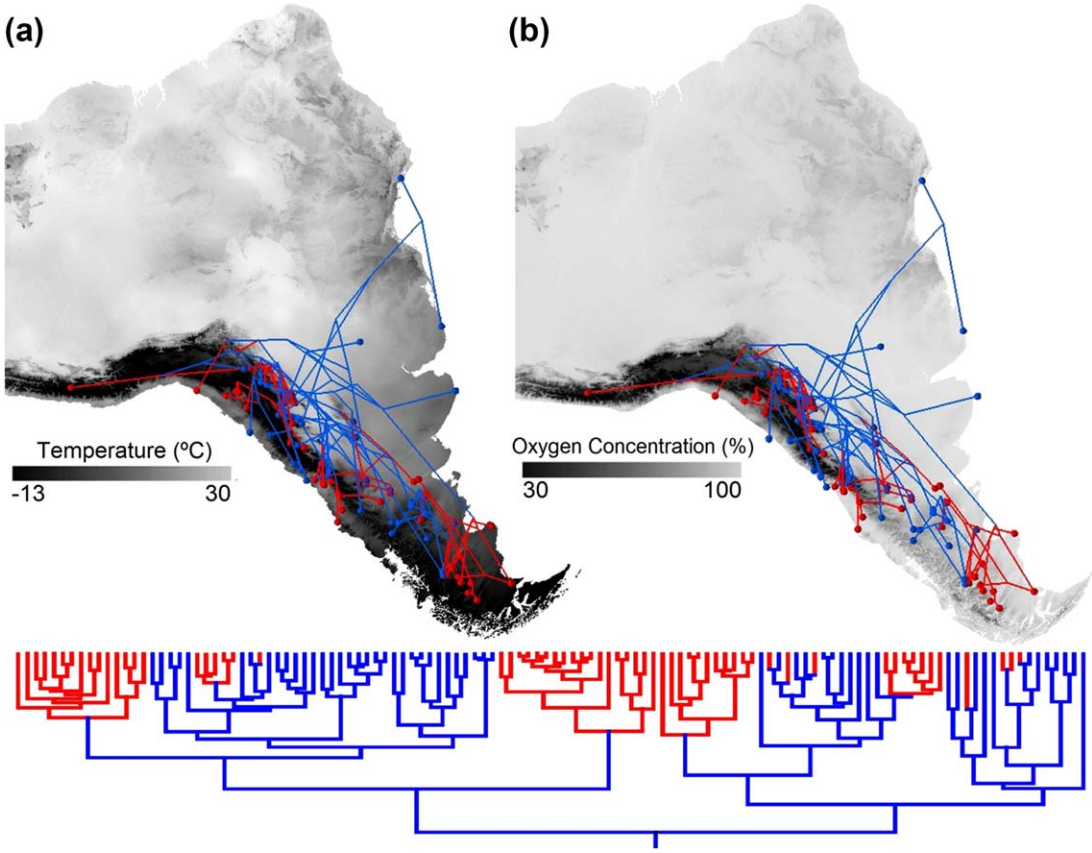

FIGURE 1 Phylogenetic frequency and distribution of oviparous (blue) and viviparous (red) *Liolaemus* species on the genus phylogenetic tree (bottom) and on climatic maps showing spatial gradients of (a) annual temperatures and (b) oxygen concentrations

based on data from the *Liolaemus* fossil record (Albino, 2008) 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 (Ma) (Pincheira-Donoso et al., 2015). 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 Ma.

For the analysis we used a lognormal prior for the treeModel.root Height parameter, and the following additional constraints (Breitman, Avila, Sites, & Morando, 2012): the stem of *Eulaemus* origin was constrained with a zero offset (hard upper bound) of 18.5 Ma, a lognormal mean of 1.0, and a lognormal *SD* of 1.5. This produced a median age centred at 21.22 Ma and a 95% prior credible interval (PCI) at 50.55 Ma.

2.4 | 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 (Supporting Information Table S1). 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, Ane, Lachlan, Tarpinian, & Ho, 2015) in R (R Development Core Team, 2016). This function replicates the phylogenetic logistic regression algorithm of Ives and Garland (2010). To conduct these analyses, parity mode was modelled as a binary response variable with logit link function. The contribution of the 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 signalled 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 χ^2 tests of the predictors' slopes.

