

Exceptional parallelisms characterize the evolutionary transition to live birth in phrynosomatid lizards

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EXCEPTIONAL PARALLELISMS CHARACTERIZE THE EVOLUTIONARY TRANSITION TO LIVE BIRTH IN PHRYNOSOMATID LIZARDS

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Abstract

Viviparity is an evolutionary innovation that enhances maternal protection of developing embryos relative to egg-laying ancestors. The behavioral, physiological, morphological, and life history pathways underpinning this innovation, however, remain unclear. We capitalized on the repeated origin of viviparity in phrynosomatid lizards to tease apart the phenotypic patterns associated with evolutionary transitions to live birth. We detected tandem reductions in mass-specific metabolic rate and mass-specific production in viviparous lineages, in turn reflecting decreases in thermal physiology and fecundity, respectively. These pathways reduce the energetic burden of viviparity without concomitant reductions in offspring body size. Although viviparous lizards are more prevalent in cold environments, transitions in thermal habitat only weakly predict parity mode evolution. Likewise, only cold tolerance adapts rapidly to thermal environment. Heat tolerance and preferred body temperatures track the thermal environment, but with a lag at million-year timescales. This lag likely reflects behavioral buffering: viviparous lizards thermoregulate to low body temperatures, regardless of ambient conditions. Rather than representing an adaptation to cold climates, the lower thermal and metabolic physiology of viviparous species are likely an energetic adjustment for reproduction that facilitated their prolific colonization of cooler environments.

Introduction

Live-bearing (viviparity) is a major evolutionary novelty in the tree of life. Viviparity is a parental care strategy that enhances physiological control and protection of developing embryos, providing higher offspring survivorship^{1,2,3,4}. The evolution of viviparity within animals is prolific, with at least 160 independent origins, particularly in squamate reptiles (>100 origins)^{5,6,7}. Complete embryogenesis within the female reproductive tract is accompanied by physiological and reproductive adjustments: compared to oviparous counterparts, viviparous species often exhibit lower metabolic rates⁸ and reduced production (understood as energy allocated into reproduction)⁹. Although the transition from egg-laying to live-bearing has repeatedly arisen, the evolutionary pathways guiding metabolic and reproductive adjustments in viviparous lineages remain unclear.

Mass-specific metabolic rate is affected by both body mass and body temperature (see equation in Methods)^{10,11}. These two traits provide three different combinations that could result in a lower ratio by which energy is acquired and allocated into survival, growth, and reproduction¹⁰ in viviparous species (**Table 1a**). In another way, mass-specific production is determined by the trade-off between offspring mass and annual fecundity (the number of hatchlings or eggs per year), normalized by maternal body mass^{9,12}. Thus, three phenotypic pathways could explain the lower mass-specific production of viviparous species (**Table 1b**). Other combinations could result in a lower mass-specific metabolic rate or a lower mass-specific production, but will depend on the magnitude of each individual response. For example, if body mass and body temperature decrease in tandem, then the effect on mass-specific metabolic rate (either to increase or decrease) depends on the relative magnitudes of those shifts.

Table 1. Three trait combinations could explain the lower mass-specific metabolic rate of viviparous species (**a**) and three other trait combinations could explain their lower mass-specific production (**b**). Note that phrynosomatids are ancestrally oviparous and there are no back-transitions to oviparity. Therefore, our hypotheses are structured around explaining the transition to viviparity (rather than the other way around).

a. Trait combinations resulting in a lower mass-specific metabolic rate

- | | |
|------------|--|
| <i>i</i> | Body mass is similar among oviparous and viviparous species, but body temperature is lower in viviparous species. |
| <i>ii</i> | Body mass is higher and body temperature is lower in viviparous species. |
| <i>iii</i> | Body mass is higher in viviparous species, but body temperature is similar among oviparous and viviparous species. |

b. Trait combinations resulting in a lower mass-specific production

- | | |
|-----------|--|
| <i>iv</i> | Offspring size is similar between oviparous and viviparous species, but annual fecundity is lower in viviparous species. |
| <i>v</i> | Offspring size and annual fecundity are lower in viviparous species. |
| <i>vi</i> | Offspring size is smaller in viviparous species, but annual fecundity is similar between oviparous and viviparous species. |

The repeated evolution of viviparity among closely-related species provides a naturally replicated framework in which to test for signatures of adaptation. Squamate reptiles (lizards and snakes) account for 75% of the evolutionary origins of viviparous vertebrates⁶. Here, we leveraged the prolific evolution of viviparity in phrynosomatid lizards, a lineage renowned for repeated transitions to live birth¹³, to investigate the evolutionary dynamics of behavior, physiology, morphology, and life history associated with viviparity. This diverse lizard family is comprised of 163 species distributed from North to Central America, and at elevations ranging from sea level to nearly 5,000 meters^{13,14,15}. Here, we addressed three goals. First, we tested whether in the transition from oviparity to viviparity, mass-specific metabolic rate and mass-specific production lower simultaneously, or whether these features can vary independently of one another in viviparous lineages. Second, we elucidated the evolutionary adjustments between body mass and body temperature resulting in the shift to lower mass-specific metabolic rate of viviparous species (**Table 1a**). Third, we clarified the combinations of offspring mass and offspring number (annual fecundity) resulting the shift to lower mass-specific production of viviparous species (**Table 1b**).

We began by building a phylogeny for phrynosomatid lizards (Supplementary Fig. 1) and reconstructing parity mode across the tree. Our reconstructions strongly support five independent shifts to live-bearing in phrynosomatids (**Fig. 1a**; Supplementary Fig. 2). We then assembled a dataset of adult body mass, adult body size (snout-vent length; SVL), thermoregulatory behavior (field-estimated body temperatures, laboratory preferred temperatures, and field-measured thermoregulatory effectiveness), thermal physiology (critical thermal limits), metabolic physiology (mass-specific metabolic rate), and life history traits (offspring mass, offspring size, clutch/litter size, and annual mass-specific

production) by gathering newly collected and previously published data from 125 phrynosomatid species (80 oviparous and 45 viviparous species) (Supplementary Data 2). To connect phenotypic variation to the local thermal environment, we also included estimates of mean annual temperature, mean temperature of the warmest quarter, and mean temperature of the coldest quarter for each species. Our dataset encompasses 77% of phrynosomatids, and includes representatives from all viviparous sub-lineages. We then fitted a series of evolutionary models to the behavioral, physiological, morphological, and reproductive data to determine the phenotypic dynamics associated with shifts from oviparity to viviparity. To contextualize these results, we explore the relationships between thermoregulatory behavior and environmental variables using evolutionary regressions.

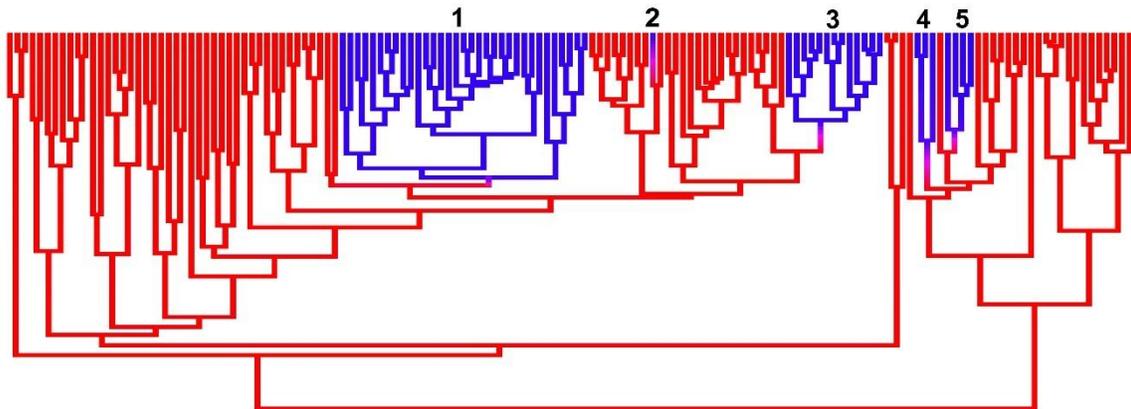
Results and Discussion

Our evolutionary models confirm that the transitions from egg-laying to live birth are consistently associated with tandem reductions in the phenotypic optimum (θ parameter; see Methods) for mass-specific metabolic rate and mass-specific production (**Fig. 1b**). Compared with egg-laying species, shifts to viviparity induced an 1.8-fold reduction in the optimal mass-specific metabolic rate and a 2.4-fold reduction in optimal annual production (Supplementary Table 2). In other words, viviparity in phrynosomatids represents a multidimensional phenotype in which the ratio and quantity of energy allocated into maintenance and reproduction is decreased.

Our results can be supported via a combination of metabolic and life history theory^{10,12,16,17}. In low-predation environments, populations evolve toward a lower metabolic rate and lower reproductive allotment¹⁶. Species with lower mass-specific metabolic rate also exhibit reduced mass-specific production, and are positioned on the

slow end of the fast-slow life history continuum¹⁷. Given these premises and assuming steady-state populations—populations in which energy invested into production (birth rates) equals energy lost by predation (death rates)^{10,12,18}—we infer that viviparity in phrynosomatids is a high-survivorship, low-fecundity phenotype positioned on the slow end of the fast-slow continuum. This notion is supported by the inherent maternal protection and mortality reduction afforded by *in utero* embryonic development (in comparison to eggs laid in nests) against abiotic and biotic hazards^{2,3,4,19,20,21,22} and with the common distribution of viviparous species in colder environments such as high elevation^{13,22}, where predation risk for ectotherms is lower^{23,24,25}. Whereas viviparous females can replace themselves each generation by allocating less energy into maintenance and production (normalized by body mass), oviparous females must instead expend greater energy into producing more offspring per year (**Fig. 1b**).

a. Evolutionary Transitions to Live Birth



b. Evolutionary Optimal Trait Values (θ)

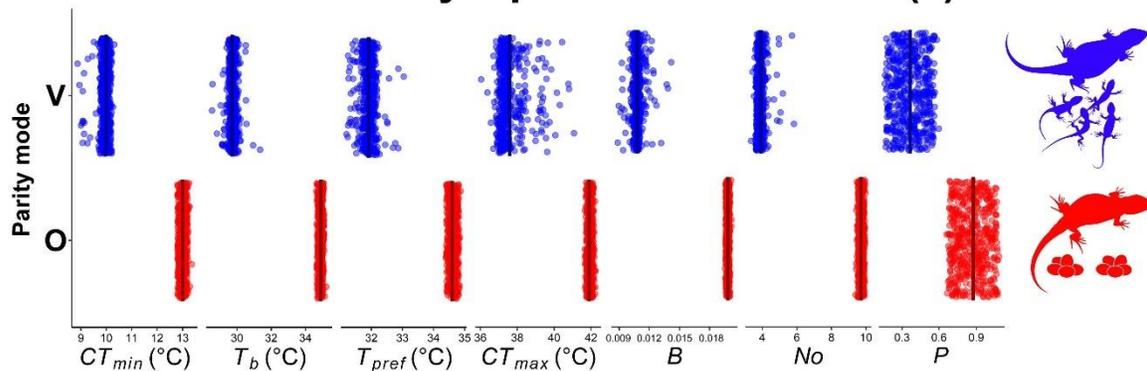


Fig. 1 Parallel reductions in physiological and life history traits are associated with viviparity in phrynosomatid lizards. **a** Five evolutionary transitions from egg-laying (red) to live-bearing (blue) occurred in phrynosomatids. **b** Viviparous lineages are characterized by reductions in the evolutionary optimal trait values (θ) for cold tolerance (CT_{min}), field-estimated body temperature (T_b), the laboratory-measured preferred body temperature (T_{pref}), heat tolerance (CT_{max}), mass-specific metabolic rate (B), the annual number of offspring (No), and mass-specific production (P). Evolutionary optimal trait values (θ) were inferred from the Ornstein-Uhlenbeck (OU) model-fitting procedure (see Methods). Each point represents a different stochastic character map from our analyses ($n=500$ per trait).

Pathways for a reduced mass-specific metabolic rate

Reductions in mass-specific metabolic rate might reflect different evolutionary pathways involving changes in body mass, body temperature, or both (**Table 1a**). We do not find any support for adaptive shifts in body mass associated with viviparity in phrynosomatids (Supplementary Table 2). Phylogenetically-corrected body size (SVL), which is strongly correlated with body mass ($\log_{10}\text{body mass} = 0.288\log_{10}\text{SVL} + 1.522$, $p=0$; Supplementary Table 4), was positively correlated with offspring size ($\log_{10}\text{offspring size} = 0.289\log_{10}\text{SVL} + 0.906$, $p<0.001$; **Fig. 2a**; Supplementary Table 4) and litter/clutch size ($\log_{10}\text{litter or clutch size} = 1.132\log_{10}\text{SVL} - 1.236$, $p<0.001$; **Fig. 2b**; Supplementary Table 4). Therefore, whether viviparous or oviparous, larger females produce larger neonates, and are more fecund. Correspondingly, we infer that during the transition to live birth in phrynosomatids, the evolutionary optimal body size in females²⁶ ($\theta = 61.1$ mm SVL; Supplementary Table 2) remains unchanged because any size shift also affects the quality and quantity of offspring.