With 14 environmental drivers to be analysed (Supporting Information Material 1), a full dredge of all model structures was not possible (there exist $2^{14} = 16{,}384$ possible combinations of main effects), and indeed, it is not recommended because of inflation of type I error rates (Burnham & Anderson, 2002). Instead, we used a hybrid model-building approach (Davies et al., 2011). We ranked the AIC values of all 14 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

TABLE 1 Ranking of best candidate phylogenetic models for the evolution of viviparity in *Liolaemus* lizards above the ΔAIC = 6 threshold

Model	Term	α	Bootstrap α	Slope	AIC	ΔAIC	*p-value*
Elevation	Elevation	.02	2.57	0.94	76.16	0	<.001
$\%O_2$	$\%O_2$	.03	2.58	−0.91	78.78	2.62	<.001
$\%O_2$ + DRTe + MTeCQ	MTeCQ	.03	2.59	−0.89	79.13	2.97	.002
$\%O_2$ + DRTe	DRTe	.03	2.58	−0.57	80.67	4.51	.035
$\%O_2$ +Latitude	Latitude	.02	2.59	−0.68	81.29	5.14	.044

Note. The other factors found to have minor significant effects are mean temperature in the coldest quarter of the year (MTeCQ, in degrees Celsius) mean diurnal thermal range (DRTe, in degrees Celsius) and latitude (in degrees South). Analyses identified elevation and concentration of oxygen in the atmosphere ($\%O_2$) as the dominant factors driving oviparity-to-viviparity transitions. See Supplementary Material 2 for a range of additional models, including secondary significant models below the change in Akaike information criterion (ΔAIC) = 6 threshold.

recognize that elevation is not a direct agent of selection; instead, elevation acts as a proxy for correlated environmental drivers (i.e., real selection pressures) that influence the relative success of oviparous and viviparous strategies with 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, Anderson, & Huyvaert, 2011), 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 because it reduces the risk of false significance caused by model dredging.

3 | RESULTS

Liolaemus species are distributed across most latitudes of South America and persist across a wide range of elevations (Figure 1). Visual assessment suggests that viviparous species are found predominantly in southerly latitudes and at high elevations; hence, in cold climates regardless of levels of oxygen. At extremely high elevations and latitudes, no oviparous species exist (Figure 1). The *Liolaemus* phylogeny (Figure 1; Supporting Information 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, although multiple independent oviparity-to-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 (Figure 1; also, figure in Supporting Information Material 5).

Our logistic regression analyses with parity mode as binary response produced a range of models ranked according to AIC values (Table 1; Supporting Information 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 (Figure 2; Table 1). 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 (Figure 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). The 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.

The phylogenetic correlation in parity mode was strong in all the candidate models, as expected based on the distribution of viviparity across the phylogeny (Figure 1; see also Supporting Information Material 5). The parameter α 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 a strong phylogenetic signal reduces the power to detect environmental predictors of trait values; hence, the conclusions that derive from these findings are robust and credible.

4 | 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; Pincheira-Donoso, Tregenza, et al., 2013; Shine, 2005). 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