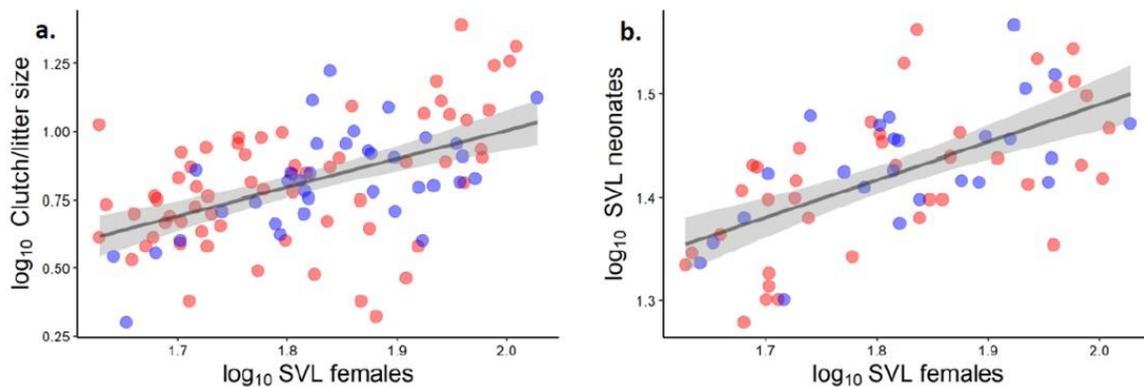


Fig. 2 Influence of dam's body size on clutch/litter size (**a**; $y = 1.1321x - 1.2362$, $p < 0.01$) and on the body size of neonates (**b**; $y = 0.2893x + 0.9064$, $p < 0.01$) in phrynosomatid lizards. Blue circles correspond to viviparous species whereas red circles correspond to oviparous species. 95% confidence bands are included around regression lines.

In contrast to body mass, however, transitions to viviparity are associated with a 5°C reduction in the optimal body temperature ($\theta = 29.7^\circ\text{C}$) when compared to their egg-laying counterparts ($\theta = 34.9^\circ\text{C}$; **Fig. 1b**; Supplementary Table 2). Therefore, reductions in core temperature, but not body mass, characterize the transition to live birth in Phrynosomatidae (corresponding to hypothesis *i* in **Table 1a**). Three non-mutually exclusive hypotheses could explain the lower body temperatures of viviparous species²⁷. First, during pregnancy (and especially during late pregnancy), body mass increases and females tend to reduce their body temperature^{28,29}. This adjustment allows pregnant females to maintain a lower mass-specific metabolic rate. Second, incubation temperature influences the phenotype and survivorship of developing embryos^{3,30}. The optimal temperature for embryonic development in nests³¹ tends to be lower than the optimal body temperature of oviparous adults ($\theta = 34.9^\circ\text{C}$). Correspondingly, it is possible that in the transition to live birth, selection favors a lower preferred body temperature, which could better match the optimal temperature for intrauterine embryonic development. These two possibilities are not mutually exclusive and could operate in tandem. Therefore, the lower body temperatures (and lower mass-specific metabolic rate) could metabolically compensate for longer gestation length, optimize embryonic development, and reduce embryonic mortality^{28,30}. A third idea centers around physiological adjustments to cold environments. The disproportionate representation of viviparous species in cooler habitats¹³ could have resulted in adaptive shifts to a more cool-adjusted thermal physiology (*e.g.*, lower cold tolerance (CT_{min}), heat tolerance (CT_{max}), preferred body temperature (T_{pref}), and body temperature (T_b)). If this were true, then we would expect a strong evolutionary match between the local thermal environment and species' thermal physiological traits.

Consistent with the hypothesis of cold-environment adaptation, we discovered that viviparous phrynosomatids also evolved a lower optimal cold tolerance ($\theta = 9.95$ °C) than the oviparous species ($\theta = 13$ °C; **Fig. 1b**; Supplementary Table 2). We also found a strong positive relationship between mean annual temperature and cold tolerance in both oviparous and viviparous species (**Fig. 3a**; Supplementary Table 4). This relationship is matched by a rapid pace of cold tolerance adaptation ($t_{1/2} = 0$ million year for viviparous and oviparous species; Supplementary Table 5). Thus, the reduced cold tolerance of viviparous species can be explained simply by their overrepresentation in cooler environments¹³. Enhanced cold tolerance in cooler environments, regardless of parity mode, fits into a broader picture of ecophysiological evolution in ectotherms. Specifically, cold tolerance is generally labile³² and often correlates tightly with the minimum temperatures ectotherms experience in their environments³³. This tight connection often reflects limited opportunities to behaviorally buffer against the cold, which results in rapid cold adaptation to prevailing conditions³⁴.

We also detected parallel evolutionary reductions in the phenotypic optimum for heat tolerance ($\theta = 37.6$ °C for viviparous and 41.9 °C for oviparous species; **Fig. 1b**; Supplementary Table 2). However, heat tolerance is uncorrelated with the thermal environment (**Fig. 3d**; Supplementary Table 4), in turn reflecting a much longer phylogenetic half-life for this trait ($t_{1/2} = 8.8$ million years for viviparous, and $t_{1/2} = 17.8$ million years for oviparous species; Supplementary Table 5). In addition to thermal limits, the preferred body temperature is lower in viviparous species than in oviparous species ($\theta = 31.9$ °C for viviparous and 34.6 °C for oviparous species; **Fig. 1b**; Supplementary Table 2). Yet, mean annual temperature is a strong predictor of preferred body temperature only in oviparous species (**Fig. 3c**; Supplementary Table 4). Likewise, the preferred body

temperature in oviparous species evolves rapidly in response to shifts in mean annual temperature ($t_{1/2}$ = 0.8 million years; Supplementary Table 5), whereas the pace of T_{pref} adaptation is considerably slower in viviparous species ($t_{1/2}$ = 13.8 million years; Supplementary Table 5). Together, these results imply that reductions in CT_{max} and T_{pref} in viviparous species are at least partially decoupled from shifts in the thermal environment itself. Rather, we suspect that viviparous species are cold-adapted for reasons besides their prevalence in colder habitats.

Consistent with this idea, the field-measured body temperature of viviparous species is poorly correlated with mean annual temperature (**Fig. 3b**), or with any of our environmental variables (Supplementary Table 4). In contrast, mean annual temperature is positively correlated with body temperature in oviparous lizards (**Fig. 3b**), although the strength of the relationship is weak. Correspondingly, body temperature exhibits a slow pace of adaptation with respect to mean annual temperature in phrynosomatids ($t_{1/2}$ = 8.5 million years for viviparous species, and $t_{1/2}$ = 13.8 million years for oviparous species; Supplementary Table 5). Whether found in relatively cool habitats or in relatively warm habitats (Supplementary Fig. 3), viviparous lizards generally exhibit a lower core temperature than their oviparous counterparts. Yet, regardless of parity mode, thermoregulatory effectiveness (the ability to maintain core temperature within the preferred range) is indistinguishable among parity modes in these lizards ($F_{1,62}$ = 0.112, p = 0.9; mean E = $0.8 \pm 0.02SE$ for both parity modes), meaning that all phrynosomatids are relatively effective thermoregulators. Even when viviparous species are found in warm habitats and oviparous species are found in cool habitats, lizards behaviorally maintain their body temperature within (or close to) their respective preferred thermal ranges.