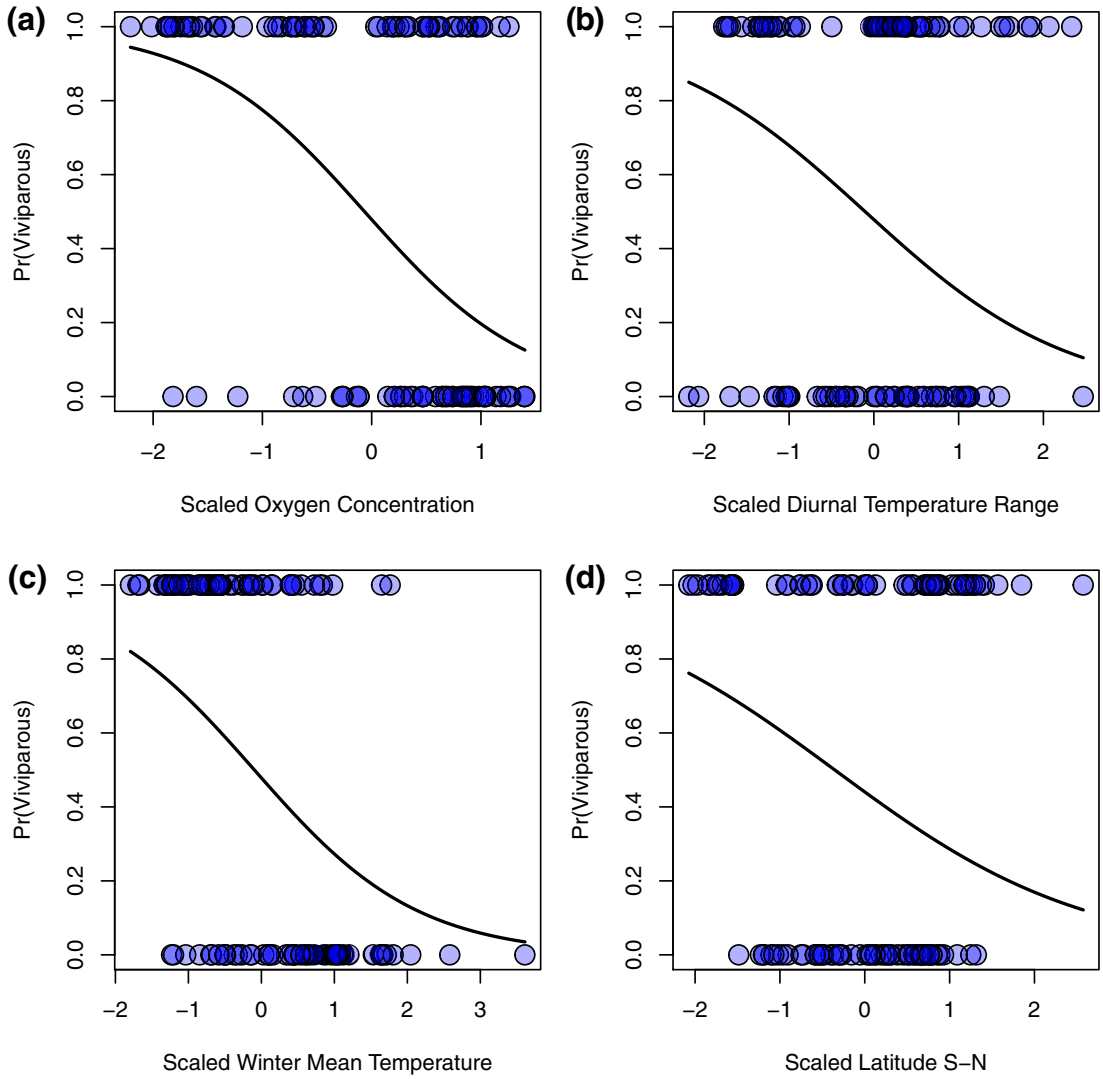

FIGURE 2 Transitions in parity mode (0 = oviparous, 1 = viviparous) within the *Liolaemus* genus as functions of environmental predictors scaled to mean zero and *SD* one. Each plot includes line of best fit calculated using phylogenetic logistic regression. (a–c) These plots include fitted lines calculated from a model containing all three predictors: percentage of 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. (d) This plot fits the impact of latitude given a mean value of the percentage of oxygen

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, 2005). 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.

4.1 | Selection from oxygen, temperature or both?

Evidence has historically shown that reptile viviparity is predominantly associated with cold climates regardless of elevation (Pincheira-Donoso & Hunt, 2017; Pincheira-Donoso, Tregenza, et al., 2013; but see Lambert & Wiens, 2013; Schulte et al., 2000; Shine, 2005, below). 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). However, given that oxygen declines with decreasing atmospheric pressure (Nagy & Grabherr, 2009), it follows that oxygen availability declines only with elevation, but not with latitude. In contrast, environmental temperatures decrease with both increasing elevation and latitude (Bonan, 2008; Nagy & Grabherr, 2009; see Figure 1), which strongly matches the

macroevolutionary patterns of viviparity in reptiles in general (Shine, 2005), including *Liolaemus* (Pincheira-Donoso, Tregenza, et al., 2013; Schulte et al., 2000).