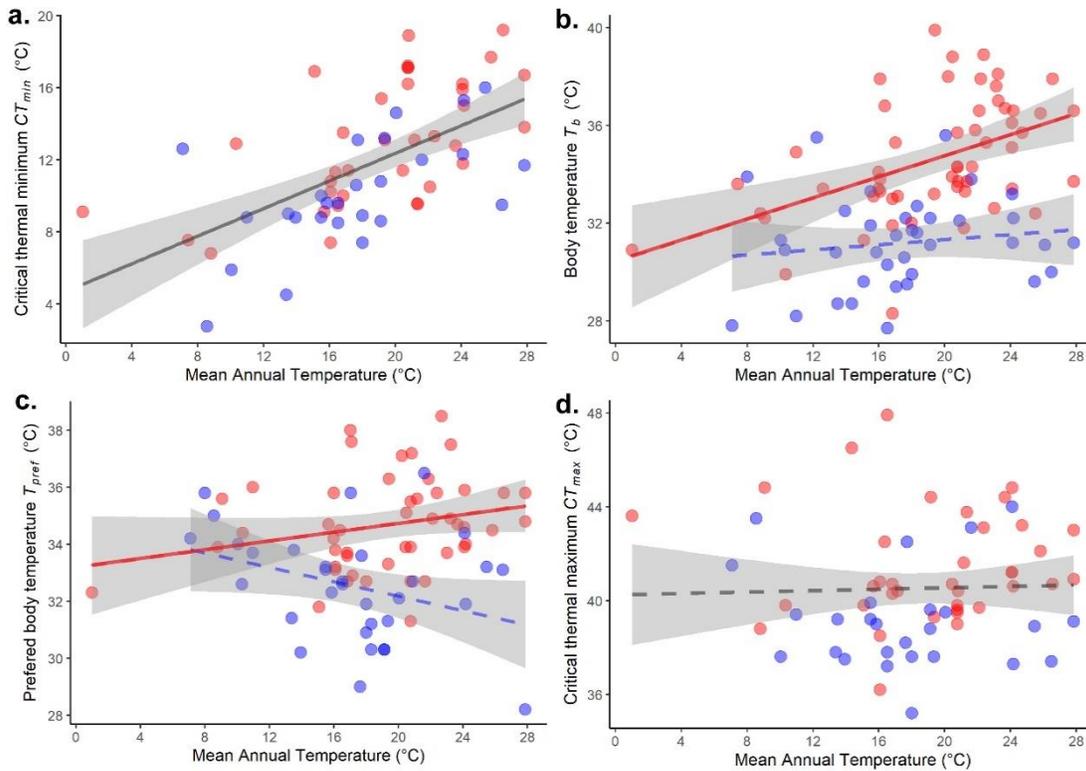


Fig. 3 Influence of mean annual temperature (MAT) on cold tolerance (**a**; $y=0.354x + 5.639$, $p=0$), field-body temperature (**b**; for oviparous: $y=0.230x + 30.3$, $p=0$, and for viviparous: $y=0.055x + 30.69$, $p=0.4$), preferred body temperature (**c**; for oviparous: $y=0.094x + 32.78$, $p=0.03$, and for viviparous: $y=-0.078x + 34.6$, $p=0.2$), and heat tolerance (**d**; $y=0.032x + 41.29$, $p=0.5$). Blue circles correspond to viviparous species whereas red circles correspond to oviparous species. Solid lines represent slopes with statistical significance (<0.05), and dashed lines represent slopes that are not statistically different from 0. 95% confidence bands are included around regression lines. PGLS results using the other macroclimatic predictors (bio10 and bio11) are given in Supplementary Table 4. Parity mode did not impact the relationship between MAT and CT_{min} (**a**) or CT_{max} (**d**); therefore, data were combined for oviparous and viviparous species (as indicated by a single gray regression line).

Furthermore, under the threshold model, we found low evolutionary covariation between environmental predictors and reproductive mode (mean annual temperature: $r = -0.205$; mean temperature of the coldest quarter: $r = -0.001$; mean temperature of the warmest quarter: $r = -0.359$), meaning that shifts in the thermal environment are not strongly associated with evolutionary transitions in parity mode. Given these results, we infer that viviparity is not an adaptation to cold climate *per se*; rather, a cool-adjusted thermal and metabolic physiology might have afforded viviparous species enhanced access into cooler environments. The notion that physiological evolution in viviparous lizards reflects life history adaptation co-opted to life in cold environments echoes ideas dating back more than half a century ago³⁵, but which lacked the behavioral, physiology, and life history trait data necessary for rigorous phylogenetic examination.

Lower fecundity reduces mass-specific production in viviparous lizards

Reductions in mass-specific production might reflect different combinations of offspring mass and annual fecundity (**Table 1b**). Yet, the optimal offspring mass ($\theta = 0.83$ g; Supplementary Table 2) and offspring size ($\theta = 26.4$ mm snout-vent-length; Supplementary Table 2) do not vary between viviparous and oviparous species. The retention of optimal offspring size³⁶ and mass in viviparous lineages could reflect the presence of a shared adaptive optimum; smaller or larger offspring sizes (based on maternal morphology), typically exhibit reduced survivorship^{37,38}. Compared with viviparous species, oviparous phrynosomatids produce ~2.5-fold more offspring per year (**Fig. 1b**; Supplementary Table 2). The lower annual fecundity and consequent lower mass-specific production in viviparous species could reflect their relatively long gestation periods, which limit most species to a single litter per year^{9,39}. Therefore, the available evidence indicates that in the

evolutionary transition to viviparity, selection favors allocating less energy into production. Because the pathway to this phenotype involves only downshifts in annual fecundity, offspring size is not compromised (corresponding to hypothesis *iv* in **Table 1b**).

The parallel evolution of viviparity in phrynosomatid lizards is accompanied by parallel phenotypic shifts in metabolic physiology, thermal physiology, thermal behavior, and fecundity. Tandem reductions in thermal physiology and fecundity clearly reduce the energetic burden of pregnancy without simultaneously impact offspring quality. Yet, even with these reductions, viviparous phrynosomatids are restricted to a single litter per year³⁹. Given that neonate size is comparable among parity modes, viviparous species are likely straddling an evolutionary tight-rope between the competing fitness demands of growth/maintenance and fecundity. The disproportionate presence of viviparous species in cold environments has fueled a decades-long interpretation of viviparity as a physiological response to lower ambient temperatures²², as opposed to a phenotype that can readily be co-opted to life in cold environments. While the inference space of our results is limited to phrynosomatid lizards, the general principles that explain evolutionary patterns in this system also characterize other ectotherm lineages, which account for nearly all origins (and species diversity) of viviparity in animals. We suspect, therefore, that the patterns we observed here might be generalizable across the animal tree of life.