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 effect of low oxygen 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 (Pincheira-Donoso, 2011; Pincheira-Donoso, Tregenza, et al., 2013; Schulte et al., 2000; our present results), 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 (Figure 1; Supporting Information 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, Tregenza, et al., 2013) suggests the advantage of viviparous reproduction in these conditions of thermal selection on life-history traits (e.g., see Shine, 2015). 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 that 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. First, many cold-climate oviparous species select exceptionally warm nesting sites, in which embryo development can be completed rapidly within a single, short, warm season (Shine, 1999; Shine, Barrott, & Elphick, 2002; Shine & Harlow, 1996). This phenomenon relies on increasing incubating temperatures to accelerate embryogenesis; hence, it is directly linked to the cold-climate model (Shine, 1999). Second, other cold-climate oviparous species adapt their physiology via adjustments of their thermal tolerances and rates of egg development, which replace strategies that avoid low temperatures via modification of developmental environments, such as viviparity (Shine, 1999). Some cold-climate reptiles, for example, have evolved significantly shorter incubation periods than their warm-climate conspecifics (Olsson, Gullberg, Shine, Madsen, & Tegelstrom, 1996; Shine, 1999, 2005). Likewise, the physiological ability to retard freezing has been observed in species that overwinter in the nest (Cagle, Packard, Miller, & Packard, 1993). Third, cooperative nesting strategies, such as (intraspecific or interspecific) communal nesting, may improve the thermal conditions experienced by developing eggs in cold climates (Doody, Freedberg, & Keogh, 2009; Pincheira-Donoso, Tregenza, et al., 2013). For example, it has often been found that nest temperature is higher in communal than in solitary nests, especially towards their centre (Håkansson & Loman, 2004; Doody et al., 2009). Also, this phenomenon has been regarded as an adaptation to prevent freezing and to accelerate incubation times in cold climates (Waldman & Ryan, 1983; Doody et al., 2009). Likewise, the only oviparous *Liolaemus* species known to inhabit an extremely high elevation (2,800–3,300 m) nests communally (Pincheira-Donoso, Tregenza, et al., 2013). 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). Regarding oxygen, interestingly, it has been observed that embryonic development can be compromised in larger reptile clutches given that oxygen concentrations decline and CO_2 increases towards the centre of the nest (Ackerman & Lott, 2004). Therefore, this effect entirely opposes the thermal advantages of a nest hosting more eggs, thus selecting against communal nesting. Nonetheless, given that temperature is a major selective agent, the thermal benefits of nesting communally might outweigh the detrimental effects of oxygen on fitness.

5 | 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 and leads to viviparous reproduction, adding a substantial conceptual layer to the longstanding 'cold-climate' hypothesis that oviparity-to-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. Nonetheless, the risk of some unmeasured environmental variables that could correlate spatially 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.

DATA ACCESSIBILITY

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

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REFERENCES

Ackerman, R. A., & Lott, D. B. (2004). Thermal, hydric and respiratory climate of nests. In D.C. Deeming (Ed.), *Reptilian incubation. Environment, evolution and behaviour* (pp. 15–43). Nottingham, U.K.: Nottingham University Press.

Albino, A. M. (2008). Lagartos iguanios del Colhuehuapense (Mioceno Temprano) de Gaiman (Provincia del Chubut, Argentina). *Ameghiniana*, *45*, 775–782.

Andrews, R. M. (2002). Low oxygen: A constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology*, *75*, 145–154.

Black, C. P., & Snyder, G. K. (1980). Oxygen transport in the avian egg at high altitude. *American Zoologist*, *20*, 461–468.

Blaustein, A. R., Hoffman, P. D., Hokit, D. G., Kiesecker, J. F., Walls, S. C., & Hays, J. B. (1994). DNA repair and resistance to solar UV-B in amphibian eggs: A link to population declines? *Proceedings of the National Academy of Sciences USA*, *91*, 1791–1795.

Bonan, G. (2008). *Ecological climatology. Concepts and applications*. Cambridge, U.K.: Cambridge University Press.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., . . . Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*, e1003537.

Breitman, M. F., Avila, L. J., Sites, J. W., & Morando, M. (2011). Lizards from the end of the world: Phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini). *Molecular Phylogenetics and Evolution*, *59*, 364–376.

Breitman, M. F., Avila, L. J., Sites, J. W., & Morando, M. (2012). How lizards survived blizzards: Phylogeography of the *Liolaemus lineomaculatus* group (Liolaemidae) reveals multiple breaks and refugia in southern Patagonia, and their concordance with other co-distributed taxa. *Molecular Ecology*, *21*, 6068–6085.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference. A practical information-theoretic approach*. New York, NY: Springer.

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*, 23–35.

Cagle, K. D., Packard, G. C., Miller, K., & Packard, M. J. (1993). Effect of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Functional Ecology*, *7*, 653–660.