Methods

Phylogeny and divergence time estimation. To estimate the phylogeny and divergence time among phrynosomatid species we used sequences of five mitochondrial and eight nuclear genes available in GenBank for 149 taxa (Supplementary Data 1). Accession numbers were the same as those used in Martínez-Méndez et al.⁴⁰ for the *Sceloporus*

torquatus, *S. poinsettii* and *S. megalepidurus* groups and the same as those in Wiens et al.⁴¹ for other phrynosomatid species. For taxa not included in the previous references, we searched GenBank for available sequences. We then performed alignments for each gene using MAFFT ver.7⁴² and concatenation and manual refinement using Mesquite ver. 3.6⁴³; obtaining a concatenated matrix of 9,837 bp for 149 taxa (the block dataset is available in the file entitled “Phrynosomatidae_Gene_Matrix.nex”, which is available in the Supplementary Material). For the relaxed clock analyses, three nodes were calibrated using lognormal distributions based on two previous studies^{41,44}. The first calibration was set for the *Sceloporus* clade (offset 15.97 million years ago (MYA)) based on a fossil *Sceloporus* specimen⁴⁵). The second calibration point was set for the *Phrynosoma* clade (offset 33.3 MYA) based on the fossil *Paraphrynosoma greeni*⁴⁶, and the last calibration point was for the *Holbrookia-Cophosaurus* stem group (offset 15.97 MYA) given the fossil *Holbrookia antiqua*⁴⁵. We conducted dating analysis with the concatenated sequences matrix, partitioned the mitochondrial and nuclear information, each gene under GTR+I+ Γ model, and allowed independent parameter estimation. We performed Bayesian age estimation with the UCLN model in BEAST version 2.5.2⁴⁷ and run on CIPRES⁴⁸. Tree prior (evolutionary model) was under the Birth-Death model, and we ran two MCMC analyses for 100 million generations each and stored every 20,000 generations. We assessed convergence and stationarity of chains from the posterior distribution using Tracer version 1.7⁴⁹. We combined independent runs using LogCombiner (version 2.5.2; BEAST distribution) and discarded 30% of samples as burn-in, obtaining values of effective sample size (ESS) greater than 200. We estimated the maximum clade credibility tree from all post-burnin trees using TREEANOTATOR ver.1.8.4⁴⁹. The ultrametric tree is available as a supplementary file “Phrynosomatidae_Tree.tre” (in the Supplementary Material). As we

describe below, we accounted for phylogenetic uncertainty in our models by re-performing analyses using 500 trees that we randomly sampled from our posterior distribution. The 500 sampled trees are available as a supplementary file “Phrynosomatidae_500_Trees.tre” (in the Supplementary Material).

Data collection. Parity mode. We categorized each species as either oviparous or viviparous based on previously published databases^{13,39,50,51}, published references, and unpublished data (Supplementary Data 2). Our assignments align with other studies, except for one species, *Sceloporus goldmani*, which has been previously considered a viviparous species^{13,52}. The only available sequence in GenBank (U88290) for that species is from a male (MZFC-05458) collected in Coahuila, Mexico⁵². However, in that same locality, one of us (F. R. Méndez-de la Cruz; unpubl. data) collected two females of the same species and both laid eggs. Thus, the population of *S. goldmani* herein included is considered oviparous.

Thermal physiology. We compiled a database of four thermal physiological traits that influence the performance and fitness of ectotherms⁵³ for 104 phrynosomatid species. These data were gathered from both published sources and from our own field and laboratory work (Supplementary Data 2). The thermal physiological traits we examined were the field body temperature (T_b) of active lizards³³, the preferred body temperature (T_{pref}) in a laboratory thermal gradient⁵⁴, cold tolerance (critical thermal minimum, CT_{min}) and heat tolerance (critical thermal maximum, CT_{max}). These latter two traits (CT_{min} and CT_{max}) describe the thermal limits of locomotion; specifically, they describe the lower and upper temperatures, respectively, at which lizards fails right itself when flipped onto their

backs^{55,32}. To minimize the confounding effects of experimental design, we limited our data selection to those that were measured with similar methods. Correspondingly, our data collection approach mirrored that of the published studies from which we extracted data. If species were measured from multiple populations, we summarized physiological traits using means weighted by sample size, and also similarly weighted environmental variables. In cases where locality details, but not coordinates, were available, we georeferenced sampling sites using Google Earth (Version 7.3.3). All physiological data correspond only to adult lizards. Some studies have found that pregnant females reduce their core temperature to better match the optimal incubation temperature for their offspring²⁸. When we detected effects of reproductive condition on thermal physiology, we excluded data from pregnant (or gravid) females. To test whether physiological traits differed between sexes, we performed *t*-tests for a sub-set of 25 species (Supplementary Table 1). We did not find significant thermal physiological differences between (non-gravid/non-pregnant) females and males in T_b ($t=0.172$, $df=48$, $p=0.86$), T_{pref} ($t=-0.482$, $df=48$, $p=0.63$), CT_{min} ($t=0.742$, $df=45$, $p=0.46$) or CT_{max} ($t=-0.407$, $df=42$, $p=0.69$), so we combined data for both sexes. Ideally, we would re-run all analyses using thermal trait data from gravid/pregnant females, but such data are still lacking. Given that, in the few cases where robust data do exist, preferred temperatures in pregnant females tend to be even lower than in non-pregnant females²⁹, we suspect that our analyses provide a relatively conservative estimate of physiological differences among parity modes.

Operative temperatures. As we describe below, we were interested in estimating thermoregulatory patterns among phrynosomatid species. Doing so requires knowledge of the environmental operative temperatures (T_e) available to lizards. T_e represents the

equilibrium temperature of an animal in the absence of behavioral thermoregulation⁵⁶. We recorded T_e using previously-calibrated pipe models (made of polyvinylchloride), which were similar in shape, size, and heat gain/loss with respect to lizards of each species³³. Into each pipe model, we inserted one temperature data logger (Thermochron iButton; model DS1921G), which recorded temperature ($\pm 0.1^\circ\text{C}$) every ten minutes during the same periods during which we were measuring field-active body temperatures (T_b) in lizards. Operative temperatures were measured during a sampling period of 1-5 days for each locality, which always occurred during times of year when lizards exhibit surface activity.

Thermoregulatory effectiveness. Several studies have found that viviparous species exhibit lower body temperatures than their oviparous counterparts²⁷. Less well known, however, is whether lower body temperatures reflect a behaviorally passive property of viviparous lizards, perhaps because of their distributions in relatively cooler habitats, or whether those low body temperatures reflect a more behaviorally active decision to thermoregulate to a cooler temperature. Therefore, we were particularly interested in the thermoregulatory patterns of oviparous and viviparous species. We calculated the effectiveness of temperature regulation (E), a ratio that describes how well lizards maintain their T_b within their T_{set} range (central 50% of data of T_{pref} , T_{set25} and T_{set75}), given the operative temperatures (T_e) available in their habitat⁵⁴. We estimated E for each species following the equation proposed by Hertz et al.⁵⁴:

$$E = 1 - \overline{(db/de)}$$

where \overline{db} is the average of the accuracy of body temperature, and indicates the deviation of T_b from T_{set} range. If each $T_b < T_{set25}$, then each $db = T_{set25} - T_b$, if each $T_b > T_{set75}$, then each $db =$

$T_b - T_{set75}$, and if each T_b is within T_{set} range, then each $db=0$. \overline{db} is the average of thermal quality of the habitat, and indicates the deviation of T_e from T_{set} range. If each $T_e < T_{set25}$, then each $de= T_{set25} - T_e$, if each $T_e > T_{set75}$, then each $de= T_e - T_{set75}$, and if each T_e is within T_{set} range, then each $de=0$. Values of \overline{db} close to 0 indicate that individuals accurately maintain their body temperature within their preferred range, and values of \overline{de} close to 0 indicate that the habitat temperatures approximate (and/or fall within) the preferred range of individuals. As both \overline{db} and \overline{de} increase, body temperatures and operative temperatures, respectively, exceed species' preferred thermal ranges. As such, E values close to 1 indicate that lizards are highly effective thermoregulators, and E values close to 0 indicate that individuals are more behaviorally passive with respect to thermal environment. E was only estimated in cases where T_e and T_b were sampled during the same period, and if T_{set} was measured from the same population of lizards from which T_b was measured. In total, we were able to gather estimates of E from 64 species (37 oviparous and 27 viviparous) of phrynosomatid lizards.

Environmental temperature. In addition to the operative temperatures, which provided a detailed (but temporally limited) snapshot of thermal environment, we gathered data on general thermal trends for each species' habitat. Specifically, we also gathered climatic measurements for each locality (Supplementary Data 2) from which any lizard trait data were gathered by extracting thermal variables from the environmental layers available in the WorldClim dataset (resolved to approximately 1km^2)⁵⁷. These variables were mean annual temperature (bio1), mean temperature of the warmest quarter (bio10), and mean temperature of the coldest quarter (bio11). We did not use these data to calculate T_e for estimates of thermoregulatory effectiveness (as E should be calculated from T_e measured

during the same time period as T_b). Instead, we used these bioclimatic variables as predictors of phenotypic trait variation using evolutionary regressions as described below.

Morphology and life-history traits. We gathered published and unpublished information for mean snout-vent length (SVL; mm), a common measure of body size in squamates, and body mass (g) of adult females and neonates. We also recorded clutch or litter size (*i.e.*, the number of offspring produced per reproductive bout), and the number of clutches or litters produced during one year (Supplementary Data 2). We multiplied these two last traits to quantify annual fecundity, which reflects the total predicted annual reproductive output of a given species. We used annual fecundity for three reasons. First, in phrynosomatids (with exception of some populations of three species^{58,59,60}), females have annual (seasonal) patterns of reproduction³⁹. Second, oviparous species are able to produce eggs in multiple clutches per year⁶¹, whereas viviparous species are typically able to produce only one litter in the same unit time³⁹. Indeed, viviparous species tend to produce only one litter per year regardless of reproductive window length. For example, both *Phrynosoma hernandesi*, a species with shorter gestation (3 months)⁶² and *Sceloporus bicanthalis*, a species with continuous reproduction^{59,63}, produce a single litter per year. Third, the maximum lifespan for phrynosomatid lizards varies considerably, but does not differ between parity modes³⁶. For some species, the lifespan is typically ~1 year (documented for the oviparous species, *Sceloporus aeneus*, and the viviparous species, *Sceloporus bicanthalis*^{59,64}), whereas the maximal lifespan is ~10 years (documented for the oviparous species *Phrynosoma asio*⁶⁵ and for the viviparous species *Sceloporus macdougalli*⁶⁶). Thus, consistent with other studies^{17,51}, we consider that by standardizing production to one year, we have an estimation of reproductive output that can be readily compared among parity modes.

Mass-specific metabolic rate. We estimated individual metabolic rate (I) for female lizards following the equation proposed by Brown et al.¹⁰:

$$I = i_0 M^{3/4} e^{-E/kT}$$

where i_0 is a normalization constant, M is mean body mass (g) of females, e = Euler's number, E = activation energy, k = Boltzmann's constant, and T = field body temperature (in Kelvin). Then, I/M can be used to obtain mass-specific metabolic rate (B). SVL of phrynosomatids lizards is more frequently reported than body mass. We built a data base of body mass and SVL of adult females for 30 phrynosomatid species (none were gravid or pregnant) via a combination of unpublished and published information (Supplementary Data 2). Using these data, we built a non-phylogenetic equation to predict \log_{10} body mass from \log_{10} SVL. Our equation is \log_{10} body mass = $3.355 \log_{10}$ SVL - 5.065 ($R^2 = 0.88$, $P < 0.001$). Then, we transformed the \log_{10} body mass value to an integer value (body mass = 10^{\log_{10} body mass}). With our equation, we predicted the body mass of females for species for which SVL and field body temperature were available. Based on this approach, we obtained a total database of mass-specific metabolic rate of females for 96 phrynosomatid species (Supplementary Data 2).

Mass-specific production. We estimated mass-specific production (P) as the product of neonate mass and annual fecundity/female body mass⁹. Therefore, P describes the amount of energy converted into reproductive effort per year, normalized by maternal body mass.

Evolutionary analyses. All evolutionary analyses were conducted using the R environment for statistical computing, version 3.6.0.⁶⁷

Stochastic character mapping of parity mode. To estimate the number of transitions between parity modes we performed stochastic character mapping⁶⁸ onto the ultrametric tree of Phrynosomatidae using the *make.simmap* function with 500 simulations and a transition model of equal rates (ER) in phytools R package⁶⁹. We selected the ER model of character evolution because was it the best-supported model ($\Delta\text{AICC}=0$, weight= 0.65) in comparison to a symmetrical model (SYM; $\Delta\text{AICC}=2.1$, weight= 0.22) and with an all-rates-different model (ARD; $\Delta\text{AICC}=3.2$, weight= 0.13).

Ancestral state reconstruction. To fit mean annual temperature through Phrynosomatidae tree and graphically show the thermal environment where each population of each species used in this study inhabits, we performed ancestral state reconstruction using *contMap* function in phytools R package⁶⁹.

Phylogenetic analyses of variance (ANOVA). To test for differences in effectiveness of temperature regulation, we performed phylogenetic ANOVAs using the *aov.phylo* function with 500 simulations in phytools R package⁶⁹.

Comparing trait evolution between viviparous and oviparous species. We were interested in whether transitions to viviparity are associated with predictable phenotypic shifts. To this end, we tested if parity mode (“oviparous” or “viviparous”) was associated with different evolutionary patterns of mass-specific metabolic rate, mass-specific production, body mass and size, thermal physiological traits, and life history traits by fitting Brownian motion (BM) and Ornstein-Uhlenbeck (OU) models. To do so, we used the R package OUwie⁷⁰ and the 500 stochastic character maps of parity mode built with the *make.simmap* function in the R package phytools⁶⁹. We fitted three different models. The simplest (BM1) is a

single-rate BM model in which a single rate of stochastic trait evolution (σ^2) was estimated for all Phrynosomatidae. The other two models were all adaptive OU models that varied in whether the estimated phenotypic optimum (θ) was either constrained to be equal among parity modes (OU1), or allowed to vary between oviparous and viviparous species (OUM). We fitted these three models separately for each physiological trait (T_b , T_{pref} , CT_{min} , CT_{max} , and mass-specific metabolic rate), each morphological variable (adult body mass, and adult body size), and each life history trait (offspring mass, offspring size, annual fecundity, and mass-specific production) (Supplementary Table 2). For these (and all) analyses, body mass, body size, offspring mass, offspring size and annual fecundity were \log_{10} transformed. We assessed model fit using a modified Akaike information criterion (AICc) that incorporates a correction for small size⁷¹. Our approach, which was based on 500 stochastic character maps derived from the MCC tree, allowed us to account for uncertainty in reconstruction across the preferred tree, but could not account for uncertainty in the phylogeny itself. Therefore, we also repeated our stochastic character mapping across 500 individually-sampled trees from the posterior distribution to account for this additional source of phylogenetic uncertainty, and repeated all of our OUwie analyses using these 500 sampled trees. Our results in this latter approach are comparable to those using the MCC tree (Supplementary Table 3). Therefore, we present our results from the analyses based on the MCC tree in the main document.