Davies, T. E., Wilson, S., Hazarika, N., Chakrabarty, J., Das, D., Hodgson, D. J., & Zimmermann, A. (2011). Effectiveness of intervention methods against crop-raiding elephants. *Conservation Letters*, *4*, 346–354.

Deeming, D. C. (2004). *Reptilian Incubation. Environment Evolution and Behaviour*. Nottingham, U.K.: Nottingham University Press.

Dethlefsen, V., von Westernhagen, H., Tüg, H., Hansen, P. D., & Dizer, H. (2014). Influence of solar ultraviolet-B on pelagic fish embryos: Osmolality, mortality and viable hatch. *Helgoland Marine Research*, *55*, 45–55.

Doody, J. S., Freedberg, S., & Keogh, J. S. (2009). Communal egg-laying in reptiles and amphibians: Evolutionary patterns and hypotheses. *Quarterly Review of Biology*, *84*, 229–252.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, *4*, e88.

Feldman, A., Bauer, A. M., Castro-Herrera, F., Chirio, L., Das, I., Doan, T. M., . . . Meiri, S. (2015). The geography of snake reproductive mode: A global analysis of the evolution of snake viviparity. *Global Ecology and Biogeography*, *24*, 1433–1442.

Håkansson, P., & Loman, J. (2004). Communal spawning in the common frog *Rana temporaria* – egg temperature and predation consequences. *Ethology*, *110*, 665–680.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978.

Ho, L. S. T., Ane, C., Lachlan, R., Tarpinian, K., & Ho, M. L. S. T. (2015). Package 'phylolm'. Retrieved from http://cran.rproject.org/web/packages/phylolm/index.html

Hodges, W. L. (2004). Evolution of viviparity in horned lizards (*Phrynosoma*): Testing the cold-climate hypothesis. *Journal of Evolutionary Biology*, *17*, 1230–1237.

Ives, A. R., & Garland, T. (2010). Phylogenetic logistic regression for binary dependent variables. *Systematic Biology*, *59*, 9–26.

Lambert, S. M., & Wiens, J. J. (2013). Evolution of viviparity: A phylogenetic test of the cold-climate hypothesis in phrynosomatid lizards. *Evolution*, *67*, 2614–2630.

Losos, J. B. (2009). *Lizards in an evolutionary tree. Ecology and adaptive radiation of anoles*. Berkeley, CA: University of California Press.

Losos, J. B. (2010). Adaptive radiation, ecological opportunity, and evolutionary determinism. *The American Naturalist*, *175*, 623–639.

McCutcheon, I. E., Metcalfe, J., Metzenberg, A. B., & Ettinger, T. (1982). Organ growth in hyperoxic and hypoxic chick embryos. *Respiration Physiology*, *50*, 153–163.

Nagy, L., & Grabherr, G. (2009). *The biology of alpine habitats*. Oxford, U.K.: Oxford University Press.

Nicholas, K. B., Nicholas, H. B., & Deerfield, D. W. (1997). GeneDoc: Analysis and visualization of genetic variation. *Embnew News*, *4*, 14.

Nylander, J. A. A. (2004). *MrModeltest v2*. (Program distributed by the author). Uppsala University, Uppsala, Sweden.

Olsson, M. M., Gullberg, A., Shine, R., Madsen, T., & Tegelstrom, H. (1996). Paternal genotype influences incubation period, offspring size, and offspring shape in an oviparous reptile. *Evolution*, *50*, 1328–1333.

Parker, S. L., & Andrews, R. M. (2006). Evolution of viviparity in sceloporine lizards: In utero Po_2 as a developmental constraint during egg retention. *Physiological and Biochemical Zoology*, *79*, 581–592.

Parker, S. L., Andrews, R. M., & Mathies, T. (2004). Embryonic responses to variation in oviductal oxygen in the lizard *Sceloporus undulatus* from New Jersey and South Carolina, USA. *Biological Journal of the Linnean Society*, *83*, 289–299.

Pincheira-Donoso, D. (2011). Predictable variation of range-sizes across an extreme environmental gradient in a lizard adaptive radiation: Evolutionary and ecological inferences. *PLoS One*, *6*, e28942.

Pincheira-Donoso, D., Bauer, A. M., Meiri, S., & Uetz, P. (2013). Global taxonomic diversity of living reptiles. *PLoS One*, *8*, e59741.