Phylogenetic Generalized Least Squares (PGLS). To know the evolutionary relationship between adult body mass and adult body size, clutch or litter size and adult body size, and offspring size and adult body size, we performed PGLS regressions using the *gls* function in the R package nlme⁷².

Threshold model for ancestral state reconstruction. We were interested in whether changes in the thermal environment were strong predictors of parity mode shifts. We tested for the evolutionary covariation between the thermal environment and reproductive mode (oviparous vs. viviparous) using the threshold model^{73,74} using *threshBayes* function in the phytools R package⁶⁹. The threshold model is used to test for evolutionary covariation between continuous and discrete traits⁷⁴. Under the threshold model, a discrete character (*i.e.* oviparity or viviparity) evolves as a function of a continuously varying feature (termed “liability”). When the value of “liability” crosses a certain threshold, the state of the discrete character evolves (*i.e.*, a transition from oviparous to viviparous occurs)^{73,74}. We ran *threshBayes* for 1.0×10^6 generations, sampling every 100 generations, and discarding the first 200K generations as burn-in. We ran separate analyses for mean annual temperature (bio 1), mean temperature of the coldest quarter (bio 10), and mean temperature of the warmest quarter (bio 11).

Stochastic linear Ornstein-Uhlenbeck models. Our OUwie analyses revealed reductions in the phenotypic optimum (θ parameter) for thermal traits in viviparous lizards (see Results and Discussion). Yet, it is unclear whether reductions in thermal physiology reflect adaptation to cool environments (given the greater representation of viviparous lineages at high elevation¹³) or, instead, reflect metabolic compensation for live birth (hypothesis *i* in **Table 1a**), which could be readily co-opted for life in cold environments. If a cool-adapted physiology reflects adaptation to cool environments, there should be a strong evolutionary association between the local thermal environment and thermal physiology. However, if a cool-adapted physiology reflects metabolic compensation for the heightened costs of pregnancy, then we expect viviparous lineages to exhibit a cool-adapted physiology

regardless of ambient conditions, which should weaken the evolutionary relationship between the local thermal environment and corresponding thermal physiology.

To test these ideas, we used the SLOUCH model of Hansen et al.⁷⁵, which simultaneously estimates an “evolutionary regression” and an “optimal” regression in an OU framework. The evolutionary regression describes the observed relationship between climatic predictors (mean annual temperature (bio 1), mean temperature of the warmest quarter (bio 10), and mean temperature of the coldest quarter (bio 11)) and physiological response variables (CT_{min} , T_b , T_{pref} , and CT_{max}), while accounting for relatedness among species. The estimated “optimal regression”, in contrast, describes the relationship between these variables predicted under an OU model, and assuming adaptation of the response variables to the predictor variables. In addition to the regressions, the model permits estimation of phylogenetic half-life ($t_{1/2}$). Phylogenetic half-life represents the amount of time required for viviparous or oviparous lineages to get halfway to their thermal physiological optimum. So, a short $t_{1/2}$ (relative to the length of tree) indicates the phylogenetic signal degrades at a rapid pace. In contrast, a $t_{1/2}$ approaching (or exceeding) the length of the tree, indicates strong phylogenetic signal.

Similarity between the evolutionary and optimal regressions is supported when $t_{1/2}$ is close to 0, which would indicate that transitions in the thermal environment are rapidly coupled with changes in thermal physiology. Differences in the slopes of these relationships, in contrast, are supported when the phylogenetic half-life ($t_{1/2}$) of the model is bounded away from zero, implying phylogenetic inertia, or a lag in physiological adaptation to the thermal environment. Under this scenario, shifts in thermal environment are not strongly associated with shifts in thermal physiology. To run the analyses, we simultaneously estimated the evolutionary regression, optimal regression, and $t_{1/2}$ for each

thermal physiological trait (CT_{min} , T_b , T_{pref} , and CT_{max}) of phrynosomatid lizards, with respect to their thermal environment (bio1, bio10, and bio11) using an OU modeling framework in the R package SLOUCH⁷⁵.

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Author contributions

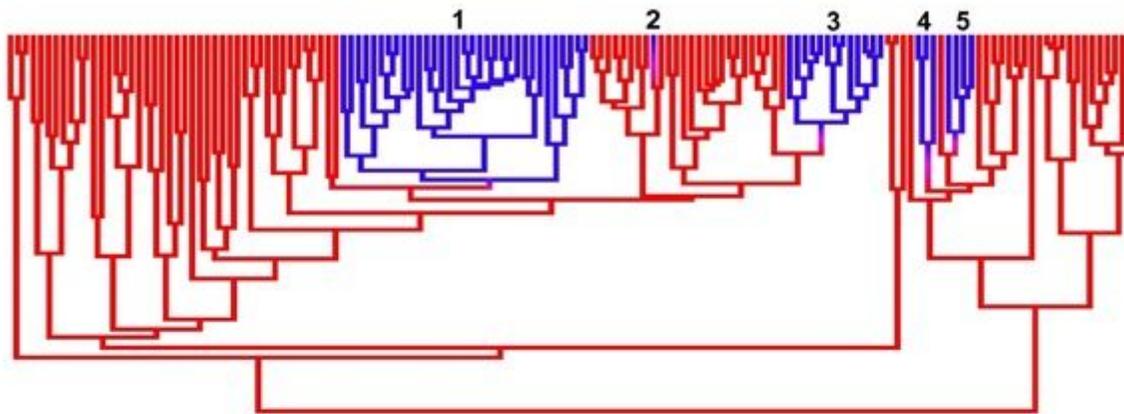
S.F.D.-G., F.R.M.-C, N.L.M.-M, M.E.O., and M.M.M. design the study. S.F.D.-G., P.G.-T., F.R.M.-C, D.M.A.-M., A.B.-M., H.G., R.A.L.-R., C.A.M.-M., F.J.M.-N., R.S.-B., J.H.V.-V., and G.A.W.-P performed field and laboratory work to obtain the physiological, morphological, and life-history data. S.F.D.-G., N.L.M.-M. and A.B.-V built the ultrametric tree. S.F.D.-G. and M.M.M. analyzed the data and drafted the manuscript. All authors contributed to subsequent revisions.

Competing interest

The authors declare no competing interests.

Figures

a. Evolutionary Transitions to Live Birth



b. Evolutionary Optimal Trait Values (θ)

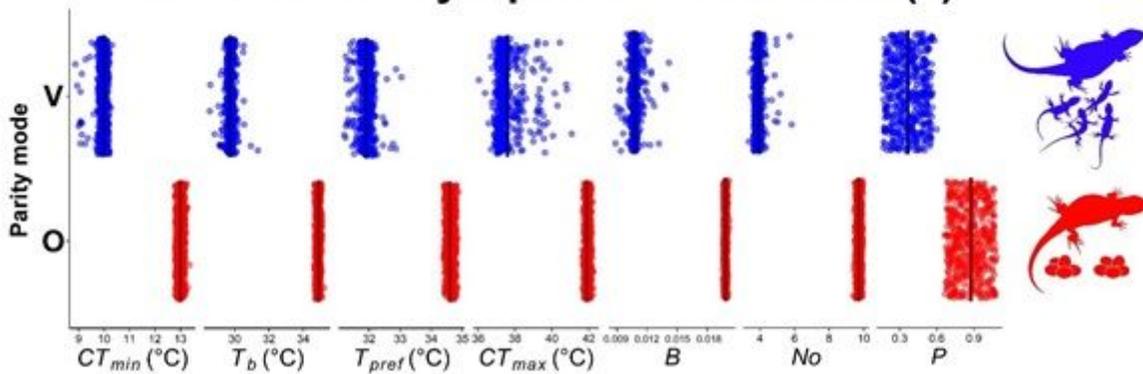


Figure 1

Parallel reductions in physiological and life history traits are associated with viviparity in phrynosomatid lizards. a Five evolutionary transitions from egg-laying (red) to live-bearing (blue) occurred in phrynosomatids. b Viviparous lineages are characterized by reductions in the evolutionary optimal trait values (θ) for cold tolerance (CT_{min}), field-estimated body temperature (T_b), the laboratory-measured preferred body temperature (T_{pref}), heat tolerance (CT_{max}), mass-specific metabolic rate (B), the annual number of offspring (No), and mass-specific production (P). Evolutionary optimal trait values (θ) were inferred from the Ornstein-Uhlenbeck (OU) model-fitting procedure (see Methods). Each point represents a different stochastic character map from our analyses ($n=500$ per trait).

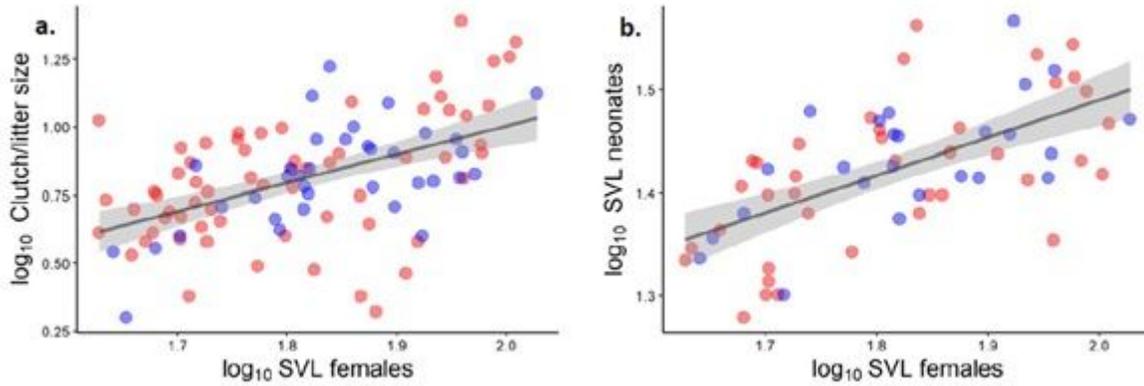


Figure 2

Influence of dam's body size on clutch/litter size (a; $y = 1.1321x - 1.2362$, $p < 0.01$) and on the body size of neonates (b; $y = 0.2893x + 0.9064$, $p < 0.01$) in phrynosomatid lizards. Blue circles correspond to viviparous species whereas red circles correspond to oviparous species. 95% confidence bands are included around regression lines.

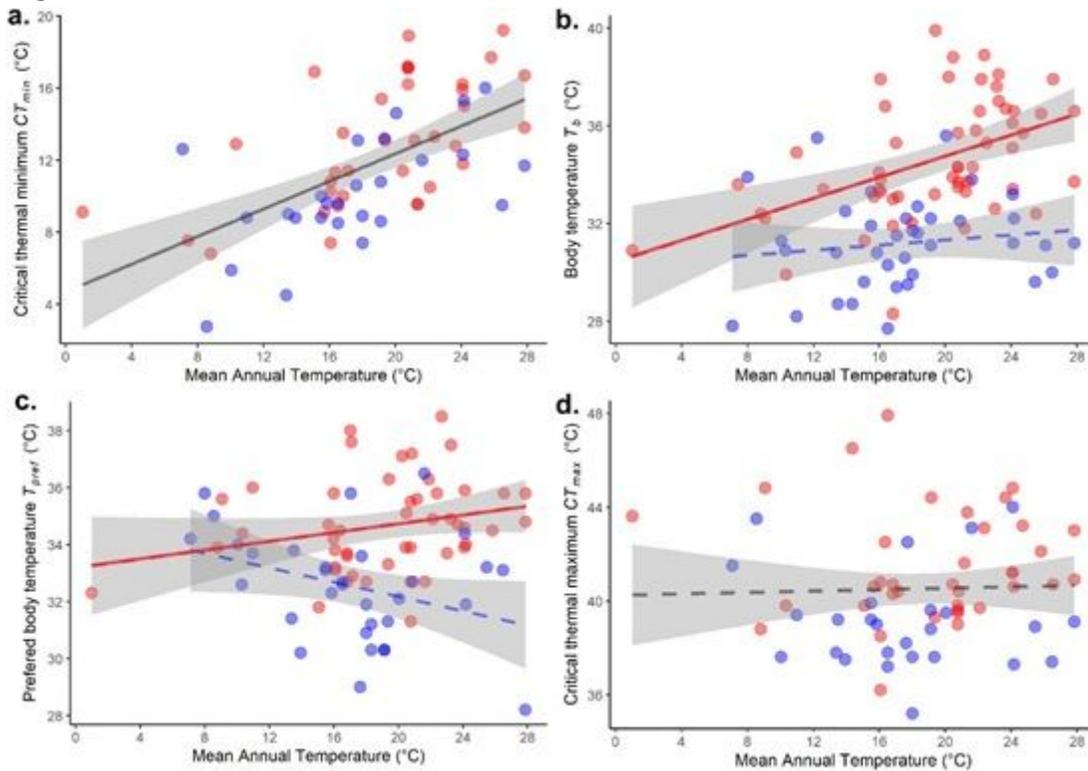


Figure 3

Influence of mean annual temperature (MAT) on cold tolerance (a; $y = 0.354x + 5.639$, $p = 0$), field-body temperature (b; for oviparous: $y = 0.230x + 30.3$, $p = 0$, and for viviparous: $y = 0.055x + 30.69$, $p = 0.4$), preferred body temperature (c; for oviparous: $y = 0.094x + 32.78$, $p = 0.03$, and for viviparous: $y = -0.078x + 34.6$, $p = 0.2$), and heat tolerance (d; $y = 0.032x + 41.29$, $p = 0.5$). Blue circles correspond to viviparous species whereas red circles correspond to oviparous species. Solid lines represent slopes with statistical significance (< 0.05), and dashed lines represent slopes that are not statistically different from 0. 95%

confidence bands are included around regression lines. PGLS results using the other macroclimatic predictors (bio10 and bio11) are given in Supplementary Table 4. Parity mode did not impact the relationship between MAT and CTmin (a) or CTmax (d); therefore, data were combined for oviparous and viviparous species (as indicated by a single gray regression line).

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