Pincheira-Donoso, D., Harvey, L. P., & Ruta, M. (2015). What defines an adaptive radiation? Macroevolutionary diversification dynamics of an exceptionally species-rich continental lizard radiation. *BMC Evolutionary Biology*, *15*, 153.

Pincheira-Donoso, D., Hodgson, D. J., & Tregenza, T. (2008). The evolution of body size under environmental gradients in ectotherms: Why should Bergmann's rule apply to lizards? *BMC Evolutionary Biology*, *8*, 68.

Pincheira-Donoso, D., & Hunt, J. (2017). Fecundity selection theory: Concepts and evidence. *Biological Reviews*, *92*, 341–356.

Pincheira-Donoso, D., & Meiri, S. (2013). An intercontinental analysis of climate-driven body size clines in reptiles: No support for patterns, no signals of processes. *Evolutionary Biology*, *40*, 562–578.

Pincheira-Donoso, D., & Núñez, H. (2005). Las especies chilenas del género *Liolaemus*. Taxonomía, sistemática y evolución. *Publicación Ocasional del Museo Nacional de Historia Natural de Chile*, *59*, 1–487.

Pincheira-Donoso, D., Scolaro, J. A., & Sura, P. (2008). A monographic catalogue on the systematics and phylogeny of the South American iguanian lizard family Liolaemidae (Squamata, Iguania). *Zootaxa*, *1800*, 1–85.

Pincheira-Donoso, D., & Tregenza, T. (2011). Fecundity selection and the evolution of reproductive output and sex-specific body size in the *Liolaemus* lizard adaptive radiation. *Evolutionary Biology*, *38*, 197–207.

Pincheira-Donoso, D., Tregenza, T., & Hodgson, D. J. (2007). Body size evolution in South American *Liolaemus* lizards of the *boulengeri* clade: A contrasting reassessment. *Journal of Evolutionary Biology*, *20*, 2067–2071.

Pincheira-Donoso, D., Tregenza, T., Witt, M. J., & Hodgson, D. J. (2013). The evolution of viviparity opens opportunities for lizard radiation but drives it into a climatic cul-de-sac. *Global Ecology and Biogeography*, *22*, 857–867.

Pyron, R. A., & Burbrink, F. T. (2014). Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. *Ecology Letters*, *17*, 13–21.

R Development Core Team (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford, U.K.: Oxford University Press.

Schulte, J. A., Macey, J. R., Espinoza, R. E., & Larson, A. (2000). Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: Multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biological Journal of the Linnean Society*, *69*, 75–102.

Seinfeld, J. H., & Pandis, S. N. (2016). *Atmospheric chemistry and physics: From air pollution to climate change*. Hoboken, NJ: John Wiley & Sons.

Shine, R. (1999). Egg-laying reptiles in cold climates: Determinants and consequences of nest temperatures in montane lizards. *Journal of Evolutionary Biology*, *12*, 918–926.

Shine, R. (2004). Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution*, *58*, 1809–1818.

Shine, R. (2005). Life-history evolution in reptiles. *Annual Review of Ecology, Evolution and Systematics*, *36*, 23–46.

Shine, R. (2015). The evolution of oviparity in squamate reptiles: An adaptationist perspective. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *324*, 487–492.

Shine, R., Barrott, E. G., & Elphick, M. J. (2002). Some like it hot: Effects of forest clearing on nest temperatures of montane reptiles. *Ecology*, *83*, 2808–2815.

Shine, R., & Harlow, P. S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology*, *77*, 1808–1817.

Sites, J. W., Reeder, T. W., & Wiens, J. J. (2011). Phylogenetic insights on evolutionary novelties in lizards and snakes: Sex, birth, bodies, niches, and venom. *Annual Review of Ecology, Evolution and Systematics*, *42*, 227–244.

Triant, D. A., & De Woody, J. A. (2007). The occurrence, detection, and avoidance of mitochondrial DNA translocations in mammalian systematics and phylogeography. *Journal of Mammalogy*, *88*, 908–920.

Waldman, B., & Ryan, M. J. (1983). Thermal advantages of communal egg mass deposition in wood frogs (*Rana sylvatica*). *Journal of Herpetology*, *17*, 70–72.

Warburton, S. J., Hastings, D., & Wang, T. (1995). Responses to chronic hypoxia in embryonic alligators. *Journal of Experimental Zoology*, *273*, 44–50.

BIOSKETCH

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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